PROCEEDINGS

United States
Environmental Protection Agency
Symposium
on

WASTE TESTING and QUALITY ASSURANCE

Volume I

July 11-15, 1988

THE WESTIN HOTEL
WASHINGTON, D.C.

Symposium Managed by American Public Works Association

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 attendent, 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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July 11 – 15, 1988

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AIR AND GROUND WATER MONITORING

PROFILE OF VOLATILE ORGANIC CHEMICALS IN NASSAU COUNTY GROUND WATER

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ABSTRACT

Statistical evaluation was conducted on the ten most common organic compounds found in Nassau County groundwater during 1987. The three most common chemicals found in the ground water were tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane. Few samples were found to contain chloroform in Nassau County (3% of drinking water samples, and 6% of non-drinking water samples). Surprisingly, bromoform was found more frequently than chloroform (11% of the drinking-water samples). However, the concentrations of bromoform were very low, usually less than 5 ppb, well below the allowed limits of 50 ppb in New York state. In addition to the frequency of occurrence of organic pollutants found in the water samples, the profile of concentration range was also studied. A possible source for bromoform and its related compounds is discussed.

INTRODUCTION

The Nassau County Health Department has been monitoring the ground water since Nov. 1977 for halogenated and non-halogenated organic compounds. The heavy workloads encountered since 1985 have produced significant quantities of data and have revealed interesting perspectives on the presence of organic chemicals in Nassau County groundwater. This paper is evaluating data collected in 1987. The ten most common organic compounds found are shown in Table 1.

DRINKING & NON-DRINKING WATER

Figure 1 shows the frequency of occurrence of these organic compounds found in the ground water samples for both drinking and non-drinking categories. In 1987, a total of 973 ground water samples were analyzed. Any samples which contained organic compounds in concentrations in excess of the detection limit were considered to be positive samples. (See table 1 for the detection limits). The histogram shown in Figure 1 indicates the percentages of the positive samples as well as the percentage of the water samples with

concentrations in excess of the allowed limit (50 ppb). The three most common organic compounds found are tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane. The frequencies of these compounds in the water samples far exceed those of all other compounds. Except for the three most common organic compounds mentioned above, the frequencies of positive samples with concentrations in excess of the allowed limit are uniformly low. The previous hypothesis presented in the literature (1) regarding the in situ formation of chloroform is not in evidence since chloroform was not one of those that were found more frequently. The frequency of occurrence for brominated compounds in public water supply samples was higher than anticipated. However, as discussed below, concentrations of bromoform and related compounds were uniformly low so that there is little concern for health effects.

DRINKING WATER

Figure 2 segregates drinking water from the sample set shown in Figure 1.

The presence of bromoform and its related brominated compounds present in the drinking water samples is significant because of their high frequency of occurrence. There was little difference in the frequency profile of the brominated compounds (present in 9.5%-12.8% of the samples) and the three chlorinated compounds (present in 9.3%-15% of the samples). Brominated compounds were found in 11% of samples tested. Their concentrations were uniformly low with 94% of the samples having concentrations less than 10 ppb (see Figure 6).

The number of the water samples which contained chloroform and carbon tetrachloride were both very low. Chloroform was present in 3% of the drinking water tested with only 1% at concentrations in excess of 10 ppb. Carbon tetrachloride was present in 1% of the drinking water tested with only 0.2% at concentrations in excess of 10 ppb. These facts suggest that formation of chloroform during the chlorination process is not significant relative to the number of samples or the concentration.

NON-DRINKING WATER

If one considers the same profile for non-drinking water, the small number (3 out of 482) of non-drinking water samples containing brominated organic compounds is striking, especially in view of the fact that many drinking water samples contained brominated compounds (53 out of 491).

This is a significant finding when we consider the possible sources for these compounds. The three chlorinated compounds, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane are found in far more abundance than other organic pollutants in non-drinking water samples (Figure 3). Note that approximately 10% of the non-drinking water samples contained trichloroethylene at levels in excess of 50 ppb. Chloroform, carbon tetrachloride and other organic pollutants are found in a relatively low percentage of the non-drinking water samples (less than 1%). In addition, the number of samples with concentrations in excess of the allowed limits is relatively small (1 out of 482).

CONCENTRATION PROFILES

Figure 4 indicate the concentration profiles for the three most common chlorocompounds (tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane) for all ground water samples. It is noted that 87% of the positive samples were in a low concentration range below 50 ppb. Approximately 45% of the positive samples in this category were less than 5 ppb. Figure 5 indicates a similar concentration profile for drinking water samples. It is noted that 58% of the samples are in the range of less than 5 ppb. Figure 6 indicates the concentration profile for bromoform and its related bromo compounds for all ground water samples. It is noted that approximately 94% of the samples were in a very low concentration range below 10 ppb. No sample had a concentration above 50 ppb. Figure 7 shows the sample concentration profile for drinking water samples. It is noted that all but three samples had concentrations below 10 ppb. No sample had a concentration above 50 ppb.

THE POSSIBLE SOURCE OF BROMOFORM

One of the important questions is where does bromoform come from? The most striking fact is that almost all the positive samples were found in drinking water and practically none was found in non-drinking water samples. This indicates that bromoform does not come from contamination such as industrial spill or an illegal dumping of the chemical. In such a case bromoform would certainly have been found in non-drinking water samples, too. Therefore, the primary suspect may be some kind of treatment done to the drinking water supplies or something happening in the distribution system, ie., on the way from the well to the houses.

In examining the source of the water samples found to contain bromoform, it was noted that only one water sample was a repeat. This indicates that the positive samples for bromoform came from a wide range of sources, not from just a few samples which were analyzed repeatedly. It was also noted that 86 percent of the samples positive for bromoform had been chlorinated. Since most of the positive samples were chlorinated, it is hypothesized that bromine may have been present as a trace contaminant in the original chemical concentrate used for chlorination and may have been responsible for the formation of bromoform. However, an assay of the chlorine gas used for chlorination showed that it was a high purity substance, 99.5% pure, and there was no indication of the presence of bromine.

Arguello, etc. (2) postulated that the probable source of bromoform and other brominated trihalomethanes may be inorganic bromides in water reacting with chlorine during the chlorination of the water supplies. There is presently no data confirming the presence of inorganic bromides in the Nassau County water supplies.

CONCLUSION

The three most common chemicals found in the ground water were tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane. Chloroform, which may have been formed during chlorination of the water supplies, was found in very few water samples. Bromoform, bromodichloromethane and dibromochloromethane were found in approximately 10 % of the drinking water samples tested. However, their concentrations were so low that there is minimal cause for public health concern. All concentrations of the brominated compounds were well below the specified safe levels in the New York State Sanitary Code. It is hypothesized that bromoform may somehow have been formed during the chlorination of the water supplies, but there is no definite evidence for this postulation.

The presence of volatile halogenated organic compounds in Nassau County drinking water is being carefully studied via an ongoing routine monitoring program. Concentrations of these compounds have typically been very low, have been well below New York State Health Department Standards and do not presently represent a significant health risk.

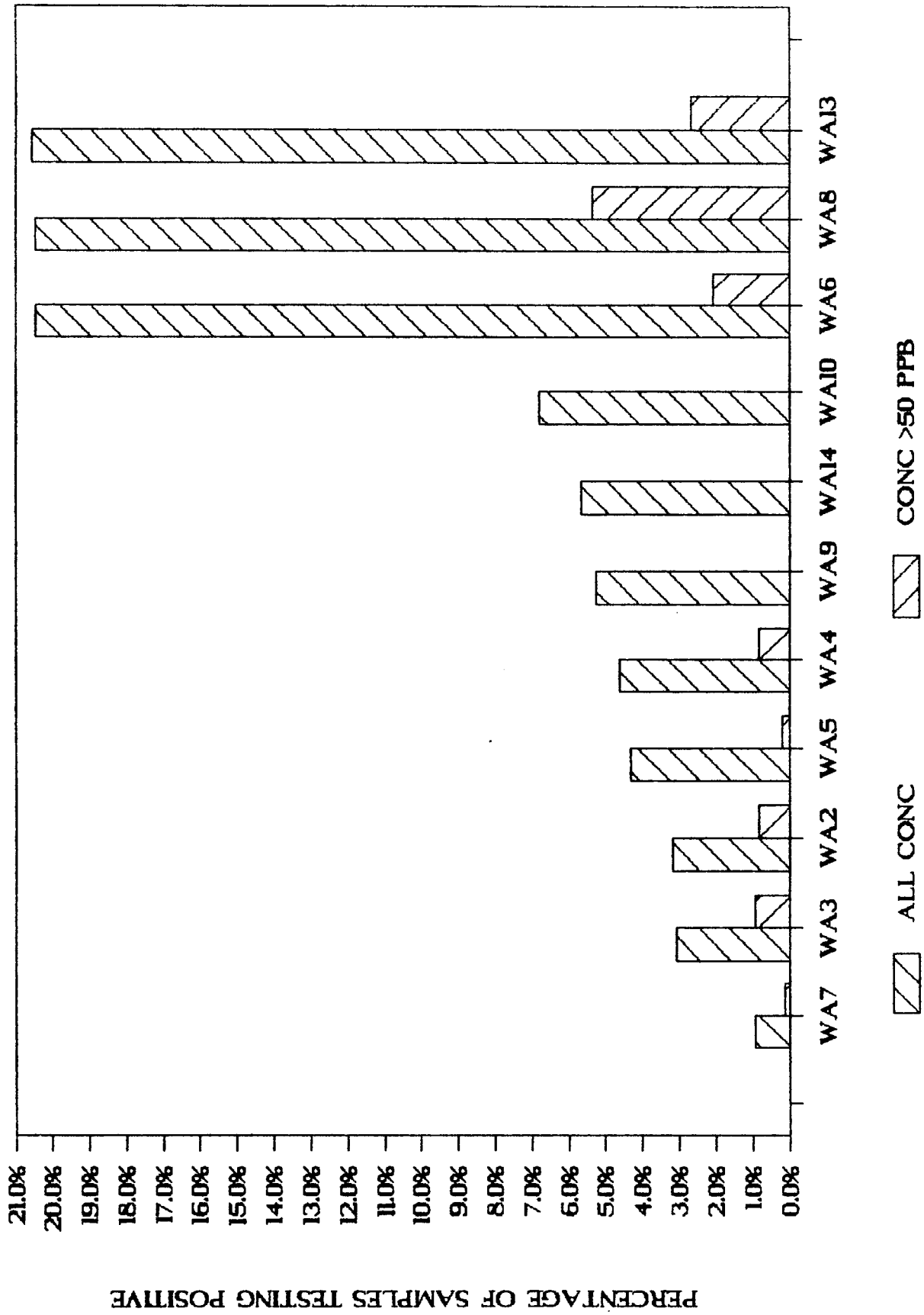
(1) Babcock, D. B. and Singer, P. C., Jour. AWWA, 1979, 71, 149.
(2) Arguello, M. D. Chriswell, C. D., Fritz, J. S., Kissinger, L. D., Lee, K. W., Richard, J. J. and Svec, H. J., Water Tech Qual, 1970, 504.

Table 1. Volatile Organic Compounds Found in Ground Water Samples

Name	Code	Detection Limit (ppb)
Tetrachloroethylene	WA13	1
Trichloroethylene	WA8	1
1,1,1-Trichloroethane	WA6	1
Dibromochloromethane	WA10	1
Bromoform	WA14	2
Bromodichloromethane	WA9	1
1,1-Dichloroethane	WA4	6
Chloroform	WA5	1
Methylene chloride	WA2	8
1,2-Dichloroethylene	WA3	11
Carbon Tetrachloride	WA7	1

FIGURE 1 POSITIVE TEST RESULTS

DRINKING & NON-DRINKING SAMPLES - 1987



A-6

FIGURE 2 POSITIVE TEST RESULTS

DRINKING WATERS TESTED IN 1987

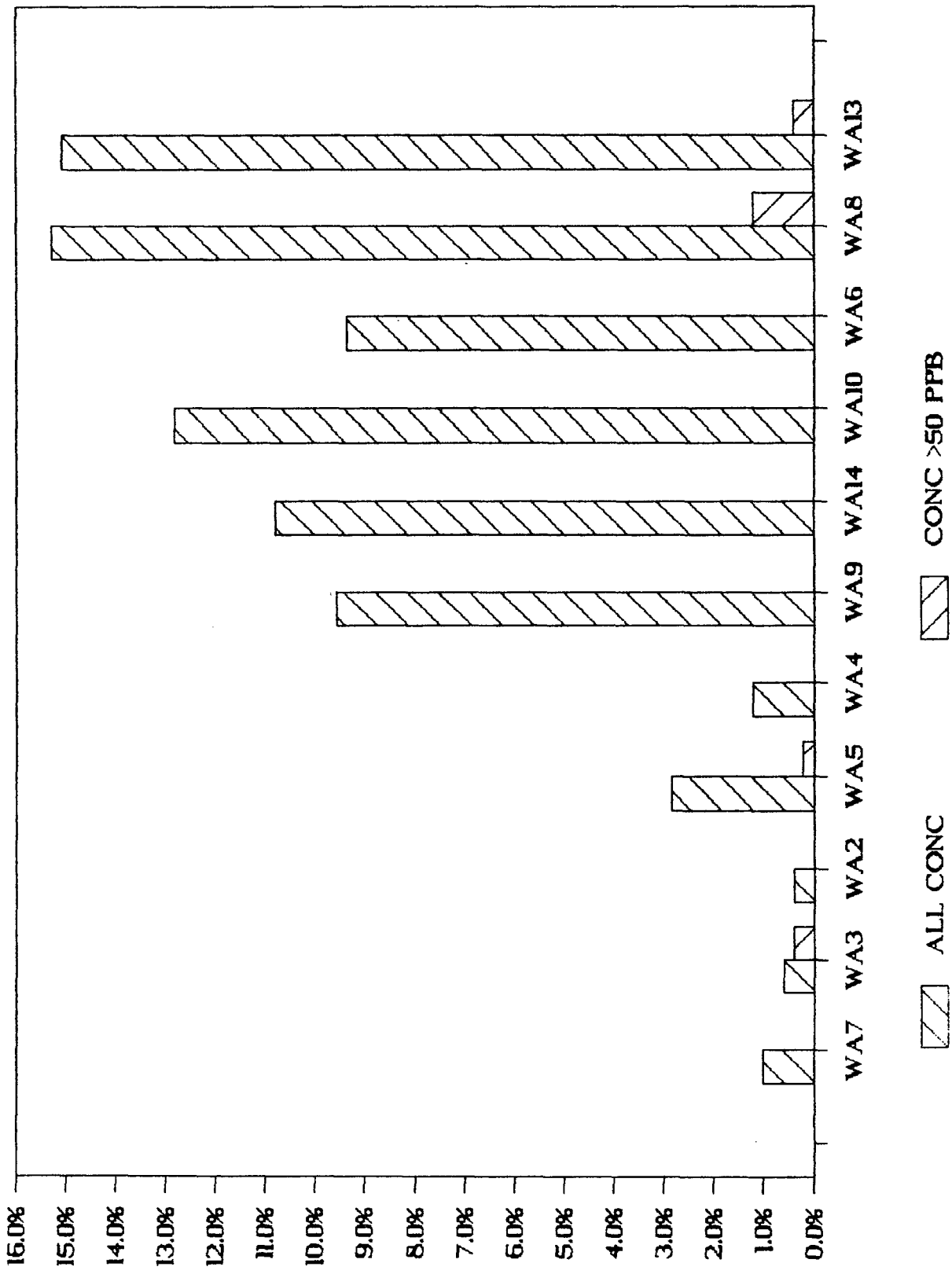


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FIGURE 3 POSITIVE TEST RESULTS

NON-DRINKING WATERS TESTED IN 1987

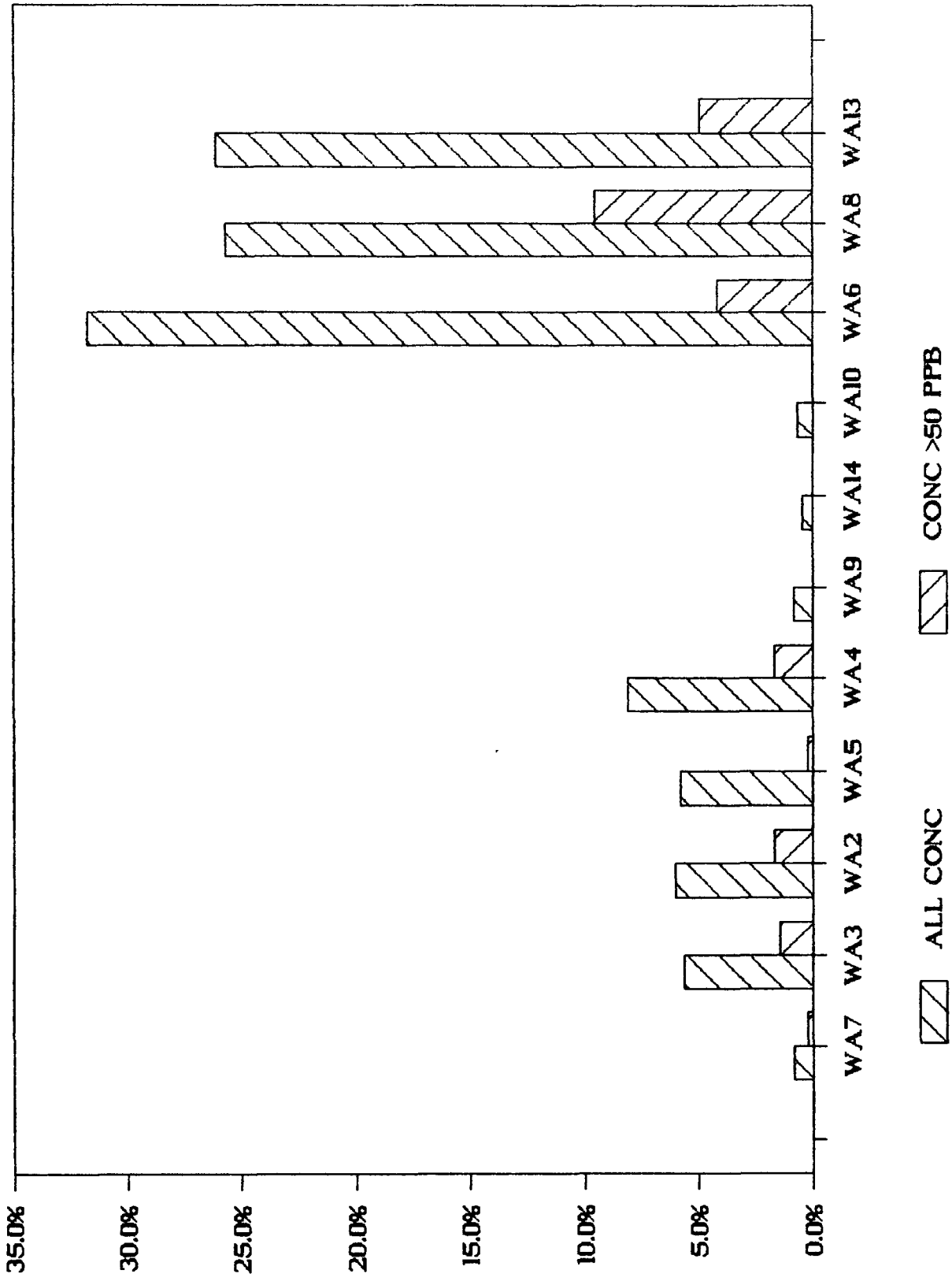

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FIGURE 4 CONCENTRATION PROFILE

DRINKING & NON-DRINKING WATERS - 1987

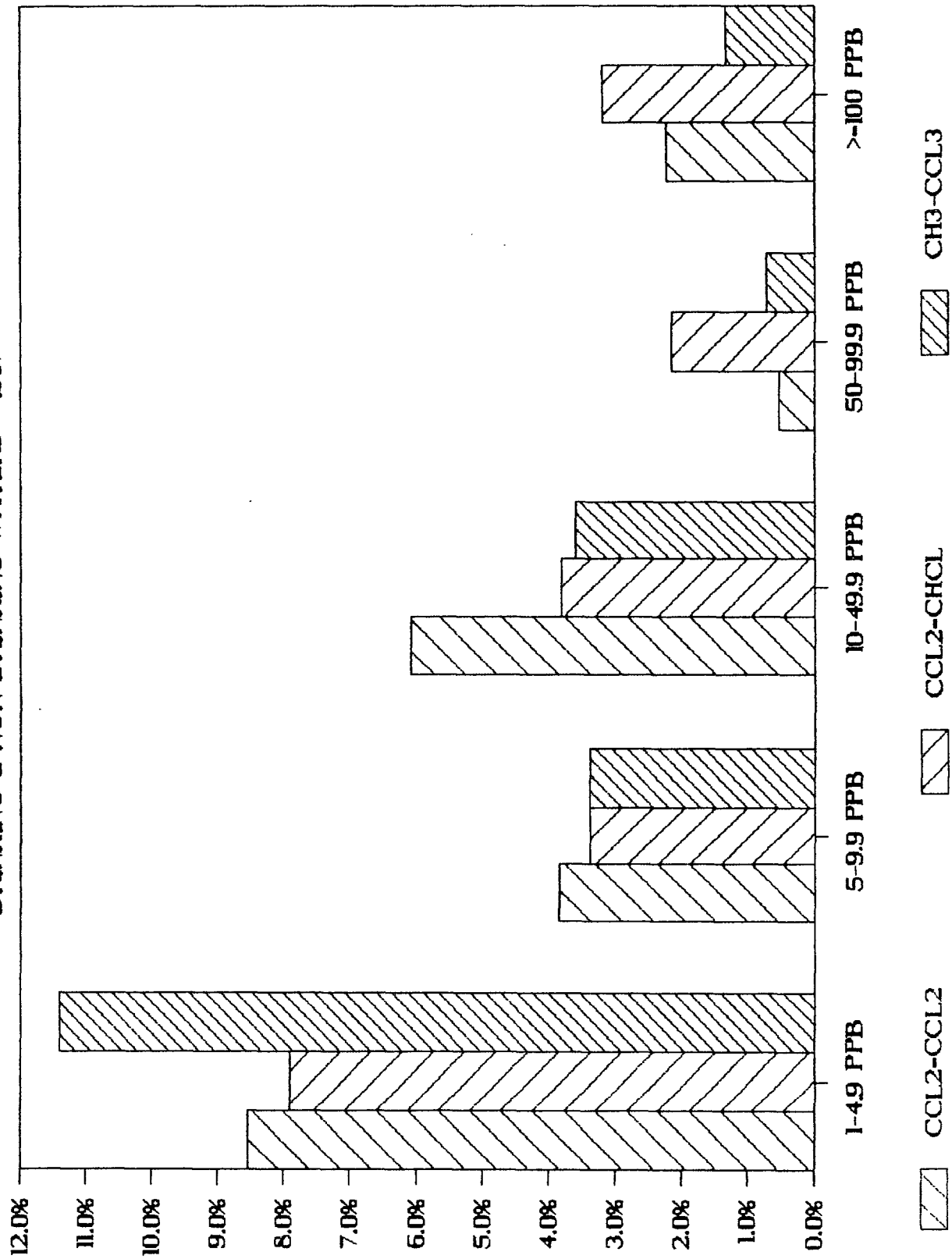
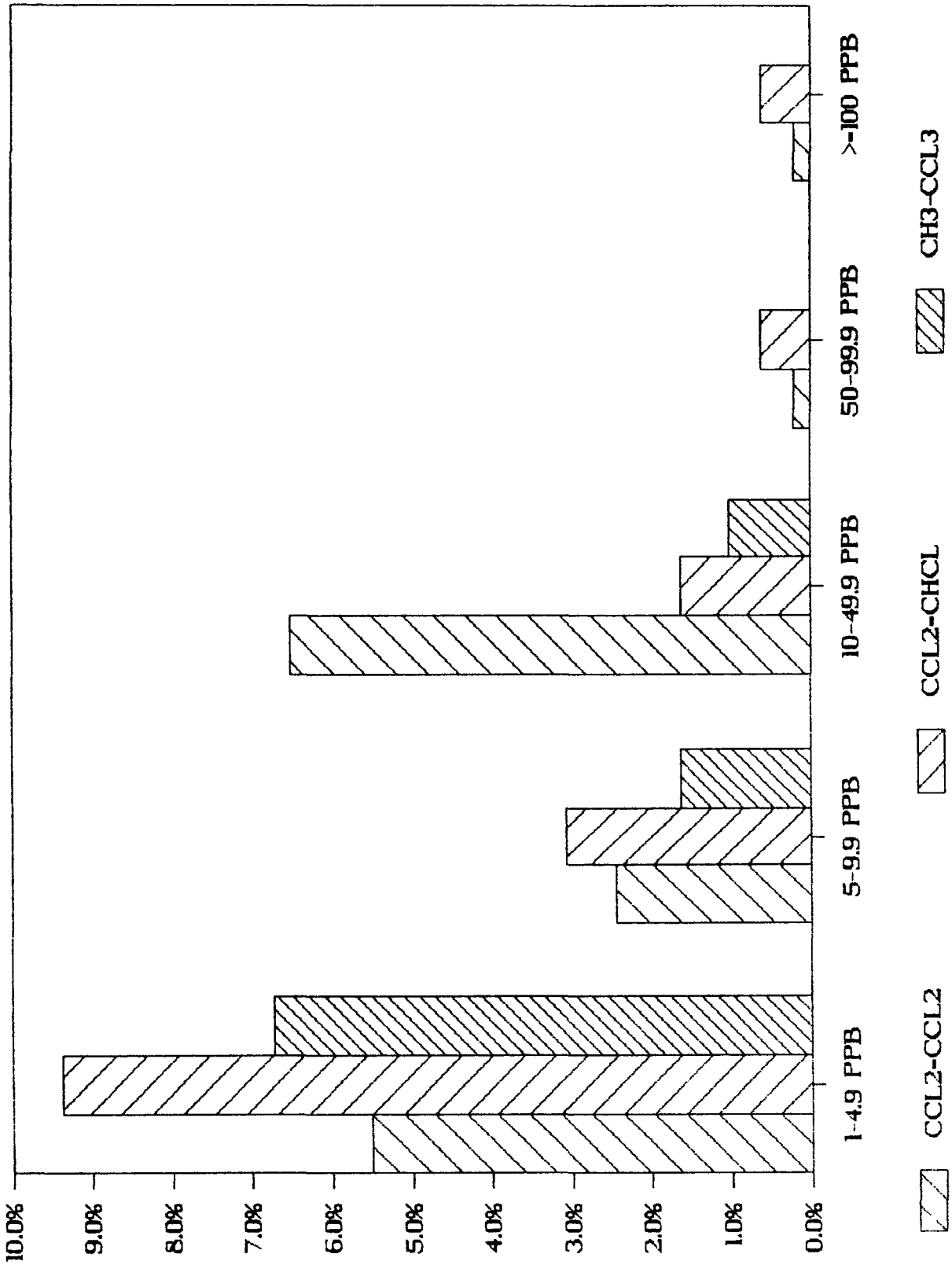


FIGURE 5 CONCENTRATION PROFILE

DRINKING WATERS TESTED IN 1987



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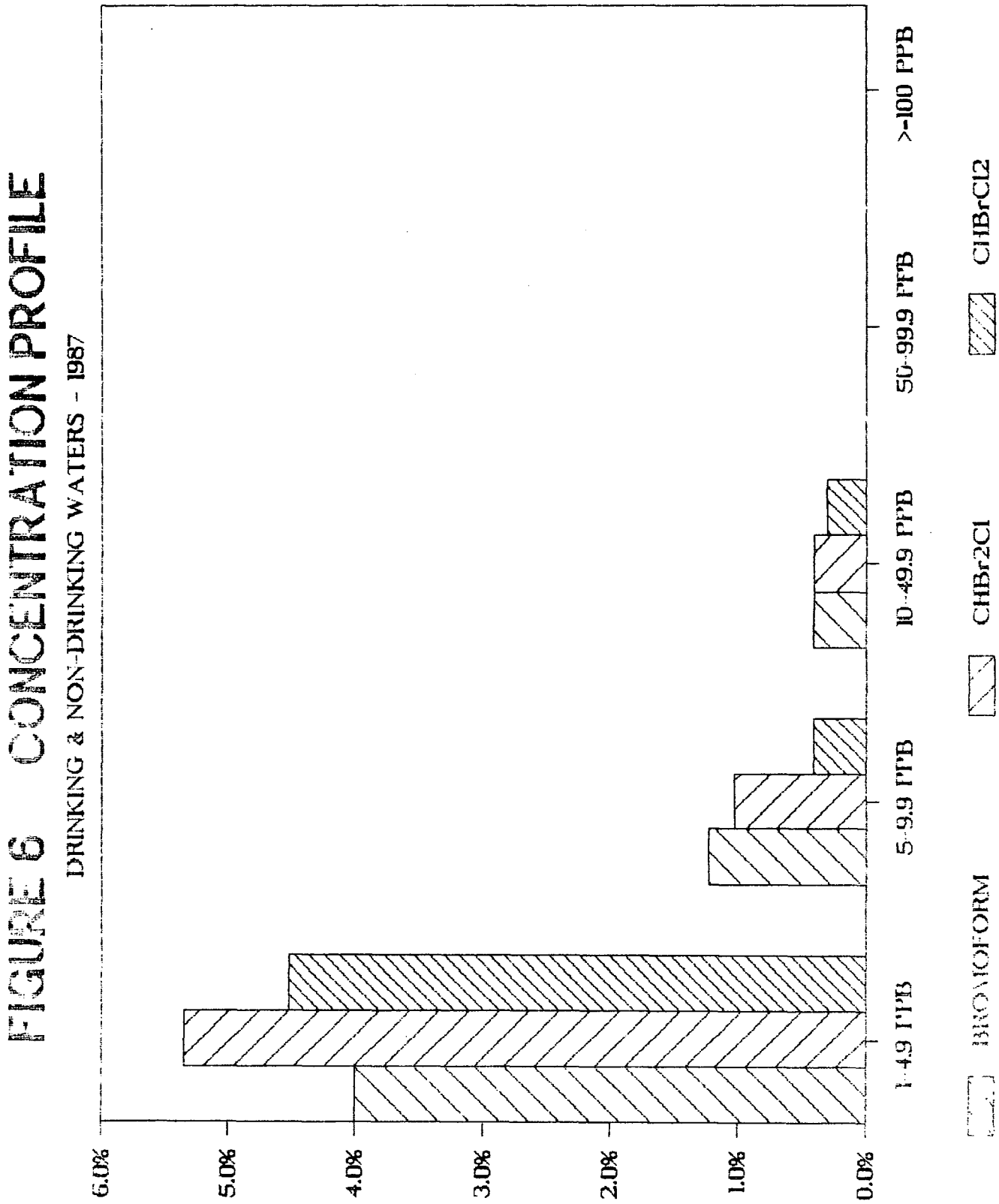
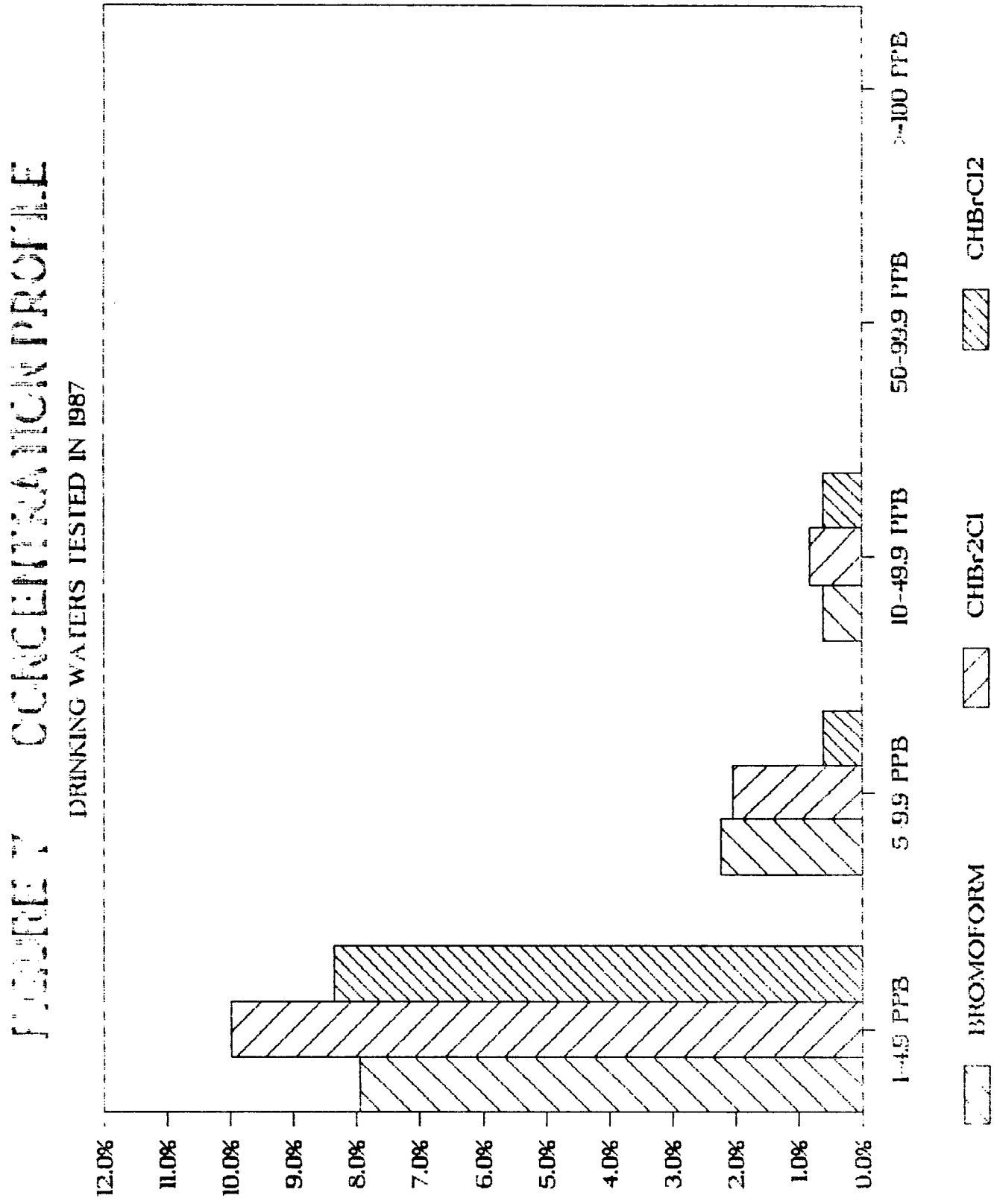


FIGURE 6 CONCENTRATION PROFILE
DRINKING & NON-DRINKING WATERS - 1987
PERCENT OF TOTAL SAMPLES
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1-4.9 PPB
5-9.9 PPB
10-49.9 PPB
50-99.9 PPB
>-100 PPB
BROMOFORM
CHBr2Cl
CHBrCl2

A-11


CONCENTRATION PROFILE
DRINKING WATERS TESTED IN 1987
PERCENTAGE OF TOTAL SAMPLES
12.0%
11.0%
10.0%
9.0%
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1-4.9 PPB
5-9.9 PPB
10-49.9 PPB
50-99.9 PPB
>100 PPB
BROMOFORM
CHBr2Cl
CHBrCl2

A-12

METHODS FOR EVALUATING GROUND-WATER MONITORING DATA FROM HAZARDOUS WASTE FACILITIES

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ABSTRACT

EPA promulgated ground-water monitoring and response standards for permitted facilities in 1982 (47 FR 32274, July 26, 1982), for detecting releases of hazardous wastes into ground water from storage, treatment, and disposal units.

The Subpart F regulations required ground-water data to be examined by statistical procedures to determine whether there was a significant exceedance of background levels, or other allowable levels, of specified chemical parameters and hazardous waste constituents. One concern was that the procedure in the regulations could result in a high rate of "false positives" (Type I error), thus requiring an owner or operator unnecessarily to advance into a more comprehensive and expensive phase of monitoring. More importantly, another concern was that the procedure could result in a high rate of "false negatives" (Type II error), i.e., instances where actual contamination would go undetected.

As a result of these concerns, EPA is amending the procedure with five different statistical methods that are more appropriate for ground-water monitoring. These amendments also outline sampling procedures and performance standards that are designed to help minimize the event that a statistical method will indicate contamination when it is not present (Type I error), and fail to detect contamination when it is present (Type II error).

BACKGROUND

Subtitle C of the Resource Conservation Recovery Act of 1976 (RCRA) creates a comprehensive program for the safe management of hazardous waste. Section 3004 of RCRA requires owners and operators of facilities that treat, store, or dispose of hazardous waste to comply with standards established by EPA that are "necessary to protect human health and the environment." Section 3005 provides for implementation of these standards under permits issued to owners and operators by EPA or authorized States. Section 3005 also provides that owners and operators of existing facilities that apply for a permit and comply with applicable notice requirements may operate until a permit

determination is made. These facilities are commonly known as "interim status" facilities. Owners and operators of interim status facilities also must comply with standards set under Section 3004.

EPA promulgated ground-water monitoring and response standards for permitted facilities in 1982 (47 FR 32274, July 26, 1982), codified in 40 CFR Part 264, Subpart F. These standards establish programs for protecting ground water from releases of hazardous wastes from treatment, storage, and disposal units. Facility owners and operators are required to sample ground water at specified intervals and to use a statistical procedure to determine whether or not hazardous wastes or constituents from the facility are contaminating ground water. As explained in more detail below, the Subpart F regulations regarding statistical methods used in evaluating ground-water monitoring data that EPA promulgated in 1982 have generated criticism. The current Part 264 regulations provide that the Cochran's Approximation to the Behrens Fisher Student's t-test (CABF) or an alternative statistical procedure approved by EPA be used to determine whether there is a statistically significant exceedance of background levels, or other allowable levels, of specified chemical parameters and hazardous waste constituents. Although the existing 40 CFR Part 264 regulations have always provided latitude for the use of an alternate statistical procedure, concerns have been raised that the CABF statistical procedure in the current regulations may not be appropriate. It has pointed out that: (1) the replicate sampling method is not appropriate for the CABF procedure, (2) the CABF procedure does not adequately consider the number of comparisons that must be made, and (3) the CABF does not control for seasonal variation. Specifically, the concerns are that the CABF procedure could result in "false positives" (Type 1 error), thus requiring an owner or operator unnecessarily to collect additional ground-water samples, to further characterize ground-water quality, and to apply for a permit modification, which is then subject to EPA review. In addition, there is concern that CABF may result in "false negatives" (Type II error), i.e., instances where actual contamination goes undetected. This may occur when the background data, which are often used as the basis of the statistical comparisons, are highly variable due to temporal, spatial, analytical, and sampling effects.

As a result of these concerns, EPA is amending both the statistical method and the sampling procedures of the regulations, by requiring (if necessary) that owners or operators more accurately characterize the hydrogeology and potential contaminants at the facility, and by including in the regulations performance standards which all the statistical methods and

sampling procedures must meet. Statistical methods and sampling procedures meeting these performance standards would have a low probability of indicating contamination when it is not present and of failing to detect contamination that actually is present. The facility owner or operator would have to demonstrate that a procedure is appropriate for the conditions at the facility and to ensure that it meets the performance standards outlined below. This demonstration holds for any of the four statistical methods and sampling procedures outlined in this regulation as well as any alternative methods or procedures proposed by facility owners and operators.

EPA recognizes that the selection of appropriate monitoring parameters is also an essential part of a reliable statistical evaluation. The Agency addressed this issue in a previous Federal Register notice (52 FR 25942, July 9. 1987).

Overview of Methodology

EPA has elected to retain the idea of general performance requirements that the regulated community must meet. This approach allows for flexibility in designing statistical methods and sampling procedures to site-specific considerations.
EPA has tried to bring a measure of certainty to these methods , while accommodating the unique nature of many of the regulated units in question. Consistent with this general strategy, the Agency is establishing several options for the sampling procedures and statistical methods to be used in detection monitoring and, where appropriate, in compliance monitoring.
The owner or operator shall submit, for each of the chemical parameters and hazardous constituents listed in the facility permit one or more of the statistical methods and sampling procedures described in today's regulations. In deciding which statistical test is appropriate, he or she will consider the theoretical properties of the test, the data available, and the hydrogeology and characteristics of potential contaminants at the site. The Regional Administrator will review, and if appropriate approve the proposed statistical methods and sampling procedures when issuing the facility permit.

The Agency recognizes that there may be situations where any one statistical test may not be appropriate. This is true of new facilities with little or no ground-water monitoring data. If insufficient data prohibits the owner or operator from specifying a statistical method of analysis, then contingency plans containing several methods of data analysis and the conditions under which the method can be used will be specified by the Regional Administrator in the permit. In many cases, the ANOVA

can be performed after six months of data has been collected. The owner or operator may propose modifying the permit at a later date when more data is available and he wishes to use a specific method of analysis.

General Performance Standards

EPA's basic concern in establishing these performance standards for statistical methods is to achieve a proper balance between the risk that the procedures will falsely indicate that a regulated unit is causing background values or concentration limits to be exceeded (false positives), and the risk that the procedures will fail to indicate that background values or concentration limits are being exceeded (false negatives). EPA's approach is designed to address that concern directly. Thus, any statistical method or sampling procedure, whether specified here or an alternative to those specified, should meet the following performance standards:

1. The statistical test is to be conducted separately for each hazardous constituent in each well. If the distribution of the chemical parameters or constituents is shown by the owner or operator to be inappropriate for a normal theory test, then the data should be transformed or distribution-free theory test should be used. If the distributions for the constituents differ, more than one statistical method may be needed.

2. If an individual well comparison procedure is used to compare an individual compliance well with background or a ground-water protection standard, the test shall be done at a Type I error level of no less than 0.01 for each testing period. If a multiple comparisons procedure is used the Type I experimentwise error rate shall be no less than 0.05 for each testing period, however the Type I error of no less than 0.01 for individual well comparisons must be maintained.

3. If a control chart approach is used to evaluate ground-water monitoring data, the upper control limit will be determined at u+zs, and the value for z (the upper alpha quantile of the standard normal distribution) shall be three (3).

4. The confidence levels and other appropriate parameters shall be established by the Regional Administrator on a case-by case basis if a tolerance or prediction interval is used to evaluate the ground-water monitoring data.

5. The statistical method will include procedures for handling data below the limit of detection. The owner or operator should

evaluate different ways of dealing with values below the limit of detection and choose the one (or more) that is most protective of human health and the environment.

6. The statistical method will consider, and if necessary control or correct for, seasonal and spatial variability and temporal correlation in the data.

In referring to "statistical methods", EPA means to emphasize that the concept of "statistical significance" must be reflected in several aspects of the monitoring program. This involves not only the choice of a level of significance, but also the choice of a statistical test, the sampling requirements, the number of samples, and the frequency of sampling. Since all of these interact to determine the ability of the procedure to detect contamination. The statistical methods, like a comprehensive ground-water monitoring program, must be evaluated in their entirety, not by individual components. Thus a systems approach to ground-water monitoring is endorsed.

The second performance standard requires further delineation. For individual well comparisons in which an individual compliance well is compared to background, the Type I error level shall be no less than 0.01 for each testing period. In other words, the probability of the test resulting in a false positive is no less than 1 in 100. EPA believes that this significance level is sufficient in limiting the false positive rate while at the same time controlling the false negative (missed detection) rate.

Owners and operators of facilities that have an extensive network of ground-water monitoring wells may find it more practical to use a multiple well comparisons procedure. Multiple comparisons procedures control the experimentwise error rate for comparisons involving multiple upgradient and downgradient wells. If this method is used, the Type I experimentwise error rate for each constituent shall be no less than 0.05 for each testing period. Here, the probability of the test resulting in a false positive is no less than 5 in 100. Again, EPA is limiting the Type I error rate for the purpose of controlling the Type II error rate. If the overall test is shown to be significant, then individual well contrasts are performed to identify which differences are statistically significant.

In conducting a multiple well comparisons procedure, if the owner or operator chooses to use a t-statistic rather than an F-statistic, the individual well Type I error level shall be no less than 0.01, rather than 0.05 divided by the total number of wells used in the comparison.

Setting these levels of significance at 0.01 and 0.05 respectively raises an important question in how the false positive rate will be controlled at facilities with a large number of ground-water monitoring wells and monitoring constituents. The Agency set these levels of significance on the basis of a single testing period, and not on the entire operating life of the facility. Further, large facilities can reduce the false positive rate by implementing a unit-specific monitoring approach. Nonetheless it is evident that facilities with an extensive number of ground-water monitoring wells and that are monitoring for many constituents will still generate a large number of comparisons and consequently false positives each testing period. At these facilities, it may be difficult to keep the false positive error rate at an acceptable level.

Such cases may require the Regional Administrator to use judgement in deciding if a statistically significant result is indicative of an actual release from the facility, or if it is a false positive. In making this decision, the Regional Administrator may note the relative magnitude of the concentration of the constituent(s). If the exceedance is based on an observed compliance well value that has the same relative magnitude as the pql (practical quantification limit), or the background level, then a false positive is more likely to be observed, and further sampling and testing may be appropriate. If however the background or an action level is exceeded by an order of magnitude, then the exceedance is more likely to be indicative of a release from the facility.

Basic Statistical Methods and Sampling Procedures

This approach specifies five types of statistical methods to detect contamination in ground water. EPA believes that at least one of these types of procedures will be appropriate for a wide variety of situations. To address situations where these methods may not be appropriate, EPA has included a provision for the owner or operator to select an alternate method which is subject to approval by the Regional Administrator.

1. A parametric analysis of variance (ANOVA) followed by multiple comparison procedures to identify specific sources of difference. The procedures will include estimation and testing of the contrasts between the mean of each compliance well and the background mean for each constituent.

2. An analysis of variance (ANOVA) based on ranks followed by multiple comparison procedures to identify specific sources of difference. The procedure will include estimation and testing of

the contrasts between the median of each compliance well and the median background levels for each constituent.

3. A procedure in which a tolerance interval or a prediction interval for each constituent is established from the background data, and the level of each constituent in each compliance well is compared to its upper tolerance or prediction limit.

4. A control chart approach which will give control limits for each constituent. If any compliance well has a value or a sequence of values that lie outside the control limits for that constituent, it may constitute statistically significant evidence of contamination.

5. Another statistical method submitted by the owner or operator and approved by the Regional Administrator.

EPA is specifying multiple statistical methods and sampling procedures and has allowed for alternatives because no one method or procedure is appropriate for all circumstances. EPA believes that the suggested methods and procedures are appropriate for the site-specific design and analysis of data from ground-water monitoring systems, and that they can account for more of the site-specific factors than Cochran's Approximation to the Behrens Fisher Student's t-test (CABF) and the accompanying sampling procedures in the current regulations. The statistical methods specified here address the multiple comparison problems and provide for documenting and accounting for sources of natural variation. EPA believes that the specified methods and procedures consider and control for natural temporal and spatial variation. The decision on the number of wells needed in a monitoring system will be made on a site-specific basis by the Regional Administrator and will consider the statistical method being used, the site hydrogeology, the characteristics of potential contaminants, and the sampling procedure. The number of wells must be sufficient to ensure a high probability of detecting contamination when it is present. To determine which sampling procedure should be used, the owner or operator shall consider existing data and site characteristics, including the possibility of trends and seasonality. These sampling procedures are:

1. Obtain a sequence of at least four samples taken at either daily, weekly, or monthly intervals. The time interval will be determined by the owner or operator after considering the rate of ground-water flow under the unit and contaminant fate and transport data. This interval must be sufficient to allow the owner or operator to obtain an independent sample and must be approved by the Regional Administrator.

2. An alternate sampling procedure approved by the Regional Administrator.

EPA believes that the above sampling procedures will allow the use of statistical methods that will accurately detect contamination. These sampling procedures may replace the sampling method present in the current regulations. Rather than taking a single ground-water sample and dividing it into four replicate samples, a sequence of at least four samples taken at intervals far enough apart in time (daily, weekly, or monthly, depending on rates of ground-water flow and contaminant fate and transport data) will help ensure the sampling of a discrete portion (i.e., an independent sample) of ground water.

The Regional Administrator shall approve an appropriate sampling procedure and interval submitted by the owner or operator after considering the hydraulic conductivity and hydraulic gradient in the uppermost aquifer under the waste management area, and the fate and transport data of potential contaminants. This information is already required to be submitted in the facility's Part B permit application under 270.14(c), and may be used by the owner or operator to make this determination. Further, the number and kinds of samples collected to establish background should be appropriate to the form of statistical test employed, following generally accepted statistical principals. For example, the use of control charts presume a well defined background of perhaps 16-30 samples. By contrast, ANOVA alternatives might require only 4-6 samples.

It seems likely that most facilities will be sampling monthly over four consecutive months, twice a year. In order to maintain a complete annual record of ground-water data, the facility owner or operator may find it desirable to obtain a sample each month of the year. This will help identify seasonal trends in the data and permit evaluation of the effects of autocorrelation and seasonal variation if present in the samples.

The concentrations of a constituent determined in these samples are intended to be used in one-point-in-time comparisons between background and compliance wells. Some facility owners or operators may want to use the concentrations to establish a "moving average" in the background well data base for comparison to the compliance well values at the frequency required in the facility permit. Using several background values to establish a "moving average" is an acceptable method of analysis, however, the number of degrees of freedom will be increased, making this method more sensitive to changes in constituent concentrations. Further, this method does not account for seasonal variation as

effectively as one-point-in-time comparisons procedures. Therefore, most owners and operators will find one-point-in-time comparisons to be the preferred method of analysis. This approach will help reduce the components of seasonal variation, by providing for simultaneous comparisons between background and downgradient well information.

The different sampling intervals were chosen to allow for the unique nature of the ground-water systems beneath hazardous waste sites. This sampling scheme will give proper consideration to the temporal variation of and autocorrelation among the ground-water constituents. The specified procedure requires sampling data from background wells, at the compliance point, and according to a specific test protocol. The owner or operator should use a background value determined from data collected under this scenario if a test approved by the Regional Administrator requires it, or if a concentration limit in compliance monitoring is to be based upon background data.

EPA recognizes that there may be situations where the owner or operator can devise alternate statistical methods and sampling procedures that are more appropriate to the facility and which will provide reliable results. Therefore, today's regulations allow the Regional Administrator to approve such procedures if he finds that the procedures balance the risk of false positives and false negatives in a manner comparable to that provided by the above specified tests, and that they meet the specified performance standards. In examining the comparability of the procedure to provide a reasonable balance between the risk of false positives and false negatives, the owner or operator will specify in the alternate plan such things as the sampling frequency and the sample size.

The methods indicate that the procedure must provide reasonable confidence that the migration of hazardous constituents from a regulated unit into and through the aquifer will be detected. (The reference to hazardous constituents does not mean that this option applies only to compliance monitoring; the procedure also applies to monitoring parameters and constituents in the detection monitoring program since they are surrogates indicating the presence of hazardous constituents.) The protocols for the specific tests, however, will be used as general benchmark to define " reasonable confidence" in the proposed procedure. If the owner or operator shows that his suggested test is comparable in its results to one of the specified tests, then it is likely to be acceptable under the "reasonable confidence" test. There may be situations, however, where it will be difficult to directly compare the performance of an alternative test to the protocols for the specified tests. In such cases the alternative

test will have to be evaluated on its own merits.

SUMMARY

EPA promulgated regulations for detecting contamination of ground water at hazardous waste land disposal facilities under the Resource Concentration and Recovery Act of 1976 (RCRA). The statistical methods used to evaluate the presence of contamination have been subject to criticism and require improvement. In an attempt to improve these current methods, this paper presents an outline of five different statistical methods of analysis and a provision for an alternate method. Additionally, a new sampling procedure is proposed along with performance standards that are designed to help minimize the event that a statistical method will indicate contamination when it is not present (Type I error), and fail to detect contamination when it actually is present (Type II error). Further, this approach offers enough flexibility to accomodate site-specific differences that may exist at hazardous waste facilities.

AIR TOXICS MONITORING PROGRAM USED DURING A BIOREMEDIATION AT A HAZARDOUS WASTE SITE

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ABSTRACT

A bioremediation demonstration project was recently conducted to test in-situ biodegradation of hazardous wastes at the 22nd NPL ranked superfund site. A comprehensive air monitoring program was implemented at the site as an integral part of the demonstration. The goals of the program were to protect health and safety on-site personnel and surrounding communities, and assess on-site and off-site air emission impacts from the process.

Air measurements included;

- Tenax sampled time-weighted-average volatile organic determinations;
- On-site 'real-time' target volatile compound determinations;
- On-site naphthalene determinations; and
- Real-time total ionizables (e.g., HNu) measurements.

Over six months of daily data were collected during the program.

Results of the study showed the air contaminant impacts to be minimal and readily controlled by site operations. The carefully constructed quality assurance parameters and a thorough analysis of the results observed yielded a great amount of detail in the data which will be of importance to future bioremediation projects.

INTRODUCTION

A 7.3 acre lagoon outside of Houston, Texas was the site of an In-situ Bioremediation Demonstration project. The site, known as French Limited, was previously a permitted industrial waste disposal facility. The lagoon is a previously excavated sand pit which industrial waste sludges were disposed. These wastes contain a variety of general organic and chlorinated organic constituents. The site was placed on the National Priorities List (NPL) due to the possible contamination of the areas groundwater with hazardous organic constituents.

Originally, incineration was chosen as the most desirable remediation technology. However, some preliminary pilot scale studies indicated a bioremediation technology could be used to clean up the organic compounds of concern. Therefore, a program was designed to test the application of the bioremediation technology to clean up the lagoon.

Based on the preliminary studies at the site several naturally occurring aerobic bacteria were identified which were thought to show promise as organic biodegraders if their activity could be stimulated and enhanced through the addition of balanced nutrients and additional oxygen to the system. The six month air monitoring program described in this paper was one part of a comprehensive environmental monitoring plan associated with the overall bioremediation program. The environmental monitoring plan encompassed air, groundwater, and health and safety issues. A more thorough description of the site and its history and the bioremediation program can be found elsewhere.[1]

The air monitoring program at the site was constructed to respond to concerns of off gassing of hazardous constituents in the sludge during the addition of oxygen in the form of air sparged into the sludge and lagoon water. The goals of the air monitoring plan for the bioremediation demonstration at the French Limited site were to:

- measure on-site and site property line impacts of air emissions from the bioremediation processes; and
- protect the health and safety of both on-site personnel and the off-site general public.

METHODS

The objectives of the air monitoring program were addressed by monitoring five separate groups of variables consisting of:

1. Continuous measurement and recording of meteorological

data (wind speed, wind direction, temperature, relative humidity, barometric pressure and precipitation);

2. "Real-time" measurements of total ionizables using photoionization detector (HNu) measurements;

3. "Real-time" syringe sampling for volatiles with on-site GC analysis;

4. "Real-time" sampling of the semi-volatile naphthalene using charcoal absorbent with on-site gas chromatograph (GC) analysis;

5. "Time-integrated" sampling for volatiles using collection on Tenax solid absorbent followed by gas chromatography/mass spectrometry (GC/MS) laboratory analysis;

Meteorological Measurements

A free-standing 10-meter tower was constructed at the site and used to determine the following meteorological parameters: wind direction, wind speed, temperature, relative humidity, barometric pressure, precipitation, and sigma theta. Meteorological data from the on-site station was used to locate sampling variations and to correlate air impact data collected with relevant wind conditions.

"Real-Time" Measurements

Three "real-time" measurements were conducted at the site during the demonstration project as a means for daily checks on air impacts within a time frame allowing mitigating actions to take place if necessary. These measurements were designed to address air program goals relating to the protection of health and safety of both on-site and off-site personnel, and to develop a data base defining instantaneous contaminant concentration levels. The real-time analyses were used by site operations managers in controlling the level of air impacts by reducing and, if necessary, shutting down operations if pre-assigned "action level" concentrations were reached. The real-time measurements include: total ionizables (e.g., HNu) measurements, on-site determination of target volatile organics, and on-site determination of the semi-volatile naphthalene.

Throughout the in-situ biodegradation demonstration, air concentrations were monitored and compared to preset

concentration limits. In the "real-time" impact monitoring program, the concentration levels of seven compounds were monitored. These compounds are shown in Table 1 with their OSHA 8-hour threshold limit values (TLVs), and the "action levels" that would require reduced intensity of aeration and/or sludge mixing.

TABLE I
Target Compounds Action Levels

Compound	TLV (ppm)	Lagoonside Action Level (ppm)
Volatiles		
Benzene	10.0	5.0
Toluene	200.0	100.0
Ethylbenzene	100.0	50.0
Trichloroethene	100.0	50.0
Tetrachloroethene	100.0	50.0
Chloroform	50.0	25.0
Semi-Volatile		
Naphthalene	10.0	5.0

The use of the term "lagoonside" refers to a sampling location at the edge of the lagoon bank, which is approximately 5-7 feet from the water's edge. The term "fenceline" refers to a sampling location at the French Limited Site property boundary.

Target compounds were selected to be representative of expected emissions based on pilot scale experiments done prior to the demonstration project.

A second curtailment criteria was based upon HNu readings taken at the fenceline sampling locations. A reading in excess of 1.0 ppm above background required immediate reduction in aeration/mixing operations, and if the 1.0 ppm reading continued for 30 minutes, system shutdown was required.

Volatile Target Compound Monitoring. Real-time volatile target compound monitoring consisted of determination of six organic compounds listed above in Table I. Two gas chromatographs equipped with photoionization detectors (Photovac 10S50) were used for the target compound determinations. These chromatographs were located in the field laboratory where the samples were analyzed and data reduced on-site. Grab samples were collected manually using 1.0 ml gas-tight syringes. The

samples once collected, were brought directly to the field laboratory for analysis.

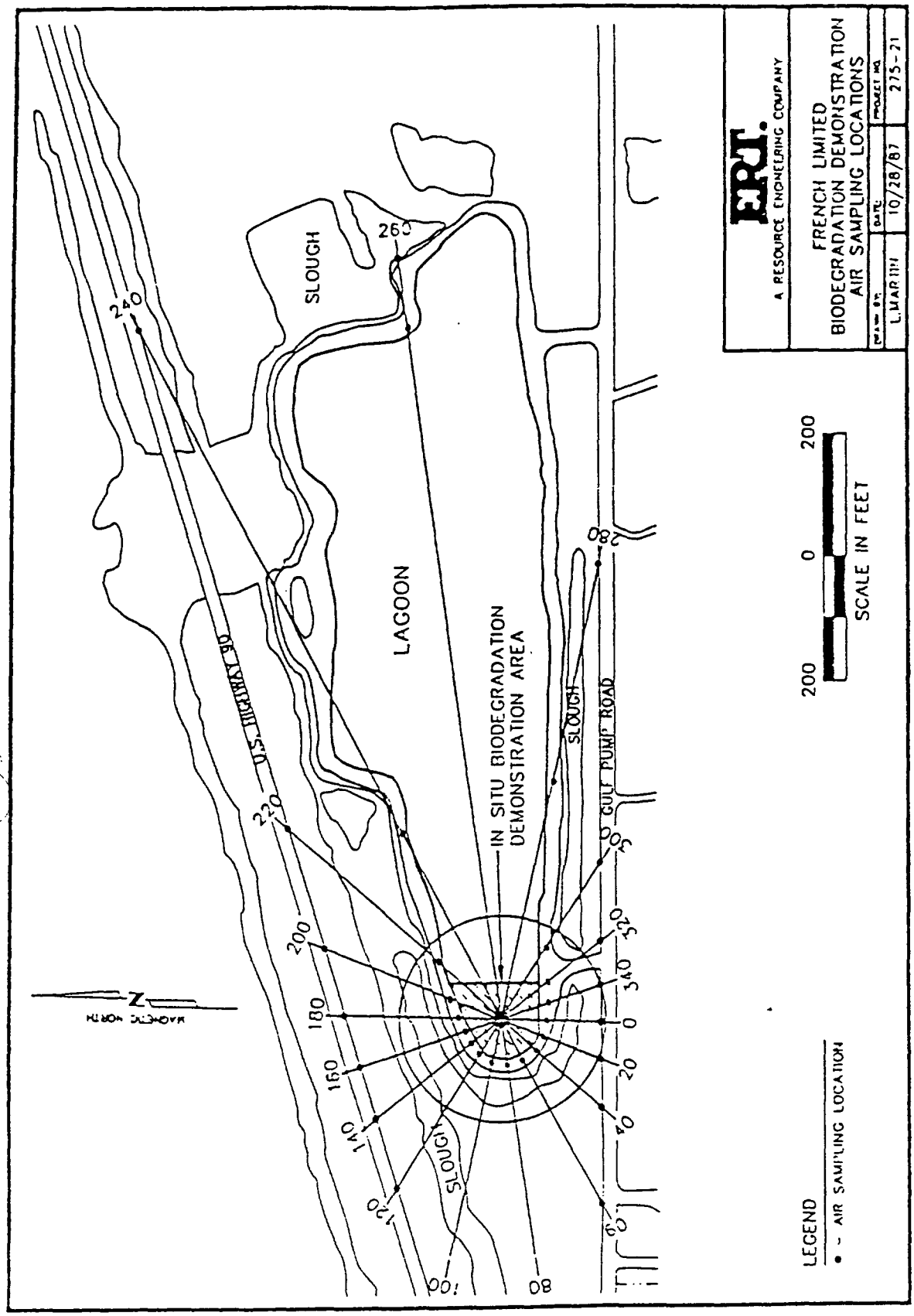
The specific sampling locations (upwind and downwind) selected for each sampling event was based upon the meteorological conditions existing at the time. Samples were taken at three 36 pre-selected sampling stations shown in Figure 1. Each specific sampling location was pre-selected, choosing the sampling location closest to the then current wind direction.

All gas chromatograph data was reduced on-site and the results were posted on a central data board located in the field operations office. Quality assurance procedures included blanks, and collocated sampling. Calibration for the six target compounds was conducted at least twice daily from a certified gas mixture of each component.

Naphthalene Monitoring. Naphthalene is the most volatile of the polynuclear aromatic (PNA) compounds. It was therefore identified as the PNA most likely to be released during the demonstration project, since naphthalene was known to be present in the lagoon sludge.

NIOSH analysis Method 1501 for aromatic hydrocarbons was used for determination of naphthalene. The naphthalene analysis was performed in the field laboratory using a gas chromatograph (Hewlett Packard 5990) equipped with a flame ionization detector. Charcoal absorbent tubes were used for absorption of naphthalene from air, sampled by a calibrated battery-operated air sampling pump. The time period for sampling was generally one hour.

The specific sample locations were selected based upon the meteorological conditions existing at the time of sampling. Samples were taken at one of the 36 pre-selected sampling stations. The specific sampling location was that pre-selected station closest to the predicted one-hour wind direction. All naphthalene data was reduced on-site and results posted on the central data board, located in the field operations office. Calibrations using prepared solutions of naphthalene were conducted at least daily. Quality assurance procedures included field and laboratory blanks, spikes and collocated samples.


ERT.
A RESOURCE ENGINEERING COMPANY
FRENCH LIMITED
BIODEGRADATION DEMONSTRATION
AIR SAMPLING LOCATIONS
DRAWN BY: L.MARTIN
DATE: 10/28/87
PROJECT NO. 275-21
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LAGOON
IN SITU BIODEGRADATION
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100
80
60
40
20
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340
320
300
280

Figure 1

A-28

Total Ionizables Measurements. A total ionizables measurement using a HNu, photoionization detector was made and recorded at the same time and at the same location as each syringe sample was taken. The HNu was calibrated with a certified standard of isobutylene at least daily.

Time-Integrated Sampling For HSL Volatiles. The time-integrated monitoring was conducted at the site during the demonstration to document the average daily impact on downwind areas on-site and at fenceline locations.

Time-integrated impact measurement samples were collected by drawing the air sample through a cartridge of pre-cleaned Tenax solid sorbent material. The sample was drawn through the Tenax tube at a measured flow rate using a battery-operated air sampling pump. The time period of sampling was generally 8 hours. The specific location of upwind and downwind sampling was based upon the meteorological conditions existing at the time. Samples were taken at one of the 36 pre-selected sampling stations shown previously in Figure 1. Each individual sampling location was selected based upon identification of the sampling location that was nearest to the 8-hour predicted downwind (or upwind) direction.

The concentration levels of the 35 volatile organic compounds from the EPA Hazardous Substance List (HSL) were determined for each sample. The samples, once collected, were packaged in accordance with EPA approved QA/QC procedures and forwarded to an off-site laboratory for analysis. This HSL was used because it represented those constituents that might be expected to evolve from the process operations based on air contaminant measurements from previously conducted pilot scale experiments. Several qualitative GC/MS analyses on collected samples proved the assumption to be true.

The air samples were analyzed by thermal desorption onto a tenax/silica gel/OV-1 trap with subsequent separation and confirmation using gas chromatography/mass spectrometry. The instrument was calibrated by spiking known concentrations of 624/HSL volatile organic compounds onto clean tenax tubes and analyzing to obtain the instrument response to the recovered compounds relative to internal standards. Field sampled tenax tubes were spiked with internal standards and surrogates, then analyzed and the results were compared to the calibrated responses to determine actual concentrations in the unknown samples.

Upon receipt, all tenax tube samples were assigned respective in-house tracking I.D.'s and stored at 4 degrees C., until the time of analysis. All tenax tubes were analyzed within

14 days of sampling. Each sample, prior to analysis was spiked with internal standards and surrogates.

The instrument was calibrated by analyzing four concentration levels of the 624/HSL compound list (50, 150, 250, and 500 ng/tube of 624 list compounds with HSL volatiles at 2.5 times the 624 concentration). After analysis of the calibration standards were completed, an average response factor, standard deviation, and % relative standard deviation (%RSD) were calculated. The validity of the calibration was judged by the % RSD (%RSD=std.dev/ave RF) of the four points analyzed. If the %RSD was less than 35%, the calibration was considered to be suitably precise. A %RSD greater than 35% suggested analytical problems such as instrument instability or insensitivity, standard preparation/dilution error, or contamination/interference. These issues were addressed and problems resolved prior to attempting another calibration.

On a daily basis, the integrity of the calibration was confirmed by analyzing a continuing calibration check standard. This was a tenax tube spiked as the mid-point of the calibration at 150 ng (HSL compounds at 2.5 times the concentration). This check standard must fall within +/- 35% of the average response factor determined by the calibration standards before analysis of samples could continue. Should the results of the check standard have fallen outside of the +/- 35% range for six or more compounds, the check standard had to be re-analyzed to confirm the disparities, or a new four point calibration had to be analyzed to account for the changing analytical responses.

In addition to the daily check standard, a blank tenax tube spiked with internal standard and surrogate compounds was analyzed on a daily basis to ensure the purity of the system. If the method blank reflected contamination of the system by any of the target list compounds, a rerun of the method blank was performed to confirm the problem. If the problem was recurrent, it was addressed before continuing with the analysis of samples.

An internal standard solution of known concentration (150 ng) was added to each tenax tube to serve as a reference for quantitation of unknowns in a sample. The internal standard also served to validate the correct and consistent operation of the instrument from sample to sample by providing a reference point to which all retention times were verified and responses confirmed. If the method blank and check standard exhibited acceptable recovery values, the accuracy of the instrument and the integrity of the analysis is confirmed.

Analysis included a variety of tubes prepared or sampled solely for the purpose of quality assurance. Collocated tubes,

i.e., duplicate tenax tubes sampled in the field, provided a comparison of precision and accuracy. In-house spiked tenax tubes sent out into the field and returned with sample batches for analysis confirmed sample stability during sampling, shipment and storage prior to analysis. Tenax tubes sampled from a gas calibration mixture confirmed the efficiency of the sampling technique as well as the accuracy of established calibration data performed in the laboratory. The results of the various quality control samples served to validate the sampling and analytical methods used in the volatile organics air monitoring program.

RESULTS

Total Ionizables Results

Total ionizables measurements were made throughout the air program totalling over 1,800 individual scheduled readings. In addition, HNu measurements were made continuously at downwind fenceline locations during actual sludge mixing or soil dredging to monitor for "action level" concentrations. The highest HNu measurement recorded was 9.5 ppm at lagoonside and 1.8 ppm at fenceline.

On two occasions, operations were curtailed due to HNu readings exceeding the 1.0 ppm above background actions limit. In both cases, aeration and mixing activities were curtailed and the fenceline readings returned to below the action limit within 20 minutes, and did not exceed the limit during the rest of the day.

Procedures required on-site personnel wear organic vapor cartridge type respirators when HNu readings exceeded 1.0 ppm above background. The occasions when this was needed were few and generally of short duration.

"Real-Time" Target Volatiles Results

Over 1,800 syringe collected samples were analyzed for the six target volatile compounds. Table II presents the summary results of the determinations. The concentrations of all target compounds remained well below their action limits for the entire demonstration project. The single highest percent of action limit was for benzene, and was only 3% of the limit at the fenceline. Therefore, there was never a need to curtail operations due to target compound concentrations level.

TABLE II
French Limited Biodegradation Demonstration
Real-time Target Volatiles Results Summary
Concentration in Parts Per Billion (ppb)

Target Compound	TLV	Action Limit	Detection Limit	Lagoonside Concentration Range	Lagoonside Maximum Concentration % of Action Limit	Fenceline Concentration Range	Fenceline Maximum Concentration % of Action Limit
Chloroform	50,000	25,000	350	BDL	- - - -	BDL	- - - - - -
Benzene	10,000	5,000	10	BDL - 1160	23	BDL - 150	3.0
Trichloroethene	100,000	50,000	10	BDL - 675	1.4	BDL - 520	1.0
Toluene	200,000	100,000	25	BDL - 1494	1.5	BDL - 590	0.6
Tetrachloroethene	100,000	50,000	30	BDL - 850	1.7	BDL - 156	0.3
Ethylbenzene	100,000	50,000	50	BDL - 810	1.6	BDL - 420	0.9

BDL - Below Detection Limit

Naphthalene Results

Over 600 on-site determinations for naphthalene were made during the monitoring program. No naphthalene was detected in any of the ambient monitoring above the method detection limit of 150 ppb. Naphthalene Action Limit was set at 5 ppm. All samples were therefore less than 3% of the naphthalene action limit for the duration of the program.

Time-Integrated Sampling Results

Over 1,500 Tenax samples were analyzed during the program period. Table III summarized the results for quantitative time-integrated determinations. As can be seen from Table III, trans-1,2,-dichloroethene had the highest lagoonside 8-hour average concentrations of any of the HSL compounds. However, this concentration represented only 0.2% of its TLV.

Concentrations determined for fenceline locations were even lower, with 30 of the 35 compounds determined to be normally below detection limits.

Figure 2 shows the 8-hour time-integrated results for benzene plotted vs. day number of sampling. As can be seen from the figure, there is substantial day to day variations in the concentration levels of benzene. These day to day variations are discussed below.

DISCUSSION

Overall, the air impacts from the bioremediation demonstration were minimal. The variation in the results observed, however, deserves a closer look. If the variations are due solely to imprecision of the measurement, then only limited information is actually contained in the details of the investigation. If this can be shown not to be the case, then a wealth of information can be gained from the thousands of determinations made. The following discussion, examines the time-integrated-results with this goal in mind. Comparison between the different analytical methods has been discussed elsewhere.[2]

As is apparent from figure 2, there is considerable day-to-day variation in even the eight-hour-average concentrations of benzene. The day to day variation in the data could have been caused by several factors:

- different site operational conditions,
- heterogeneousity of the sludge,

Table III

FRENCH LIMITED BIODEGRADATION DEMONSTRATION
TIME-INTEGRATED IMPACT MONITORING RESULTS SUMMARY
Concentration in Parts Per Billion (ppb)

			Lagoonside			Fenceline	
Compound	TLV	Detection Limit[1]	Actual Concentration Range[2]	Highest Concentration % of TLV	Most Frequent Concentration Range[3]	Actual Concentration Range[2]	Most Frequent Concentration Range[3]
Choloromethane	50,000	0.6	BDL–2.4	0.005	BDL	BDL	BDL
Bromomethane	5,000	0.3	BDL	–	BDL	BDL	BDL
Vinyl Chloride	5,000	0.4	BDL–132	3	BDL	BDL–1.8	BDL
Chloroethane	1,000,000	0.4	BDL	–	BDL	BDL	BDL
Methylene Chloride	50,000	0.3	BDL–7.7	0.015	BDL	BDL–3.9	BDL
Acetone	750,000	0.5	BDL–46	0.006	BDL–10	BDL–31.1	BDL–10
Carbon Disulfide	10,000	0.4	BDL–134	1	BDL	BDL–56	BDL
1,1-Dichloroethene	5,000	0.3	BDL–3.5	0.7	BDL	BDL	BDL
1,1-Dichloroethane	200,000	0.3	BDL–225	0.1	BDL–10	BDL–5.9	BDL
Trans-1,2-Dichloro-ethene	200,000	0.3	BDL–483	0.24	10–50	BDL–16	BDL
Chloroform	10,000	0.2	BDL–9.4	0.009	BDL–10	BDL–3.4	BDL
1,2-Dichloroethane	10,000	0.3	BDL–214	2	BDL–10	BDL–9.6	BDL
2-Butanone	200,000	0.4	BDL–122	0.06	BDL–10	BDL–61.4	BDL
1,1,1-Trichloroethane	350,000	0.2	BDL–1.4	0.0004	BDL	BDL–0.5	BDL
Carbon Tetrachloride	5,000	0.2	BDL–1.1	0.02	BDL	BDL–1.1	BDL
Vinyl Acetate	10,000	0.3	BDL–9.8	0.1	BDL	BDL–1.0	BDL
Bromodichloroethane		0.2	BDL	–	BDL	BDL	BDL
1,2-Dichloropropane	75,000	0.2	BDL–110	0.15	BDL–10	BDL–2.0	BDL
Trans-1,3-Dichloro-propene	1,000	0.3	BDL	–	BDL	BDL	BDL
Trichloroethene	50,000	0.2	BDL–88	0.18	BDL–10	BDL–1.2	BDL
Dibromochloromethane		0.1	BDL	–	BDL	BDL	BDL

Notes:

1. Based on normal 20-liter air volume.
2. BDL entries indicate levels were below detection limits.
3. Concentration level ranges used: BDL, BDL–10 ppb, 10–50 ppb, >50 ppb.

TABLE III (Continued)

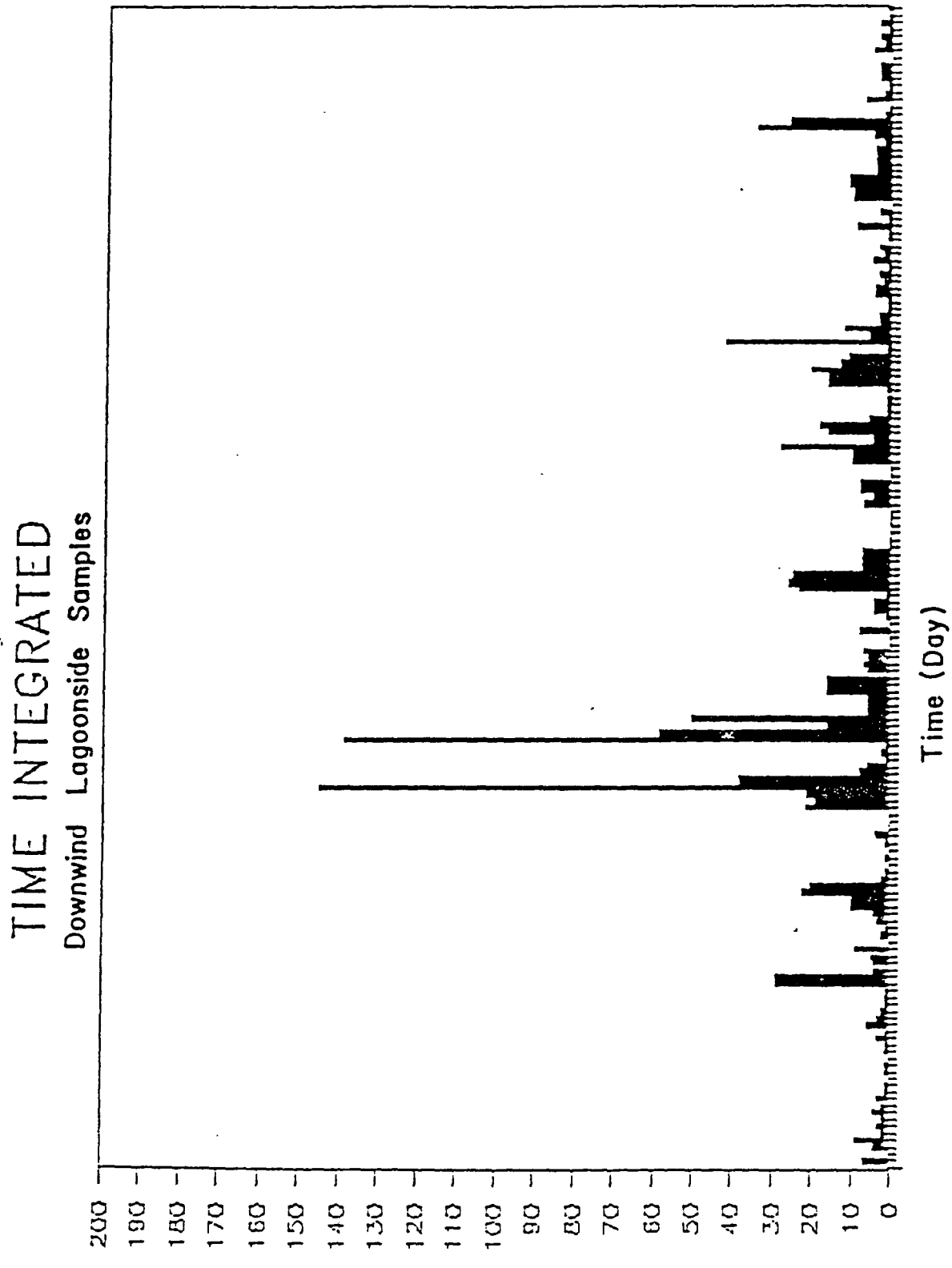
FRENCH LIMITED BIODEGRADATION DEMONSTRATION
TIME-INTEGRATED IMPACT MONITORING RESULTS SUMMARY
Concentration in Parts Per Billion (ppb)

Compound	TLV	Detection Limit[1]	Lagoonside			Fenceline	
			Actual Concentration Range[2]	Highest Concentration % of TLV	Most Frequent Concentration Range[3]	Actual Concentration Range[2]	Most Frequent Concentration Range[3]
1,1,2-Trichloroethane	10,000	0.2	BDL-11.2	0.11	BDL	BDL-1.1	BDL
Benzene	10,000	0.4	BDL-255	3	BDL-10	BDL-11	BDL-10
Cis-1,3-Dichloro-propene	1,000	0.3	BDL	-	BDL	BDL	BDL
2-Chloroethyl Vinyl Ether		0.3	BDL-3.8	-	BDL	BDL	BDL
Bromoform	500	0.1	BDL	-	BDL	BDL	BDL
2-Hexanone	5,000	0.3	BDL-1.3	0.02	BDL	BDL	BDL
4-Methyl-2-Pentanone	50,000	0.3	BDL-3.7	0.007	BDL	BDL	BDL
Tetrachloroethene	50,000	0.2	BDL-6.1	0.01	BDL	BDL-0.2	BDL
1,1,2,2-Tetrachloro-ethane	1,000	0.2	BDL	-	BDL	BDL	BDL
Toluene	100,000	0.3	BDL-121	0.1	BDL-10	BDL-24	BDL-10
Chlorobenzene	75,000	0.2	BDL-19.8	0.025	BDL	BDL-1.0	BDL
Ethylbenzene	100,000	0.3	BDL-152	0.1	BDL-10	BDL-5.8	BDL-10
Styrene	50,000	0.3	BDL-52.9	0.1	BDL	BDL-1.1	BDL
Total Xylene	100,000	0.3	BDL-112	0.1	BDL-10	BDL-7.0	BDL-10

Notes:

1. Based on normal 20-liter air volume.
2. BDL entries indicate levels were below detection limits.
3. Concentration level ranges used: BDL, BDL-10 ppb, 10-50 ppb, >50 ppb.

Figure 2


TIME INTEGRATED
Downwind Lagoonside Samples
ppb Benzene
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Time (Day)

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- varying meteorological conditions, or
- quality control parameters of the measurement itself.

Each factor will be discussed.

Operational Conditions Effects. Figure 2 shows that levels of benzene remained low during the initial stage of the bioremediation process and did not significantly increase until day 20 when sludge pumping began. Reducing the level of mixing was found to reduce air emissions. It was also noted that when new areas of sludge were mixed, emissions impacts increased for the first day of mixing. The high reading on days 53 and 56 correspond to mixing days after a thick mat of black foam formed and covered the lagoon surface. Physical characteristics of the foam layer caused reduction of mixing and air sparging operations and air concentrations dropped accordingly. The intensity of mixing and aeration were generally functions of attempts to reach and stabilize other operation parameters such as oxygen or substrate concentration, or as the result of mechanical constraints of equipment used. These day to day and even hour to hour changes had direct effects on air emission impacts, and were in fact used to control air emissions when necessary.

Sludge Heterogeneousity Effects. The wide variety in the types of wastes may also be a cause of the day to day fluctuations in the emission concentration. The wastes are not evenly distributed throughout the lagoon due to disposal trucks having dumped different wastes at different locations randomly around the site. As new areas of sludge were mixed, different areas of different types of sludge were stirred up. These areas of different sludges were for example, sometimes high in waste oil and sometimes high in chlorinated solvents. Figure 3 shows the downwind lagoon side concentration of toluene vs the concentration of benzene for the same sample. As can be seen, even though there is great day to day variation in the benzene concentrations (Figure 2) the toluene concentrations correlate very well with benzene concentrations. This would be expected when there is a common source of the chemicals, such as waste oil, in the sludge. Figure 3 also suggest that toluene concentrations are effected by the same factors in approximated the same strength as factors effecting benzene.

This correlation would not be expected for compounds supposedly from different source "patches" in the lagoon. Figure 4 shows the same plot for three different time periods for benzene vs trans-1, 2-dichloroethene. Early in the demonstration 4/21-6/21 the benzene and the chlorinated solvent show little or no correlation. The mixing presumably stirs up different amounts

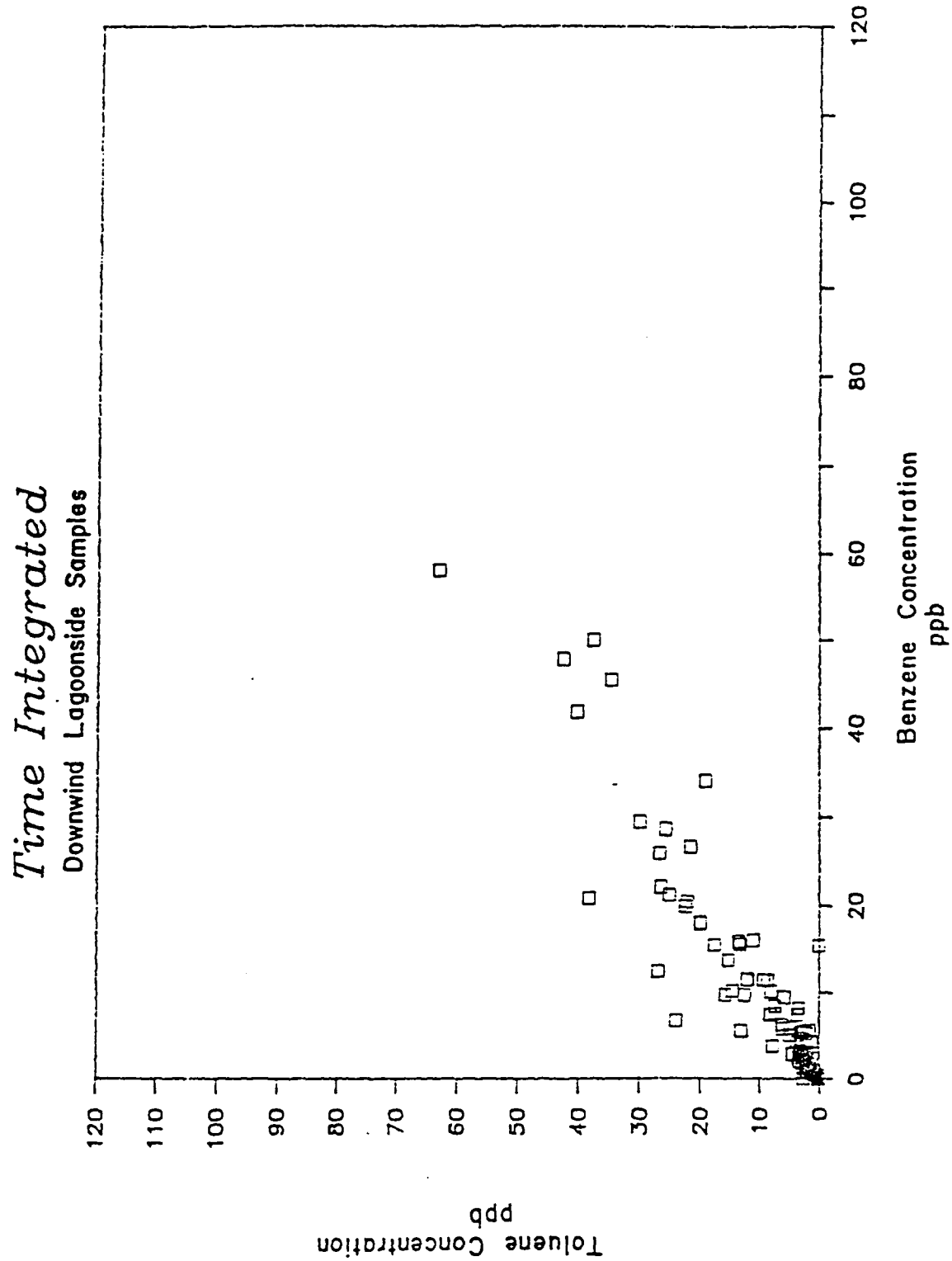
Time Integrated
Downwind Lagoonside Samples
Benzene Concentration
ppb
Toluene Concentration
ppb
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100
110
120

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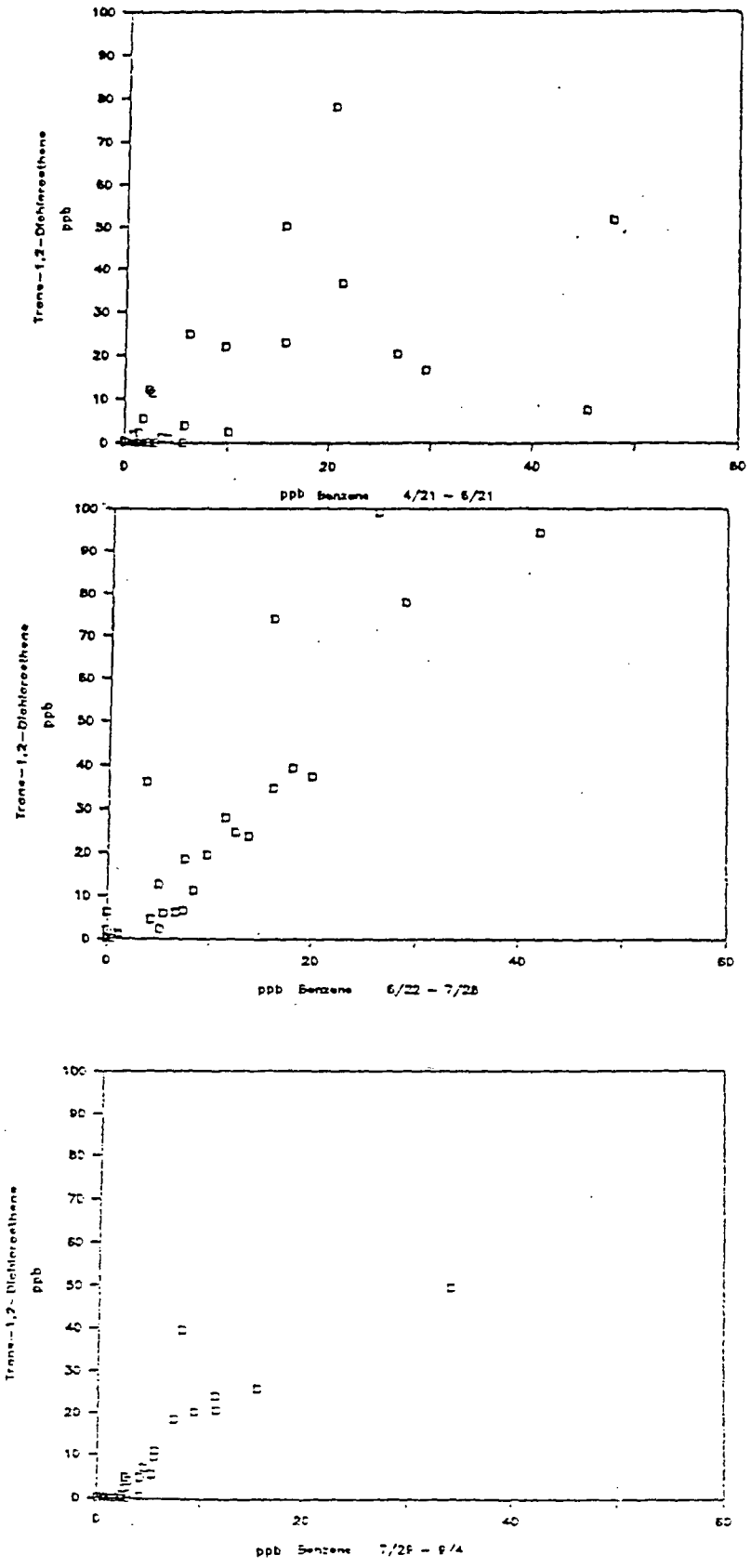
Figure 4

Time Integrated Samples



of benzene as mixing goes from one area to the next. As the mixing continues 6/22-7/28 and 7/29-9/4 the sludge becomes more homogeneous throughout the lagoon and the trans-1, 2-dichloroethene concentration becomes more and more correlated with benzene concentrations.

Meteorological Effects. One of the drawbacks to time-integrated sampling is the requirement that the wind direction needs to be predicted for the integrated sampling period, such as 8 hours. If the wind changes direction early in the sampling period, or winds are light and variable throughout the day it will have a direct effect on the concentrations experienced by the sampling location. In the present study 61% of the downwind sampling locations were within $\pm$ 20^{o} of the average wind direction for that day. Nearly all remaining samples were collected on highly variable days on which no fixed location was downwind the majority of the sampling period.

Measurement Precision. Variability superimposed upon all other effects is the imprecision of the collection and analysis of the sample. Precision can be optimize by using proven laboratory and field procedures and be closely following the quality assurance procedures specified for the method used. Precision in this study was good. Even for minor species detected at low ppb or sub ppb concentrations there was good agreement between measurements. For example, a relative standard deviation for the 25 collocated measurements of tetrachloroethene of 22%.

In summary, all the above affects added to the day to day variability in the impact concentration of contaminants. Site operations, such as mixing, seemed to be the most important effect. This is actually preferred since it allows emissions to be readily controlled by modifications to the operations.

CONCLUSIONS

The air monitoring program successfully achieved its objectives of measuring the on-site and off-site impacts of air emissions and protecting the health and safety of on-site personnel and the off-site general public. The air emissions impacts during the in-situ bioremediation demonstration were minimal and placed few limitations on day-to-day operations. When impacts did occur, they were found to be readily controllable, as they would decrease immediately upon reducing the intensity of sludge aeration or mixing.

The comprehensive air monitoring program used at French Limited allowed answers to air impact questions to be available in time to assure proper safe operations. It also allowed the flexibility to decrease air monitoring intensity once air impacts

were documented to be minimal. The carefully constructed quality assurance program and a thorough analysis of the results observed yielded a great amount of detail in the data. This data should aid in the continuing effort at the French Ltd. site, and future bioremediation projects.

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EPA's METHOD DESCRIPTION FOR MONITORING VOLATILE ORGANIC COMPOUNDS

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ABSTRACT

The U.S. Environmental Protection Agency provides information on monitoring of air quality as a general service to regional, state, and local organizations. Method TO14 documents a monitoring methodology for sampling and analysis of volatile organic compounds in air. This methodology incorporates a sampling strategy based on the use of Summa(R)-passivated stainless steel canisters to collect whole-air samples. Analysis of these air samples for individual organic compounds is accomplished by gas chromatographic separation combined with a variety of specific or non-specific detector options. The specific detector option or options used can be chosen to provide species identification and quantitation compatible with resource constraints.

INTRODUCTION

The Environmental Monitoring Systems Laboratory (EMSL) of the U.S. Environmental Protection Agency has prepared a new method for sampling and analysis of volatile organic compounds in ambient air. The method, identified as Method TO14, is titled "Determination of Volatile Organic Compounds (VOCs) in Ambient Air Using Summa(R) Passivated Canister Sampling and Gas Chromatographic Analysis."(1) Developed from the results of recent EMSL methods development and standardization activities, Method TO14 includes the experimental findings and technical contributions of many researchers in the field, and it has been extensively peer-reviewed. This method will join several other similar methods for measurement of toxic organics contained in the EPA publication *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air.*(2,3) The purpose of this paper is to outline the content of Method TO14 and to discuss various aspects and applications of this new method.

SAMPLING

Method TO14 is based on collection of whole-air samples at the

monitoring site, with subsequent VOC analysis in the laboratory. The air samples are collected and stored prior to analysis in specially designed stainless steel canisters. The interior surfaces of these sample canisters are passivated via the Summa(R) passivation process (Molectrics, Carson, CA), in which a pure chrome-nickel oxide is formed on the surface of the stainless steel. Numerous compounds, many of which are chlorinated VOCs, have been tested to verify storage stability and quantitative analytical recovery in these sample canisters.(4,5) Many compounds have been successfully measured in canister-collected air samples at the parts-per-billion (ppbv) level.(6) The Summa-passivated sample canisters are commercially available from several suppliers.

Collection of whole-air samples in the canisters offers several advantages over field-extractive methods using solid sorbents such as Tenax(R). These include convenient integration of ambient samples over a wide range of specific sample periods, ease of storing and shipping air samples, application to a wide range of organic compounds, lack of need for precise sample volume measurement during sample collection, and opportunity for multiple analyses of the air sample. However, care must be exercised in selecting, cleaning, and handling the sample canisters and sampling apparatus to avoid losses or contamination of the sample.

Air samples collected and contained in sample canisters may be at subatmospheric pressure or they may be pressurized above atmospheric pressure.(7) Pressurized samples provide greater sample volume for analysis for a given canister size and may be somewhat easier to extract from the canister for analysis. However, a sample pump is required, which could contaminate the air sample, and moisture condensation inside the canister could be a potential problem. Subatmospheric pressure samples may be collected into an initially evacuated canister without a pump and normally avoid moisture condensation in the canister. However, both sample collection into the canister and subsequent extraction of the sample air out of the canister for analysis may be slightly more difficult than for pressurized samples.

Sampling apparatus for sample collection in canisters varies depending on the air sample requirements. Grab samples may be obtained by simply opening the valve on an initially evacuated canister. Integrated samples require a timer and solenoid valve to control the start and duration of the sample period as well as some means of flow control to maintain a constant flow rate over the sample period. Pressurized samples require a non-contaminating-type of pump such as a

metal bellows pump, while subatmospheric pressure samples require a low-pressure-drop type of flow rate control device such as an electronic mass flow controller. Other sampler variations include weatherization and internal temperature control for outdoor samplers, and auxiliary pumps used to ventilate sample inlet lines when very low flow rates are used (required for long integration periods) or to precondition inlet lines prior to the sample period.

As noted earlier, the sample canister and all components in the sample collection apparatus must be clean and free of contaminating organic compounds as well as substances that may cause losses of the sampled compounds of interest. Use of stainless steel components and chromatographic grade materials is recommended, and special cleaning procedures are given in TO14 for cleaning pumps, solenoid valves, and flow rate control elements. After assembly, the sample apparatus should be "certified" as clean. Certification includes tests for contamination with zero air and tests for compound losses using compound concentration standards.(8)

Canisters must also be cleaned before sample collection. Procedures and apparatus suggested for cleaning canisters include repeated evacuation to a vacuum of 0.05 mm Hg or better for up to an hour, purging with humidified zero air, and optional application of tepid heat. Cleaned canisters should be tested with zero air (blanked) to verify cleanliness before use.

ANALYSIS

Following collection of the air sample, the canister valve is closed, and the canister is transported to a predetermined laboratory for analysis. Upon receipt at the laboratory, the canister is attached to the analytical system. During analysis, water vapor is reduced in the gas stream by a Nafion(R) dryer (9) (if required by the analytical system), and the VOCs are then concentrated by collection in a cryogenically-cooled trap.(10) The cryogen is then removed and the temperature of the trap is raised. The VOCs originally collected in the trap are revolatilized, separated on a GC column, then detected by one or more detectors for identification and quantitation.

The analytical strategy of Method TO14 involves using a high-resolution gas chromatograph (GC) coupled to one or more appropriate GC detectors. Detectors for GCs may be divided into two groups: non-specific detectors and specific detectors. Non-specific detectors

include the nitrogen-phosphorus detector (NPD), the flame ionization detector (FID), the electron capture detector (ECD), and the photo-ionization detector (PID). Specific detectors include quadrapole mass spectrometers (MS) operating in either the selected ion monitoring (SIM) mode or the SCAN mode, and the ion trap detector.

The analytical finish chosen by the analyst should provide a definitive identification and precise quantitation of the volatile organic compounds of interest. In a large part, the actual approach selected to achieve these two objectives is subject to equipment and resource availability. Figure 1 indicates some of the favorite options that are used as an analytical finish. The section references in Figure 1 correspond to sections in TO14. The GC-MS-SCAN option uses a capillary column GC coupled to a MS operated in a scanning mode and supported by spectral library search routines. This option offers the nearest approximation to unambiguous identification and covers a wide range of compounds, as defined by the completeness of the spectral library. GC-MS-SIM mode is limited to a set of target compounds that are user-defined and is more sensitive that GC-MS-SCAN by virtue of the longer dwell times at the restricted number of m/z values. Both of these techniques, but especially the GC-MS-SIM option, can use a supplemental, general, non-specific detector to verify or identify the presence of non-targeted VOCs. Finally, the option labeled GC-multidetector system uses a combination of retention times and multiple general detector verification to identify and quantify compounds. However, interference due to nearly identical retention times (co-elution) can affect both quantitation and identification when using this option.

Due to the low concentrations of VOCs encountered in urban air (typically less than 4 ppbv and the majority below 1 ppbv) along with their complicated chromatograms, Method TO14 strongly recommends the specific detectors (GC-MS-SIM or GC-MS-SCAN) for positive identification and for primary quantitation to ensure that high quality measurement data are acquired. For the experienced analyst whose analytical system is limited to the non-specific detectors, Section 10.3 of TO14 covers the GC analytical system utilizing the non-specific detectors (GC-FID-ECD-PID) as the primary quantitative technique and provides guidelines, example chromatograms showing typical retention times and calibration response factors.(11)

A list of some of the advantages and disadvantages associated with non-specific and specific detectors may assist the analyst in the decision-making process.

Non-Specific Multidetector Analytical System

Advantages	Disadvantages
o Somewhat lower equipment cost than GC-MS	o Multiple detectors to calibrate
o Less sample volume required for analysis	o Compound identification not positive
o More sensitive - ECD may be up to 1000 times more sensitive than GC-MS	o Lengthy data interpretation (up to one hour each for analysis and data reduction)
	o Interference(s) from co-eluting compound(s)
	o Cannot identify unknown compounds outside range of calibration or without specific standards
	o Does not differentiate targeted compounds from interfering compounds

GC-MS-SIM

o Positive compound identification	o Can't identify non-specified compounds (ions)
o Greater sensitivity than GC-MS-SCAN	o Somewhat greater equipment cost than multidetector GC
o Less operator interpretation than for multidetector GC	o Greater sample volume required than for multidetector GC
o Can resolve co-eluting peaks	o Universality of detector sacrificed to achieve enhanced sensitivity
o More specific than the multidetector GC	

GC-MS-SCAN

o Positive compound identification	o Lower sensitivity than GC-MS-SIM
o Can identify all compounds	o Greater sample volume required than for multidetector GC
o Less operator interpretation	o Somewhat greater equipment cost than multidetector GC
o Can resolve co-eluting peaks	

A more specific discussion of detector characteristics is given in the TO14 method description.

CONCLUSIONS

Method TO14 describes an alternative to the use of solid sorbent methodology for the monitoring of volatile organic compounds in ambient air. The method represents the cumulative developmental efforts at the U.S. Environmental Protection Agency to combine a VOC sampling strategy based on whole-air collection in Summa(R)-passivated stainless steel canisters with an analysis strategy based on reduced temperature preconcentration followed by gas chromatographic separation and specific/non-specific detector combinations. The method is available from EPA, along with a companion Technical Assistance Document.(12)

ACKNOWLEDGMENTS

We gratefully acknowledge the work of Mr. William T. Winberry and Ms. Norma T. Murphy of Engineering-Science, Inc., who researched and authored Method TO14.

DISCLAIMER

The research described in this article 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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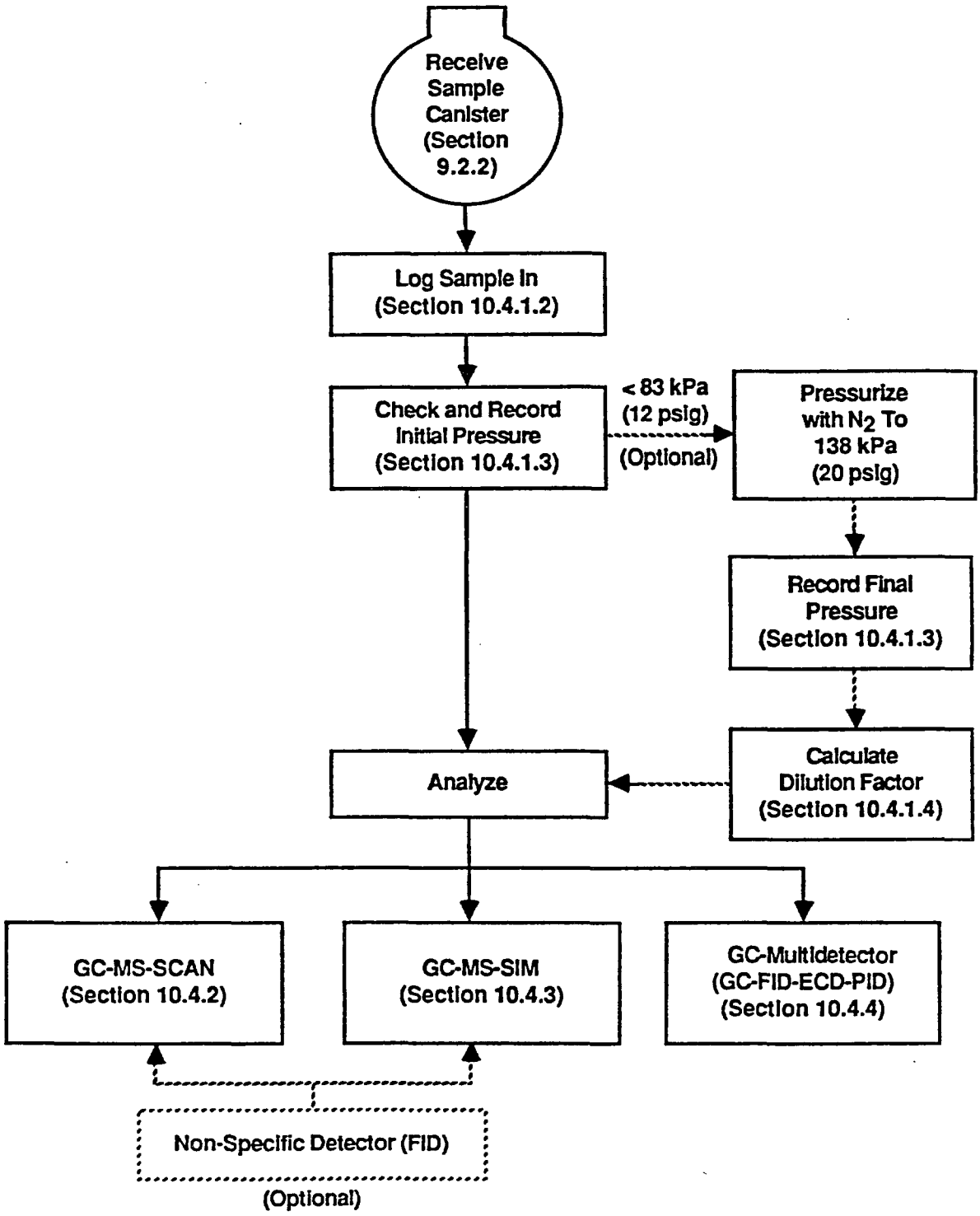

FIGURE 1. ANALYTICAL SYSTEMS AVAILABLE FOR CANISTER VOC IDENTIFICATION AND QUANTITATION

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EXAMINATION OF AIR SAMPLES NEAR HAZARDOUS WASTE SITES: CONCENTRATIONS, RISKS, METHODS, AND FINDINGS

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ABSTRACT

This paper reviews findings of air concentrations near hazardous waste sites/facilities. Information on concentrations, available methods, and risks are addressed as reported for situations that fall within the realm of both the Hazardous and Solid Waste Program as well as the Superfund Program. The paper addresses approaches that are under development to support screening air concentrations for potential Superfund sites and efforts to characterize air concentrations near RCRA facilities. The implications for limitations in measurement methodologies for assessing potential risks and use of modeling information to complement and support monitoring efforts is discussed.

BIOLOGICAL TEST METHODS

THE USE OF BIOMONITORING TECHNIQUES TO CHARACTERIZE THE TOXICITY AND ASSESS THE ENVIRONMENTAL IMPACTS OF WASTES FROM UNCONTROLLED HAZARDOUS WASTE SITES

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ABSTRACT

Chemical and physical data obtained during preliminary site assessments and remedial operations are often insufficient to evaluate the environmental impact of uncontrolled hazardous waste sites. Factors contributing to such data insufficiencies include the inability to routinely test for all potentially toxic chemicals, and the failure of the analyses to provide information on synergistic and additive effects of toxic substances in the leachate. Two CERCLA sites where biomonitoring techniques (specifically, acute and chronic toxicity tests, and Ames Test) were used demonstrate the utility of these techniques in characterizing the toxicity and evaluating the environmental impact of complex waste mixtures. At both sites, chemical characterization of the wastes did not clearly identify compounds at levels which would suggest significant aquatic or human health impacts.

At the first site, acute toxicity tests indicated that the leachate was extremely toxic to aquatic life (96 hour LC50 - 0.13% by volume). An additional acute toxicity test conducted on the same sample after storage for two months resulted in a 96 hour LC50 of 0.20%, indicating that the toxicity of the leachate was persistent. Chronic toxicity tests performed on the leachate indicated an estimated no observable effect level (NOEL) of 0.0095% by volume (95 ppm). The NOEL for a complex mixture is equivalent to the water quality criterion (continuous exposure) for a single chemical. This information formed the basis for a major regional enforcement action.

At the second site, acute toxicity testing and Ames Tests were conducted of both leachate and receiving water samples. Acute toxicity tests indicated that the leachate was very toxic (96 hour LC50 - 4.6%). Ames Tests of both the leachate and downstream samples indicated a significant mutagenic response. Tests were also performed three years later, just prior to remedial activities at the site, to assess the persistence of the toxicity/mutagenicity of the leachate stream and test several new leachate streams from another part of the landfill. These tests indicated that the previously tested leachate stream was still as acutely toxic (96 hour LC50 - 7.3%) and mutagenic, while the new leachate streams were less acutely toxic and were not mutagenic. On the basis of this information, significant changes in the planned remedial actions at the site ensued.

UTILITY OF IN SITU ASSAYS FOR DETECTING ENVIRONMENTAL POLLUTANTS

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INTRODUCTION

The environment contains a wide variety of man-made and naturally occurring toxic agents. Traditionally the toxicity of environmental contaminants is assayed in the laboratory. However, adverse biological effects induced by environmental chemicals result, for the most part, from complex interactions among various chemical and physical components of a given environment. In the natural environment, no biological system is acted on by a single chemical. The induced toxic effects are often the result of synergistic and antagonistic interactions among various chemicals in complex mixtures and their interaction with physical factors that may be impossible to reproduce in the laboratory.

For the last ten years interest has developed in the assessment of the effects of environmental pollution to man and to the environment in the biological, chemical and physical complexity of real world conditions in contrast to the controlled conditions of the laboratory. The methods of evaluating the induced biological effects on the site to be evaluated is termed *in situ* assessment. With *in situ* assay the indication organism, either special strains developed for the laboratory or indigenous resident species, are introduced to or are present at the test site. The data are collected at the test site or the samples of the environmentally exposed test organisms are preserved and analyzed in the laboratory.

In this presentation, we concentrate on currently available assays for assessing mutagenicity, but also discuss other assay systems as appropriate. In addition, we review the utility of *in situ* monitoring for environmental assessment. Three recent reviews discuss some aspects of *in situ* mutagenicity monitoring [1-3].

IN SITU MONITORING METHODOLOGIES

Of the many species that could be used for *in situ* environmental mutagenicity assessment, certain ones have been used more often than others because they are readily available, easily manipulated, and exhibit easily recognizable genetic markers. The organisms most commonly used for *in situ*

* This manuscript 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.

mutagenicity testing have been flowering plants, wild and captive terrestrial mammals and aquatic vertebrates.

Plant systems are particularly suitable for in situ assessment. They are relatively easily maintained, and allow evaluation of effects induced by individual or simultaneous multiple exposures to contaminants in air, water, and soil. Progress in use of in situ plant test systems has been slow; however, significant efforts have been made since the mid-1970's to use a few species of plants to determine levels of mutagens in the air, water, soil, and a variety of industrial situations.

Several species, hybrids, and strains of Tradescantia have been to investigate environmental mutagenicity. Two Tradescantia assay systems have been employed; one for detecting in single-gene mutation in stamen hair cells and the other for chromosomal aberrations as micronuclei in pollen mother cells.

A series of in situ studies using the Tradescantia stamen hair assay to monitor ambient air for genetic hazards at 18 U.S. sites was instituted by Brookhaven National Laboratory from 1976 through 1980. One of the sites monitored was Elizabeth, NJ, which has a large number of petrochemical refineries and at which mutation frequency in plants exposed to ambient air was 90.6% above the control level. Two clean sites, the Grand Canyon, AZ, and Pittsboro, NC, registered no increase in mutations above the control level [4, 5]. Part of each air sample was analyzed by GC/MS, and a part was also tested for mutagenicity in Salmonella typhimurium. The results obtained in the S. typhimurium showed close agreement with those obtained from the Tradescantia assay. However, no compound or class of compounds could be associated with the mutagenic responses observed and the mutagenic response at a particular site was dependent upon the ambient temperature and wind direction relative to the monitoring site.

The Tradescantia stamen hair assay has also been used to determine the mutagenicity of sewage sludges, polluted water, bottom sediment from a drinking-water reservoir, diesel emission exposures, air pollution from petrochemical plants, soil and air pollution from a lead smelter, chemical smokes at a military installation, effluent from a titanium factory and the environment around nuclear power plants [6-14] and to evaluate the hazards of peroxyacetyl nitrate and related photo-oxidants in the ambient environment and under laboratory conditions [15]. Most of the efforts to use this system have been yielded positive results although one, using clone KU-7 to monitor the area around a nuclear power plant for three years in Japan, did not show no significant changes in mutation frequencies as compared to the concurrent negative control.

The Tradescantia micronucleus assay detects chromosomal aberrations as micronuclei at the tetrad stage in meiotic cells of pollen. This uniquely sensitive assay developed by Ma [16] has been used for testing several in situ monitoring sites, as well as sewage sludges, pesticides, diesel exhaust, and obscurant smokes [3, 14, 16-18].

Another plant system used for in situ mutagenesis studies is the single-gene waxy locus of corn (Zea mays). This bioassay system, promulgated by M. Plewa [19], involves the synthesis of amylopectin and amylose, in pollen

cells and detects both forward and reverse mutations. The waxy corn pollen system in combination with the Tradescantia stamen hair test and observations of nitrogen fixation of soybean (Glycine max), corn height at anthesis, percent pollen abortion of corn, and excretion of urinary δ-aminolevulinic acid in Peromyscus leucopus/maniculatus (white-footed/deer mouse) have been used to evaluate toxicity in the vicinity of a lead smelter [7, 20-23]. This test system in concert with S. typhimurium, the Tradescantia micronucleus test, and the maize yellow/green (yg-2/yg-2) leaf spot assay has also been used to evaluate the mutagenicity of a number of city sewage sludges [24] and with the Tradescantia stamen hair test to evaluate environmental mutagenicity near an oil refinery and a petrochemical complexes [6].

A natural population of Osmunda regalis (royal fern) growing in a river heavily contaminated with paper recycling waste was reported to have a high incidence of chromosomal aberrations [25]. Solvent extracts of samples of the paper recycling waste collected from the wastewater treatment facility and tested in the laboratory also showed mutagenicity in the Ames test with S-9 fraction and in the soybean meitotic crossing-over assay.

Other plant assays that could be further developed for in situ mutagen monitoring of soil, water, and possibly air include the Hordeum (barley) chlorophyll-deficient-mutation assay and Hordeum chromosome aberration assay [26,27], Arabidopsis embryo mutation assay [28], Vicia faba (broad bean) root-tip cytogenetic assay [29], and Allium cepa (onion) root-tip cytogenetic assay [1].

The use of feral, wild and laboratory mammals to indicate the presence of environmental mutagens and carcinogens has been investigated. However, emphasis on this type of research is recent, and limited work has been done.

Nayak and Petras [30] studied sister chromatid exchanges (SCE) in feral Mus musculus (house mouse) caught in corn cribs at various distances from industrial sites in southern Ontario, Canada. The frequencies of SCEs were compared between (1) feral mice and inbred mice reared in laboratory and (2) feral mice kept in the laboratory for nine months and inbred mice kept outdoors for nine months; the results suggested that environmental toxic contaminants may induce significantly higher levels of SCE's under natural conditions. An inverse relationship between SCE frequency and the distance of the sample collection site from the nearest major industrial center also suggested that industrial atmospheric pollutants contributed to the increased levels of SCEs and that SCEs may be useful for detecting environmental mutagens.

From a preliminary cytogenetic study of bone marrow and germ cell damage in wild populations of P. leucopus from a hazardous waste site and uncontaminated areas, Tice et al. [31] concluded that these mice can be used to monitor the toxicity of waste sites. A similar but larger study in Texas used Sigmodon hispidus (cotton rat) and P. leucopus to demonstrate significant differences in the incidence of chromosomal aberrations between a control site and two petrochemical waste disposal sites [32-34]. From work at a military facility in California, Schaeffer et al. [14] detected significantly higher frequencies of SCEs in the native rodents, Dipodomys merriami (kangaroo rat), exposed to obscurant smokes than in the unexposed animals.

Man should play an active role in environmental monitoring. At times, it may be more efficient to directly monitor man himself rather than a surrogate

species, particularly when human health is the prime concern. Monitoring humans for genetic damage due to occupational, accidental, or recreational exposure to mutagens is becoming more common. This is in contradistinction to purposefully using humans as environmental monitors, although, in most cases, the end result may be the same. The occurrence of chromsome abnormalities including SCEs in bone marrow cells, lymphocytes and sperm abnormalities are increasingly used for evaluating potential genetic damage in humans due to exposure to environmental contaminants. Such work on SCEs and chromosomal aberrations is described in an extensive review by Lambert et al. [35] and recently discussed by Dulout et al. [36]. These reviews show that, in general, smoking is the prime contributor to an increase in SCEs, but many drugs, particularly anticancer drugs, also cause an increase in SCEs. The frequency of chromosome aberrations in alcoholics, heavy smokers and workers exposed to vinyl chloride and pesticides were significantly higher compared to those not exposed to these agents [37-40]. The potential role of paternal exposure to mutagens on birth defects, spontaneous abortions and inherited childhood cancer is discussed by Narod et al. [41]. They point out the need for definitive epidemiological data and drawing parallels with animal models to define the role of exposure to enviornmental chemicals from dioxin to anesthetic gases on somatic and germinal mutations in man.

Testing for mutagenicity of feces and body fluids (such as urine and blood) in the Ames assay and other tests has been reviewed [42]. The formation of DNA adducts and monoclonal antibodies to these adducts are are also now used as indicators of exposure for human populations to hazardous chemicals.

Aquatic animals have been used more often than terrestrial animals to identify and characterize the mutagenic effects of environmental pollution. Since late 1970, a number of studies have been reported on various mutagenic effects on aquatic animals from coastal areas and continental rivers, lakes, and ponds.

With the discovery of SCE as a sensitive indicator of exposure to potentially harmful agents, studies have been performed to evaluate the effects of chemicals on induction of SCE in mudminnows [43], mussels [44-46], and benthic worms [47]. The SCE assay was extended to complex environmental mixtures in a study of SCE formation in mudminnows exposed to Rhine river water [48]. Induction of other chromosomal aberrations by chemicals has been studied in the fish Boleophthalmus dussumieri [49] and in purple sea urchin [50]. Micronucleus counts, along with the incidence of various red and white blood cell abnormalities were higher in white croakers from San Pedro and Santa Monica Bays than those from Dana Point, California [51]. An in vivo assay for micronuclei in the erythrocytes of the mudminnow and brown bullhead has been developed [52], and an assay for micronuclei in bullfrog tadpoles has been developed for genotoxicity studies of environmental pollutants, but has not been applied in situ [53].

Several of the studies cited above provide examples of both the simultaneous measurement of different biological functions within an organism as well as the use of several species for bioassay within a given study. For example, the study by Brookhaven of air pollution involved both the stamen hair mutation test of Tradescantia and mutagenicity testing with S. typhimurium and GC/MS analysis of air samples [4, 5]. The three year study of an oil refinery complex at Wood River, Illinois, and a one year study of the petrochemical complex at Beaumont, Texas, employed the stamen hair test of Tradescantia and

the pollen mutation test of Z. mays, with length of exposure of 4 to 12 weeks. The Tradescantia and Z. mays at Wood River, planted in the ground and exposed to both soil and air born pollution, showed consistently elevated mutation frequencies with respect to controls. The same plants at Beaumont, Texas planted in pots of Pro Mix, a commercial potting mixture, also registered air pollution and showed consistent statistically significant elevated mutation frequencies compared to controls. The determination of mutation frequencies each day of Tradescantia for 11 consecutive days showed consistently elevated daily mutation frequencies.

In the study in the vicinity of a lead smelter plants and animals, Z. mays, Tradescantia, G. max, P. leucopus/maniculatus, were all used, and a variety of endpoints were measured in Z. mays; mutation frequency of pollen, height of plant at anthesis and percent pollen abortion. All of the measured endpoints in all of the species indicated toxic effects and corroborated one another [7, 19-22]. In this study, chemical analyses of soil content of lead, cadmium, zinc and copper as well as the various biological endpoints were measured with distance from the lead smelter. The independent variable distance was of far less interest than metal concentration, because the concentration of metal was the cause of the observed biological changes. A statistical method was devised to consider lead concentration, and by implication the other metals also as the independent variables with multiple observations of lead concentration at each distance. This test, an indirect test of correlation, permitted the comparison of results from the series of experiments designed to determine changes measured in the biological functions of the plants with respect to concentrations of lead in the soil at different distances from the smelter without loss of information on the variance of multiple measurements of lead at each distance [21, 22].

Along with mutagenesis, the electron transport system of photosynthesis, a physical-chemical, non-mutagenic test which measures the oxidation-reduction state of the Photosystem II primary acceptor, was also measured in leaf segments of Tradescantia and Z. mays exposed to water reservoir bottom sediment and water from a cooling tower [8, 9]. In both studies the pertubations of the electron transport system were negatively correlated with and corroborated the increase in mutation frequencies.

The study of chemical smokes used as obscurant smoke screen involved two strains of Tradescantia, 4430 and 03, with measurements of stamen hair mutations, production of micronuclei, pertubations of the electron transport system of photosynthesis and flower production. Measurements were also made of SCE induction in D. merriami and pertubations of the electron transport system of photosynthesis of the native plant Franseria dumosa (burrobush). All species and measurements indicated varying degrees of lethal, physiological and mutagenic effects in the assay systems.

DISCUSSION AND CONCLUSION

Assessment of environmental toxicity is made difficult by the complex involvement of many unknown factors in producing the observed biological effects. A few attempts have been made to establish quantitative relationships between the chemical concentration at a particular test site and the manifestation of biological effects. In studies cited above using Tradescantia [4, 5] to monitor ambient air quality at 18 U.S. sites it was not possible to correlate levels of mutagenicity or carcinogenicity with the concentrations of specific

compounds or classes of compounds. Even the total concentration of organic chemicals was not correlated with the levels of the observed genotoxic effects. Several factors, some discussed below, contribute to the absence of correlation between biological effects and chemical concentrations at a particular site.

Our understanding and technology for detecting transformed chemicals (e.g., transformed by photochemical oxidation or biochemical processes), is primitive, and few studies have been made of the role of photochemical oxidation in modulating the biological effects of chemicals. In addition, although significant progress has been made since the mid-1970's in the field of analytical chemistry, particularly in the application of capillary gas chromatography, high-performance liquid chromatography, and multi-dimensional chromatography, we still are not able to identify the majority of the chemicals in complex mixtures. Identity of the chemicals would not change the conclusions of studies discussed, but would identify the causative agents and probably aid in the development of methods to decrease their concentration in the environment. It is desirable to know the cause of an observed mutation or carcinoma.

An observed biological effect is a result of complex interactions between biological, chemical, and physical factors. Certain chemicals, innocuous in themselves, may be very effective in potentiating or otherwise affecting the toxicity of other chemicals, and it frequently is not possible to ascribe a specific biological effect to a particular compound present in a complex chemical mixture. Compounds are not taken up with equal efficiency by all organisms, and the bioavailability, transformation, and fate of a compound must be known before its complete biological effects can be ascertained.

Attempts to reproduce or verify the field observations in the laboratory have not been very successful. Difficulties in establishing a quantitative relationship between laboratory and field observations are due to lack of control over the physical environmental components, the inherent variability and interactions of test organisms, and differences in or lack of control over dosages. In the laboratory, physical factors are better controlled and not subject to the variability of the natural environment. There may also be less variation among tester organisms used in the laboratory than among those employed for field monitoring, especially if native wild species are used as the biological indicators.

Another difficulty in relating field to laboratory observations is the frequent lack of concurrent negative in situ field controls. In addition, it is often difficult to define and identify clean sites due to the common presence of unknown contaminants and the pervasive occurrence of agricultural chemicals. The use of historical controls is not considered adequate. More than one negative in situ control site may be necessary, particularly if there is any concern or suspicion that a proposed control site may be contaminated. A laboratory control is also useful to estimate the contribution of the ambient environment of the in situ control site on the biological endpoint measured, but it is not a substitute for an in situ control.

Many of the problems of in situ monitoring can be partially alleviated by careful planning of the experimental design and statistical analyses before the field studies are started. By their very nature, in situ studies are likely to have less clear-cut results than laboratory studies, and require well planned experimental design and statistical methods for their interpretation.

Two general categories of in situ genotoxicity assay systems are (1) those that can be used both in the laboratory and in situ and (2) those that can be used only in situ. The majority of species and techniques used for in situ mutagenicity monitoring have been developed for the laboratory and secondarily tested and applied in situ.

In situ assays may be used simply to determine whether an effect is present at a given site, to establish dose-response relationships (e.g., response as a function of distance from a contamination source, duration of exposure, and so forth), or to survey an area to locate and characterize possible sources of genotoxicity.

The utility of in situ bioassay systems can be enhanced in several ways. For example, taking advantage of the fact that organisms have many biological functions with different sensitivities to toxic agents, we can assess several endpoints in the same organisms; a small increase in effort and cost can greatly increase the information derived from such a test system. The use of a battery of tests in one organism decreases the chance that adverse effects will escape detection, and the use of a single species to evaluate a variety of media (e.g., air, water, soil, food), individually or in various combinations, increases the efficiency of monitoring efforts. The simultaneous use of several species would provide further information on the diversity of toxic effects [7, 8, 13, 17, 18]. Finally, although direct testing of an organisms is the most common approach, testing of excreta, secretions, and extracts of material collected in the field in bacterial and other test systems in the laboratory will further extend the potential of in situ toxicology.

It would be desirable ultimately to develop a repertoire of in situ mutagenic and non-mutagenic test systems from which tests could be selected to meet specific environmental assessment needs. This may be a formidable task, because various environments such as terrestrial, freshwater, brackish water, salt water, air, desert, etc. may require many different tests and species.

ACKNOWLEDGEMENTS

We wish to thank Kathy Shaw and Debra Schuster for help in preparation of the manuscript.

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A HEXAPLOID VIRESCENT WHEAT ASSAY FOR DETECTING CYTOGENETIC EFFECTS FROM HAZARDOUS WASTE SITES*

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ABSTRACT

A simple short-term assay using Chinese spring hexaploid wheat (Neatby's strain) for detecting environmentally induced clastogenicity and/or aneuploidy is described. In the tester strain, diploidy of $\underline{v}_1$ alleles located on the short arm of chromosome 3B causes leaves of the wheat seedling to be virescent. Trisomy of these alleles completely inhibits chlorophyll development; whereas, monosomy of these alleles does not interfere with chlorophyll development. Normally, the seed of $\underline{v}_1\underline{v}_1$ genotype are treated with the test agents. Therefore, appearance of white sectors ($\underline{v}_1\underline{v}_1\underline{v}_1$) or green sectors ($\underline{v}_1$o) on the virescent leaves indicates the loss or gain of chromosome or a segment of chromosome bearing $\underline{v}_1$ allele. The presoaked seed of Neatby's strain of wheat were exposed to varying doses of gamma rays (5 to 20 kR), ethyl methanesulfonate (20 to 2000 µg/ml), or vinblastin sulfate (3.12 to 50.0 µg/ml). In addition to scoring green and white longitudinal sectors on the virescent leaves, cytogenetic analysis was performed on root tip cells obtained from the wheat seed treated with gamma radiation, ethyl methanesulfonate, or vinblastin sulfate. These test agents produced a concordant increase in the leaf sectors and chromosome aberrations in the root cells suggesting the cytogenetic basis for the leaf sectors.

INTRODUCTION

The significance of aneuploidy as a factor in human mortality and morbidity has been only recently realized. Since 1979, efforts have been made to increase our knowledge on the incidence, etiology, mechanisms, and methods to detect aneuploidy (de Serres, 1979; Bond and Chandley, 1983; Dellarco et al., 1985, 1986; Vig and Avery, 1987). With improvements in cytological techniques and renewed interest in the role of chromosome aberrations in the etiology of human diseases, it has become evident that malsegregation of chromosomes in the mitotic and meiotic cells is much more prevalent than previously believed. Coupled with the high frequency of aneuploidy is the concern that the environmental agents, especially manmade chemicals, may be involved in the induction of aneuploidy.

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* This manuscript 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.

A large number of short-term assays are available for detecting mutagens and clastogens. However, there is a lack of short-term assays for utilizing higher eucaryotes for identifying chemicals capable of inducing aneuploidy (Dellarco et al., 1986). A few methods that are currently available for detecting aneuploidy in higher organisms rely on cytogenetic observations which are highly labor intensive and as a consequence limited to the analysis of a rather small number of cells treated at high doses.

We have previously described a simple assay for detecting environmentally induced aneuploidy using Neatby's virescent strain of Chinese spring wheat (Triticum aestivum, 2n = 6x = 42) (Redei et al., 1985; Redei and Sandhu, 1988). Neatby's virescent mutant was isolated in 1933 from a F_5 progeny of an intervarietal cross of Chinese spring wheat (Neatby, 1933). By backcrossing with each of 21 wheat monosomics, v_1 alleles responsible for virescence were found to be located on the short arm of 3B chromosome. The development of chlorophyll in this strain is dependent on allelic dosage and temperature regimentation.

At temperatures below 26°C, the v_1v_1 condition interferes with chlorophyll development and induces virescence; plants of $v_1v_1v_1$ genotype are albinos, and v_1o are green. Nondisjunction generally results in green-white twin sectors, and deletions and duplications cause single sectors when appropriately marked chromosome(s) are involved. At higher temperatures it develops normal green color. Obviously, nondisjunction is not limited to chromosome 3B, and such events involving 3A (V_2) and 3D (V_3) have the phenotypic consequences (in the presence of homozygosity for v_1) as indicated in Table 1. The frequency of green or white sectors on virescent background is considered as a phenotypic expression of nondisjunction or the duplication/deletion of the segment of short arm of chromosome 3B carrying v_1 alleles responsible for antimorphic expression in Neatby's strain. Thus, the two events are frequently distinguishable by the arrangement of the sectors. A white sector is not always detectable, however, on a virescent background. Chinese spring wheat is a hexaploid in which chromosomal aberrations lethal to diploids may only have minor effects on its viability.

The purpose of this study was to evaluate the ability of certain model agents to cause chlorophyll variants as white or green sectors on the leaves of virescent wheat seedlings grown at low temperature and to compare their frequency with the cytological analysis of chromosome alterations in the root cells of wheat seed treated with these compounds.

This assay is very simple to perform and is suitable for evaluating the ability of liquid effluents, soil, and leachate samples to induce chromosome breaks and aneuploidy.

MATERIALS AND METHODS

Test Agents

Ethyl methanesulfonate (EMS), vinblastin sulfate, and gamma rays were employed in this study. The EMS and vinblastin have been recommended by the Aneuploidy Committee of EPA as standard compounds for validating the assay systems for detecting aneuploidy (Liang and Brinkley, 1985). Treatment with gamma rays was included in the experiment because gamma rays are known to induce clastogenicity and gene mutation, and interfere with kinetochore and microtubule related mitotic

Table 1. Chromosomal complements of the chlorophyll variants observed in Neatby's virescent wheat seedlings grown at 18-26°C

Genotype			Phenotype
$\underline{v_1v_1}$	$\underline{V_2V_2}$	$\underline{V_3V_3}$	virescent
$\underline{v_1v_1}$	$\underline{V_20}$	$\underline{V_3V_3}$	extreme virescent
$\underline{v_10}$	$\underline{V_2V_2}$	$\underline{V_3V_3}$	green
$\underline{v_1v_1}$	$\underline{V_2V_2}$	$\underline{V_3V_3V_3}$	light green
$\underline{v_1v_1}$	$\underline{V_2V_2V_2}$	$\underline{V_3V_3}$	light green
$\underline{v_1v_1v_1}$	$\underline{V_2V_2}$	$\underline{V_3V_3}$	white

From Redei and Sandhu (1988).

functions (Sega, 1984). The chemicals (purity greater than 98%) were obtained from Sigma Chemical Co. (St. Louis, MO). Dry seed were exposed to gamma radiation at Brookhaven National Laboratory, Upton, NY.

Both EMS and vinblastin are soluble in water and tested at pH 7.0. The seed (100, 70, and 20 for gamma rays, vinblastin, and EMS, respectively) were presoaked for 8 h in distilled water at room temperature. Treatments were carried out in 50-ml flasks placed on a rotary shaker. The temperature during the treatment was maintained at 25°C. After 20 h of treatment, seed were washed for 15 min in distilled water. Some seed were placed in petri dishes containing wet filter paper to obtain roots for cytogenetic analysis. Often seed were planted in a mixture of gravel and vermiculite (1:1). The plants were grown in growth chambers at Duke University, Durham, NC, under 16-h daily light (intensity 340 m Mol $S^{-1}M^{-2}$) at 18° C, and watered daily with hoagland solution.

The types of green and white sectors scored on the second and third leaves are shown in Figure 1. There is usually a better expression of genetic damage (in terms of leaf sectoring) on the fourth leaf than the preceding leaves. However, most of the virescent plants take a much longer time to reach the fourth-leaf stage because they have exhausted most of their food reserves by the third-leaf stage; further growth is much slower, hence sectors on the fourth leaf are rarely scored.

Cytogenetic Analysis

Cytogenetic analysis was performed in an attempt to see if a chemical that causes a high frequency of leaf sectors also enhances structural and numerical chromosomal abnormalities in the roots. We realize that the concordance of green/white sectors in the leaves and the incidence of chromosome aberrations in the roots is only secondary evidence in support of the cytogenetic basis for leaf sectoring.

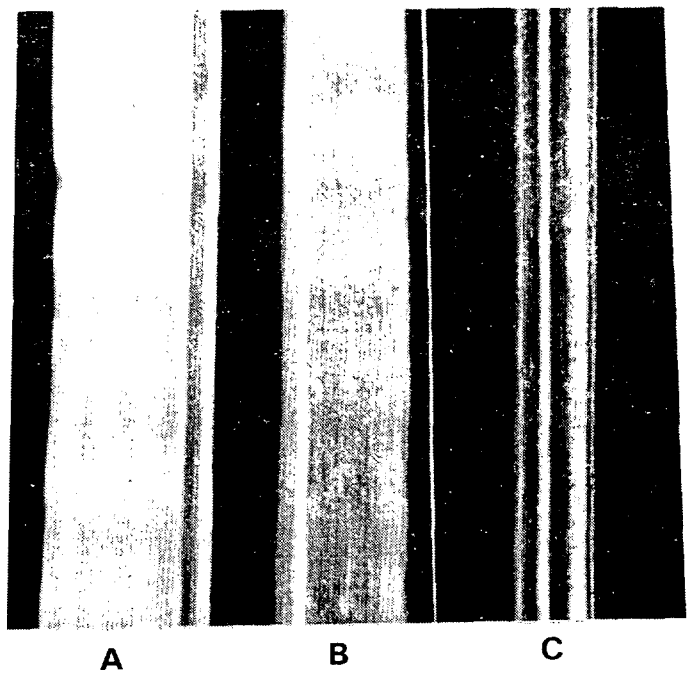

Figure 1. Wheat leaves showing (a) a single green sector due to loss of a chromosome, (b) a white sector indicating trisomy, and (c) multiple sectors resulting from independent nondisjunctional events.

The definitive evidence may be provided by the cytological analysis of tissues from virescent, green, and white leaf sectors. This is a difficult task to accomplish at this time.

The roots were excised from the seed when they were 0.5-1.00 cm long and kept in a saturated solution of monobromonapthalene for 4 h at room temperature, fixed in 3:1 (ethyl alcohol:glacial acetic acid) for 24 h, and then placed in 70 percent ethanol. For cytogenetic analysis, roots were hydrolyzed in 1 N HCl for 10 min at 60°C. After washing with distilled water, roots were placed in a solution of acetocarmine for 30 min before squashing. At least five slides for each chemical treatment and untreated controls were prepared for scoring numerical and structural chromosomal aberrations. For evaluating the concordance between chromosome alterations (both numerical and structural) and leaf sectors, the statistical analysis of Amphlett and Delow (1984) was applied.

All incidence rates of mutagenicity were analyzed the same way. The incidence of induced leaf sectors and chromosome alterations for each treated group was compared to the respective control rate. A statistic was calculated based on the likelihood ratio and assuming an underlying Poisson distribution. When the null hypothesis holds, the statistic has a standard normal distribution. These statistics were compared to Dunnett's Table to allow for multiple comparisons due to the presence of more than one dosed group. Sectors were analyzed on a per plant basis, and the other observations were analyzed on a per cell basis.

RESULTS

The data on leaf sectors induced in the tester strain by gamma radiation, vinblastin sulfate, and EMS are shown in Table 2. It must be pointed out that most of the data listed in this table are based on the occurrence of green sectors; in most of the situations, white sectors were difficult to distinguish from the virescent background of the leaves.

Of the three test agents used in this study, gamma radiation produced the most striking response in terms of proportionate increase in the frequency of leaf sectors with increasing radiation dose. There was a 45-fold increase in the leaf sectors in plants treated with 15 kR (2.69 sectors per plant) as compared to the control (0.06 sector per plant). There was a slight decrease in the induction of leaf sectors at 20 kR as compared to irradiation at 15 kR. The frequency of multiple sectors and twin sectors was strikingly higher in plants grown from irradiated embryos (data not shown) as compared to treatment with chemicals.

Table 2. Induction of leaf sectors by gamma rays, vinblastin sulfate, and ethyl methanesulfonate in hexaploid wheat

Test agents	Total sectors	Number of plants	Sectors per plant
Gamma Rays (kR)			
0	6	94	0.06
5	25	89	0.28
10	80	75	1.07
15	218	81	2.69
20	131	70	1.87
Vinblastin Sulfate (µg/ml)			
0.00	10	61	0.16
3.12	9	57	0.16
6.25	9	56	0.16
12.50	6	52	0.12
25.00	25	53	0.47
50.00	20	57	0.35
Ethyl Methanesulfonate (µg/ml)			
0.00	3	14	0.21
2.00	6	16	0.37
20.00	4	14	0.29
200.00	8	16	0.50
2000.00	22	15	1.47

Vinblastin is an antitumor alkaloid and is considered to be a spindle poison. It has been reported to cause aneuploidy in Drosophila (Sorsa et al., 1980), mammalian diploid cells in culture (Athwal and Sandhu, 1985), and mammalian transformed cells in culture (Palyi, 1976; Hsu et al., 1983). In the virescent wheat assay, it yielded a positive response within a narrow range (10 to 50 µg/ml).

EMS is a classical genotoxin that causes gene mutation, chromosome breaks, and aneuploidy in a variety of organisms (Sega, 1984). This compound did not produce as clean a response as was obtained with vinblastin or gamma rays. However, at the highest dose tested (2000 µg/ml), it yielded a sevenfold increase in the sectors as compared to the control. One of the problems of using EMS in our experiment was that the EMS-induced sectors were rather diffuse, and it was difficult to distinguish these from the chlorophyll development as it occurs at temperatures above 26°C and in the older leaves. Similar difficulty with EMS has been experienced by other investigators working on the nature of wheat sectors (Redei, personal commun.).

Cytogenetic analyses of root tip cells from the embryos treated with the test agents are presented in Table 3. The gamma rays induced the highest frequency

Table 3. Aneuploidy and chromosome aberrations in the root cells obtained from seed of hexaploid wheat treated with gamma rays, vinblastin sulfate, and ethyl methanesulfonate

Test agent	Number of cells analyzed	Percent of metaphases with			Percent chromosome aberrations
		<42 chromosomes	Normal (42)	>42 chromosomes	
Gamma Rays (kR)					
0	48	6.3	93.8	0	0
5	70	15.7	62.9	4.3	17.1
10	63	19.1	44.4	4.8	31.8
15	56	5.4	35.7	8.9	50.0
20	76	11.8	39.5	2.6	46.1
Vinblastin Sulfate (µg/ml)					
0	82	4.9	91.5	0.0	3.7
3.12	96	2.1	92.7	2.1	3.1
6.25	80	2.5	97.5	0.0	0
12.50	94	3.2	92.6	0.0	4.3
25.00	102	5.9	85.3	2.9	5.9
50.00	137	14.6	78.1	5.1	2.2
Ethyl Methanesulfoante (µg/ml)					
0	107	6.5	88.8	3.7	0.9
2.0	95	5.3	84.2	5.3	5.3
20.0	84	13.1	82.1	1.2	3.6
200.0	95	12.6	82.1	1.1	4.2

of aneuploids at 10 kR; whereas, the maximum yield for chromosome breaks was at 15 kR. Vinblastin did not cause chromosome breaks but did induce about a fourfold increase in the aneuploid cells at 50 µg/ml. The EMS induced both aneuploidy as well as chromosome breaks.

From Figures 2 and 3 it is evident that there is a close relationship between the chromosome aberrations (aneuploidy and chromosome breaks) and the incidence of leaf sectors induced by gamma rays (r^2 = 0.97) and EMS (r^2 = 0.68). For vinblastin sulfate, the correlation coefficient between leaf sectors and chromosome aberrations was 0.76 (graph is not shown).

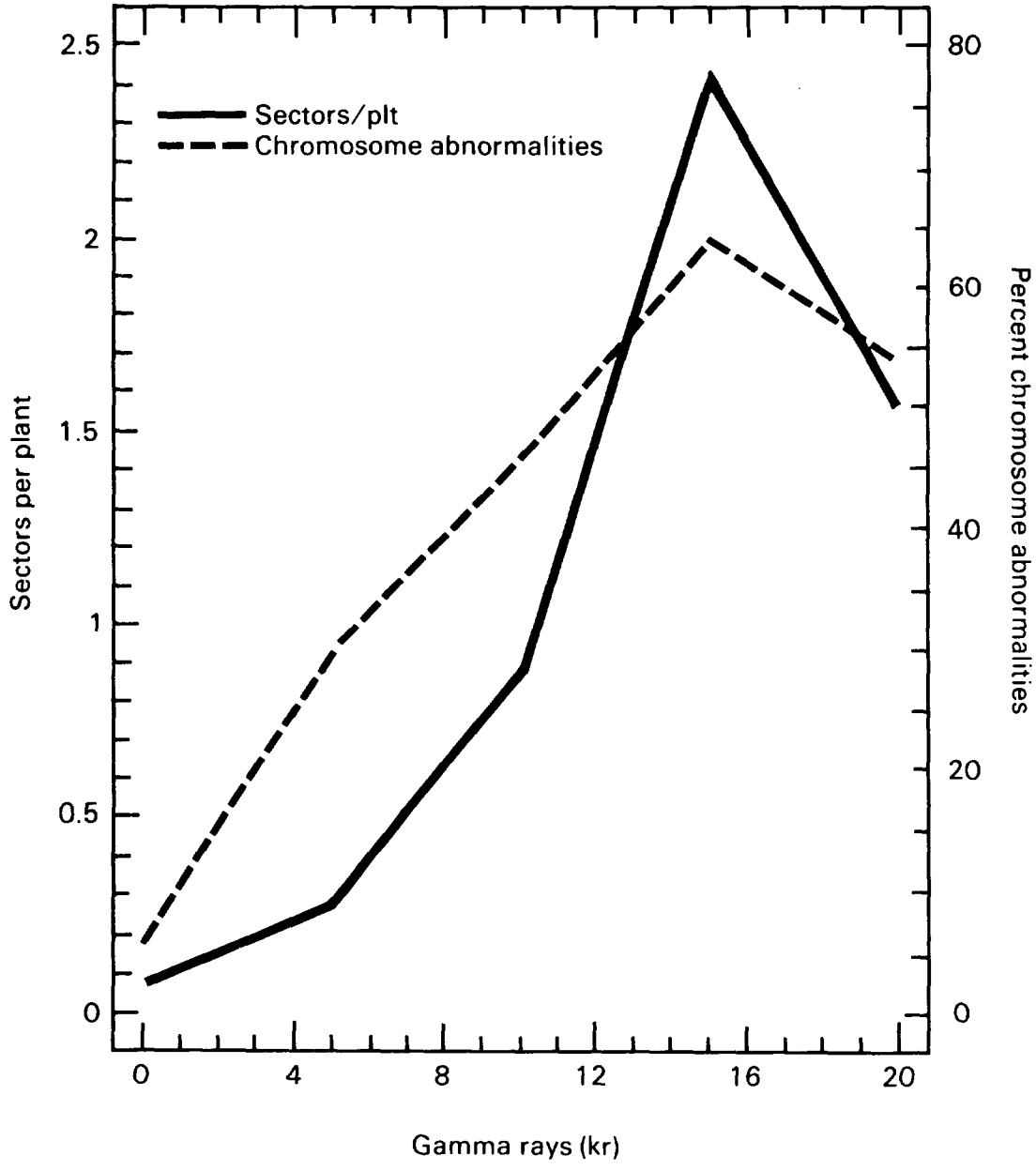

Figure 2. The relationship between chromosome abnormalities and sectors induced by gamma radiation in hexaploid wheat.

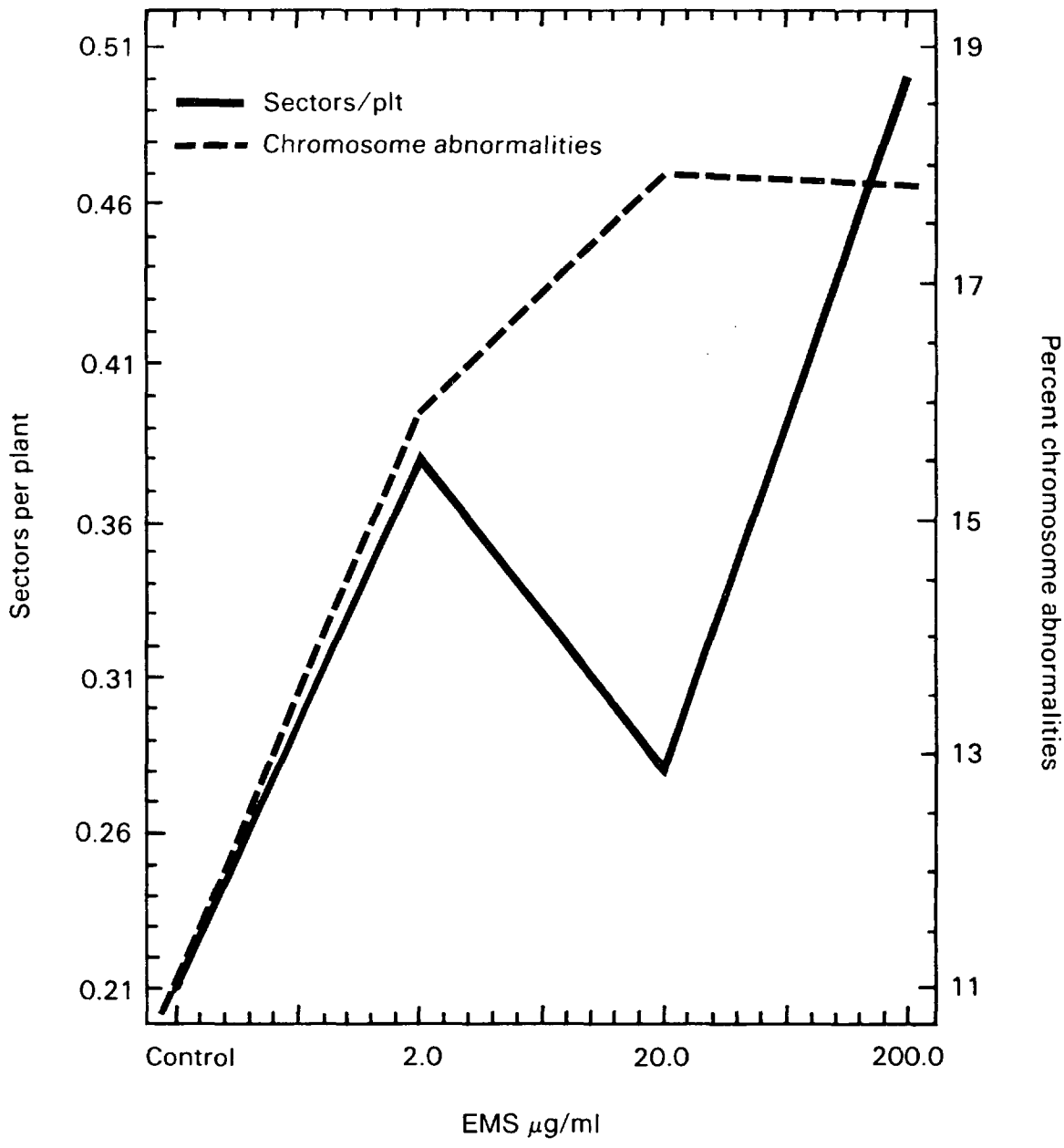

Figure 3. Relationship between chromosome abnormalities and leaf sectors induced by EMS in hexaploid wheat.

A composite graph based on the frequencies of leaf sectors and chromosome abnormalities induced by the three test agents used in this study is shown in Figure 4. The correlation coefficient between the chromosome aberrations and leaf sectors on the basis of the composite data for all the test agents was 0.86.

DISCUSSION

We have observed significantly enhanced frequencies of green and white sectors on virescent leaves induced by two chemicals and gamma radiation. Both the twin sectors (green and white sectors adjacent to each other) as well as single

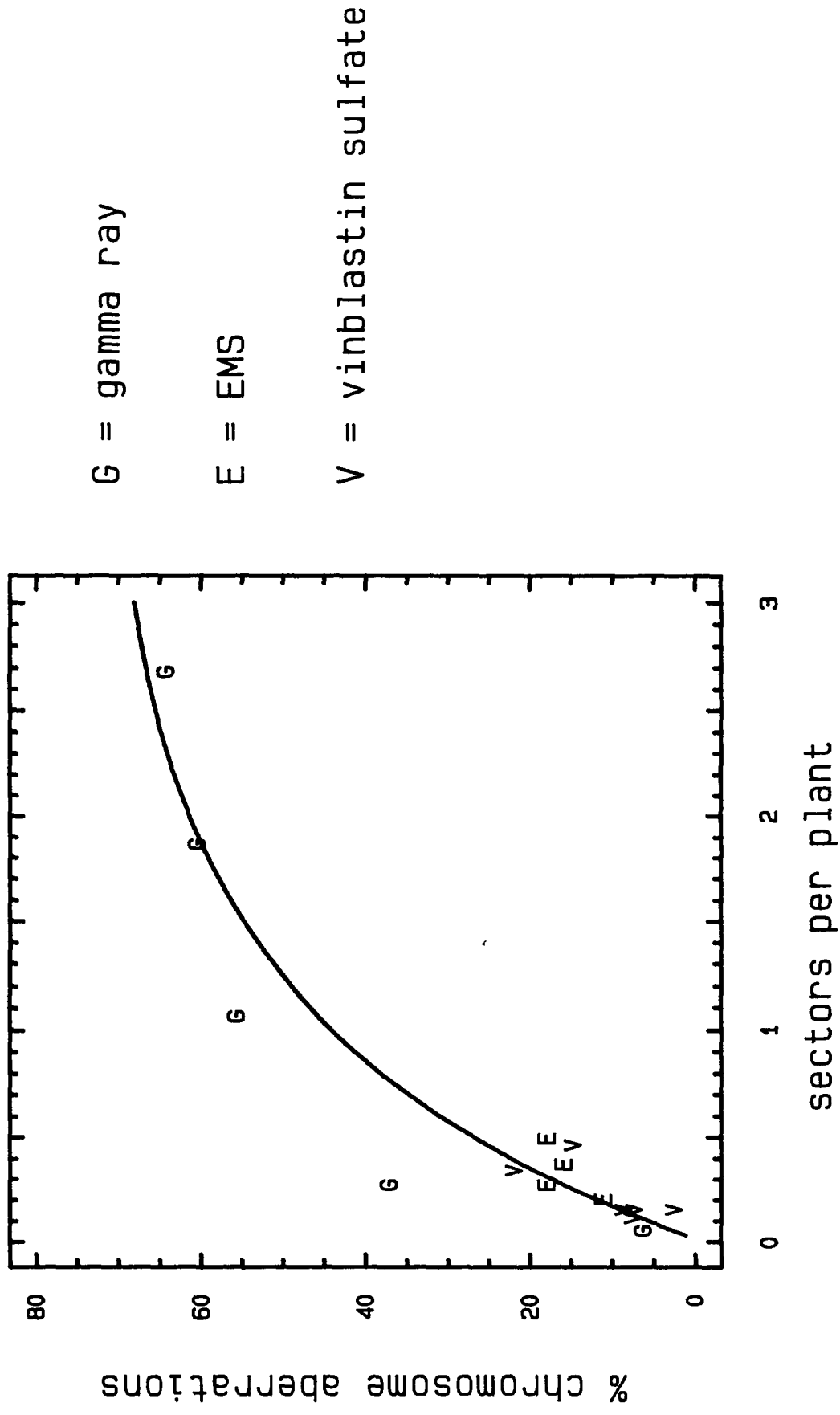

Figure 4. Composite graph showing relationship between leaf sectors and chromosome abnormalities induced by gamma rays, EMS, and vinblastin sulfate.

sectors were observed. Conceivably the green and white sectoring on the virescent leaves can be caused by gene mutation or by structural and/or numerical chromosomal aberrations.

Spontaneously occurring gene mutations are rare events in diploid species of higher organisms. With chemical and physical agents enhanced frequencies of induced gene mutations in M_1 and successive generations of diploid animals and plants have been obtained. Since in hexaploid wheat every gene is present in triplicate, the recovery of visible induced gene mutations is very rare. In our control population, 7 to 10 of the plants showed chlorophyll leaf sectors, and their frequency was greatly enhanced with physical and chemical agents. It therefore does not appear probable that the high frequency of the observed sectoring could be caused by gene mutation.

However, induction of leaf sectors in the M_1 generation of monosomic lines of Chinese spring wheat with EMS has been reported by Shama Rao and Sears (1964). But the frequency of such sectoring in the disomic line of this strain was very low. These authors recovered one EMS-induced chlorophyll mutant (chlorina-1). The marker gene for this mutant was assigned to chromosome 7A. In this mutant, like Neatby's virescent, the nullisomics were fully green, disomics were chlorina, and trisomics were extremely virescent or gold.

Work reported earlier, most significantly by Steinitz-Sears (1966), Sears and Sears (1968), and Sears (1969) on Neatby's virescent wheat show that the development or lack of development of chlorophyll in this strain grown under low temperature is dependent upon the gene dosage of $\underline{v_1}$ alleles at the 3B locus. The gene dosage may be caused either by the loss or gain of an entire chromosome or a segment of 3B chromosome bearing $\underline{v_1}$ alleles. The data presented by Steinitz-Sears (1966) show that the centromere of a line of Neatby's strain of Chinese spring wheat has intrinsic instability for chromosome 3B, on which $\underline{v_1}$ alleles are located. The centromere in this strain undergoes misdivision with significantly greater frequency than in the other strains resulting in telocentric chromosomes. The telocentric chromosomes are usually lost in the cell division, giving rise to monosomy. The intrinsic instability of centromeres can be further enhanced by physical and chemical agents (Brinkley et al., 1985).

It appears that most of the green sectors induced by the test agents in the leaves of virescent wheat may be due to the enhanced instability resulting in the nondisjunction of chromosome 3B or 3A and 3D. The development of green-white twin sectors in which a loss and gain of a chromosome or a segment of a chromosome occurs further supports the assumption of the nondisjunctional basis of chlorophyll sectors induced by the test agents.

During the process of cell division, chromosome loss can occur either due to nondisjunction or by chromosome breaks. In this study, irradiation induced a dose-related increase for leaf sectors, chromosome breaks, and chromosome loss. Therefore, the mechanism of chromosome loss and consequently appearance of green sectors for gamma rays may be caused by chromosome breaks as well as nondisjunction.

In absence of concrete evidence to the contrary, we assume that leaf sectors in our test organisms are caused by nondisjunction or by deletions and duplications. The strong concordance ($r^2 = 0.86$) for the composite sample between leaf sectors

and chromosome aberrations provide further support for the assumption that the leaf sectors are indeed caused by chromosome alterations. The nondisjunction may be caused by disruption of any of the very complex mechanisms related to the normal distribution of chromosomes during cell division. The accurate distribution of chromosomes depends upon integrity of genes controlling the cell division process and the production and the proper functioning of cell organelles involved in cell division. Although there are chemicals which cause aneuploidy without causing detectable gene mutations, and, vice versa, most chemicals are capable of inducing both gene mutation and aneuploidy.

The detection of aneuploidy in higher organisms is mostly confined to cytological observations. Recently genetic methods for identifying chemically induced aneuploidy in Drosophila and mouse (see Bond, 1985 for review) and mammalian cells in culture (Sandhu et al., 1988) have been reported. The use of genetic methods for detecting aneuploidy has the advantages in that it eliminates the labor intensive cytological methods and that target population can potentially be very large. Consequently, very low doses of environmental agents can be evaluated for their potential aneuploidy inducing effects. In common wheat for example, there are 150,000 cells in the apical meristem (Redei and Redei, 1955) of the embryo of the mature seed. We assume that there are at least 20,000 cells in the primordia of leaf numbers two and three. Thus, our target population for chemically induced chromosomal effects is 2×10^6 cells (100 seed per treatment). An additional advantage of this system is that all products of chromosome aberrations such as aneuploidy, deletions, and duplications, and translocations which may cause selective disadvantage to the diploid cells can be recovered in the hexaploid genome.

Thus, after adequate validation, this assay may offer a very simple technique for evaluating the potential of environmental chemicals to induce chromosomal alterations.

ACKNOWLEDGMENT

We wish to thank Mrs. Shirley Milton for assisting in the preparation of this manuscript.

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VALIDATION OF TRADESCANTIA-MICRONUCLEUS (TRAD-MCN) BIOASSAY FOR WASTE DUMP SITE POLLUTION MONITORING*

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ABSTRACT

Tradescantia-micronucleus (Trad-MCN) test is a short term in vivo bioassay for liquid, gaseous and radioactive agents in the environment. It can be applied to the pollutants on site or the agents in the laboratory setting. Although the target organell is the meiotic chromosome of the pollen mother cells, the physiological and morphological damage of the meiotic cells and the leaves of the plant cuttings can also be used as indicators of the toxicity of the agents. When agents impinge on the highly synchronized early prophase chromosomes (pachytene or diplotene when chromatids undergo the process of crossingover), acentric fragments are formed through the excision or fusion interference mechanism. These fragemnts in turn result in micronuclei in the synchronized early tetrads of the same meiotic cycle 24 - 30 hr after treatment. Frequencies of the MCN in a large population of tetrads of the meiotic pollen mother cells are indicators of the clastogenicity of the agents. Present study is designed to use Trad-MCN bioassay to conduct laboratory tests on 11 chemicals commonly found in waste dump sites, and the on site monitoring of a chosen dump site. Currently, test results of arsenic oxide and 1,2-benzanthracene are presented in this report. Plant cuttings of Tradescantia clone #4430 were treated with very low concentration solutions of these agents for 30 hr. Young inflorescences were fixed immediately after treatment without the recovery time for preparation of microslides. Positive results were obtained from the test of arsenic oxide which yielded 36.99 ± 11.80 MCN/100 tetrads at 100 μM concentration, and from the test of 1,2-benzanthracene which yielded 30.73 ± 9.71 MCN/100 terads at 100 μM concentration. The negative control (tapwater) yielded 2.56 ± 1.16 MCN/100 tetrads, and the positive controls yielded 12.30 ± 0.96 MCN/100 tetrads with 50 μM of sodium bisulfite and 15.5 ± 1.52 MCN/100 tetrads with 50 μM Benzo(a)pyrene. Both of these chemicals tested also exhibited high toxicity and caused meiotic delay in the pollen mother cells.

*Research supported by HERL, RTP, US EPA contract #H73768

MEASUREMENT OF THE MUTAGENICITY OF ENVIRONMENTAL SAMPLES

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ABSTRACT

Protocols have been validated for the preparation of environmental and waste water, drinking water, and soil/sediment samples for the Ames Salmonella mutagenicity assay. These protocols are proposed for use with environmental samples in "Guidelines for Preparing Environmental Waste Samples for Mutagenicity (Ames) Testing: Interim Procedures and Panel Meeting Proceedings, EPA 600/4-85-058 (ICAIR, 1985) and are validated essentially as written. Environmental and waste water samples are extracted with dichloromethane, concentrated, and solvent exchanged to dimethylsulfoxide (DMSO). Drinking water samples are prepared by passing them through stainless steel columns packed with polymeric resins (XAD-2 and XAD-7, Rohm and Haas). The absorbed organic compounds are recovered by backflushing the columns with hexane/acetone; the solvent is concentrated and exchanged to DMSO. Soil/sediment samples are extracted using EPA Method 3540 or 3550, concentrated, and the solvent is exchanged to DMSO. Mutagenicity of extracts is measured using dose-response determinations for strains TA98 and TA100 with and without S-9 activation.

The performance of each method is optimized using environmental samples; experimental variables include sample size, volume of adsorption columns, and extraction solvent. Bias of the methods is established by using samples fortified with reference mutagens; precision is determined by testing replicate aliquots of a variety of environmental samples. This work is an on-going effort sponsored by EMSL-LV to produce robust, reliable procedures for the measurement of mutagenicity in environmental media.

INTRODUCTION

There is increasing evidence that environmental mutagens are a cause of cancer and of genetic birth defects (Ames, 1979; Hartman, 1983). Of particular concern are toxic and hazardous chemical wastes that are produced in quantities of over 250 million metric tons a year in the United States. The chemical complexity of hazardous wastes and their residues precludes complete chemical analysis of toxic components. Furthermore, toxicological synergism and antagonism may occur between chemical waste components. These interactions make risk assessment of

hazardous wastes on a chemical-by-chemical basis extremely difficult (Federal Register, 1986b). A critical need is to develop short-term biological methods to assist in assessing the potential hazards of chemicals in complex waste samples (Brusick and Young, 1981).

While short-term bioassays such as the Ames test are now used by many public and private laboratories in screening of complex mixtures for mutagenic activity (Hollstein and McCann, 1979; Sugimura and Nagao, 1982), no standardized procedures for the preparation of environmental samples have been adopted by the USEPA. A set of draft procedures suitable for the preparation of air particulates, drinking water, environmental and waste water, soil and sediment, waste solid, and nonaqueous liquid waste was proposed (Marsden et al., 1987) in a meeting co-sponsored by the EPA's Environmental Monitoring Systems Laboratory-Las Vegas (EMSL-LV) and the US Army Medical Bioengineering Research and Development Laboratory (MBRDL). This is a report on the validation of the proposed procedures for environmental and waste water, drinking water, and soil and sediment.

MATERIALS AND METHODS

ENVIRONMENTAL AND WASTE WATER PROTOCOL (Wang et al., 1987)

Environmental and waste water samples are extracted by liquid-liquid partitioning with pH adjustment. The pH of two 1500 mL aliquots of the sample is adjusted to 11 with 5N NaOH, then extracted with 150, 100, and 100 mL of dichloromethane (Burdick and Jackson, Muskegon, Michigan). The pH of the aqueous phase is adjusted to less than 2 using a diluted (1:1) sulfuric acid solution, then extracted three times with dichloromethane. All the organic extracts are combined and concentrated to approximately 10 mL using a Kuderna-Danish (K-D) concentrator or a rotary evaporator. The extract is transferred to a tared, 3-dram glass vial, and the remaining dichloromethane is evaporated under nitrogen. The residue is resuspended in DMSO prior to Ames assay.

The Ames assay is performed according to the standard plate incorporation assay procedure (Ames et al., 1975; Maron and Ames, 1983) without modification, using Salmonella typhimurium strains TA98 and TA100. All samples are initially assayed in the presence and the absence of an exogenous metabolic activation system (2% and 10% of the S-9 fraction (V/V) in the total S-9-cofactor mix) derived from the livers of Aroclor 1254-induced Sprague-Dawley rats (Litton Bionetics, Kensington, Maryland). The protein concentration of the S-9 used is 25 mg/mL as determined by the method of Lowry et al (1951). All samples are evaluated by using a minimum of three dose levels at half-log intervals and by using duplicate or triplicate plates per dose whenever possible. Doses as high as 6 mg extract/plate are used when the sample quantity is sufficient. The strain genotype function tests and other quality control tests are performed for each experiment to ensure the validity of the test (Claxton et al., in press; deSerres and Shelby, 1979; Williams, 1985; Williams and Preston, 1983).

Samples are collected which include a wide range of environmental and waste waters, including: (1) an effluent from a municipal waste water treatment plant (AIHL-85-0403); (2) an effluent from an industrial waste water treatment plant (AIHL-85-0406); (3) a surface runoff from a class I hazardous waste landfill (AIHL-85-0405); (4) two brackish estuarine surface waters receiving industrial effluents and areal runoff, one collected at a city marina (AIHL-85-0404) and the other collected at the discharge site of an industrial waste water treatment plant in the San Francisco Bay (AIHL-85-044A); (5) two contaminated groundwater samples from a closed industrial waste disposal facility, one from an on-site well with the highest contamination (AIHL-85-0402) and another from an upgradient well with minimal contamination (AIHL-85-042A); and (6) an aqueous leachate made in our laboratory using a National Bureau of Standards (NBS) reference sludge sample (AIHL-85-0401). Preparation of the aqueous leachate is based on the Toxicity Characteristics Leachate Procedure (TCLP), one of the solid waste extraction procedures developed under the Resource Conservation and Recovery Act (RCRA). The TCLP (Federal Register, 1986a) is a modification of the Extraction Procedure (40 CFR 261.24, specified in RCRA Section 3001) developed for the Office of Solid Waste, EPA.

DRINKING WATER PROTOCOL (Wang et al., in press)

Organic residues are extracted from 30-L drinking water samples using a specially designed apparatus (Tabor and Loper, 1985) consisting of a reservoir (Amicon Corporation, Danvers, Massachusetts), a filtration unit (Millipore Corporation, Bedford, Massachusetts), and three stainless steel chromatography columns, each with a 25-cc bed volume (Tristate Controls, Inc., Cincinnati, Ohio). The three columns are each packed with a different material: the first contains silanized glass wool (Supelco, Inc., Bellefonte, Pennsylvania); the second contains a rigorously cleaned polymeric stationary phase, Amberlite XAD-2 (Rohm and Haas, Philadelphia, Pennsylvania); and the third, Amberlite XAD-7 (Rohm and Haas, Philadelphia, Pennsylvania). Organics are extracted from water by passing a 30-L sample through the extraction train at a flow rate of 50 to 100 mL per minute.

Following passage of the sample through the apparatus, the adsorbed residual organics are removed by backflushing each of the three columns with 300 mL hexane:acetone (85:15) (Burdick and Jackson, Muskegon, Michigan). The small amount of residual water from the columns is combined and extracted in a separatory funnel with three times its volume of dichloromethane. The glass wool is unpacked from the column, and Soxhlet extracted with 400 mL hexane:acetone (85:15). The filters from the filtration unit are extracted three times with 100 mL hexane:acetone (85:15).

All residues are concentrated in acetone, are brought to required volume with DMSO, and then are tested for mutagenic activity. The organic residues are stored under nitrogen in amber or foil wrapped glass vials at -20°C until the Ames testing is conducted. DMSO is added at the time of mutagenicity bioassay in order to bring the acetone concentrate (or dried residue) to the total volume required for the mutagenicity testing.

The Ames assay is performed according to the standard plate incorporation assay procedure and testing scheme described above. Fifty μL DMSO per plate are applied. Preliminary tests are carried out in two Salmonella typhimurium strains, TA98 and TA100, in the presence and absence of 10% S-9 mix. The preliminary tests are used to determine the assay condition for optimum detection of drinking water mutagens as described below.

Samples are assayed by using (1) a minimum of three dose levels at half-log intervals and (2) duplicate or triplicate plates per dose whenever the amount of residue was sufficient. Doses up to 6 mg extract/plate or 11 L equivalent/plate are used when the sample quantity is sufficient.

Samples are obtained from five drinking water collection sites in the San Francisco Bay area; each contains surface or ground water, or a mixture of these two source types. Samples from each of the five collection sites are grouped and coded together. The five sites are identified as AIHL-86-0301 to -0305. Three sites, AIHL-86-0301 to -0303, provide drinking water samples from three different surface water sources. Multiple samples are collected at each of these three sites. From site AIHL-86-0301, duplicate samples are collected on one date and single samples taken on five other dates. From sites AIHL-86-0302 and AIHL-86-0303, single samples are collected on two separate dates. Ground water is the source for a single sample collected at site AIHL-86-0304. Two samples are taken on separate dates from site AIHL-86-0305. This site provides a combination of both surface and ground waters. The samples from all sites have undergone various degrees of regular water treatments; all samples are processed by chlorination and meet California domestic water quality standards.

SOIL AND SEDIMENT PROTOCOL

The extraction method for soil evaluated in this report is Method 3540 (Soxhlet), several solvents are tested to determine the maximum extraction efficiency for mutagens. The choice of an extraction solvent is based on three requirements, (1) the solvent must remove both toxic and growth-stimulating substrates (e.g. histidine) that could interfere with the bioassay, (2) it must not generate artifacts which increase or decrease the mutagenic potential of the soil extract, and (3) it must be neither carcinogenic nor toxic to the test system. The solvents which meet these criteria, and are used in the protocol evaluation, include dichloromethane, hexane:acetone (2:1), dichloromethane:acetone (1:1), and hexane:isopropanol (3:1) (Burdick and Jackson, Muskegon, Michigan).

Soil samples, in 10 g aliquots, are mixed with 10 g of prepared sodium sulfate and placed in a cellulose extraction thimble, 33 mm x 94 mm (Whatman International Ltd, Maidstone, England). The mixture is stirred with a glass stirring rod for even distribution. A glass wool plug weighed with a glass stopper is used to cover and contain the mixture. Then each thimble is Soxhlet extracted for sixteen hours with 300 mL of HPLC grade solvents. Following extraction, solvent is removed by rotary evaporation to a volume of 5 to 10 mL. The extract

is filtered by passage through a 10 cm column filled with solvent washed anhydrous sodium sulfate.

The final concentration step employs a solvent exchange using DMSO. The volume of initial solvent extract is reduced to 2 mL by drying the sample under a stream of nitrogen at 40°C. At this point, a 200 μL aliquot is removed for gravimetric determination. The solvent extract is then reduced to a volume of 1 mL. 1 mL of the DMSO is added and the total sample volume is reduced to 1.5 mL. Another 1 mL aliquot of DMSO is added and the volume is reduced to 2.25 mL. The exchange is repeated with another 1 mL of DMSO and the volume is reduced to a final volume of 3 mL.

The soil sample utilized in this study, AIHL-87-0601, is obtained from a closed non-agricultural hazardous waste landfill site. As in all environmental sampling, a record form documenting sample collection, transportation, and storage is initiated by the field manager at the time of collection. This form includes information on collection procedures, sample type and appearance, any necessary treatments and shipment and storage methods. A copy of the form accompanies the sample through all phases of processing to insure chain-of-custody.

The soil sample is obtained from a well at a depth of 21-21.5 ft. The cross sectional soil/pH contour shows that the sample pH at the time of collection was 2.2. A chemical profile of this sample is listed as follows: sample dry ignition weight loss at 85°C was 213 mg/g, dry ignition weight loss at 550°C was 149 mg/g, pH at sampling was 2.2, pH at testing time was 4.1, conductivity was 11,700 μmhos/cm, extractable organic halogens was 1,660 μgcl/g, and the humus content was low. The soil is a mix of mottled dark yellow brown, dark brown, black, and brownish yellow. It consists of fine to coarse sand with trace silt. The particulates are spherical to subdiscoidal, angular to subrounded. The sample is friable, compact, and damp. The 100 lb. soil sample is stored at room temperature in an air tight polystyrene tub, and protected from light exposure.

For the first experiment, the subsample is taken from the surface of the container and processed using the procedures described above. The soil sample is then mixed to insure homogeneity by placing it in a clean stainless steel pan, repeatedly quartering with a clean stainless steel spatula, and re-mixing, for 15-30 minutes. The mixed soil is stored in the original polystyrene tub at room temperature, protected from light, until next processing. For all other experiments, the subsamples are taken from the mixed soil sample.

RESULTS AND DISCUSSION

ENVIRONMENTAL AND WASTE WATER PROTOCOL

As listed in Table 1, mutagenic activity is detected in all eight environmental and waste water samples. All the samples are more mutagenic in bacterial strain TA98 than in TA100. The optimum S-9 condition for each sample is listed in Table 1.

The on-site contaminated groundwater sample (AIHL-85-0402) from a hazardous waste disposal facility produces approximately 3600 revertants/L. The residue contains both direct- and indirect-acting mutagens. TA98 with 2% S-9 is the optimum Ames testing condition. Mutagenicity is also observed in TA100. Toxicity is not observed at doses up to 3 mg/plate in the initial screening experiment. The sample is fairly acidic (approximately pH 3.5) on receipt and it precipitates heavily when the pH is raised. In order to reduce this sample preparation problem, a modification of the procedure is tested which uses an initial acid extraction of the sample. This results in most of the mutagenicity partitioning into the acid-neutral fraction. Unfortunately, no strong conclusions can be drawn because a large portion of the mutagenicity observed in the initial measurement has been lost during storage. The cause for the decreased mutagenicity in this contaminated groundwater sample may be any combination of chemical reactions, microbial activities, or shipping and storage conditions (Alexander, 1974).

The sample prepared by Method 1311 (TCLP) (AIHL-85-0401) exhibits high mutagenic activity in both TA98 and TA100 in the initial experiment. Both direct- and indirect-acting mutagens are detected. No toxicity is observed at doses up to 0.3 mg/plate. The optimum testing is in TA98 with 2% S-9 mix. The addition of 2% S-9 increases the direct-acting mutagenicity by approximately 45 percent.

A GC/MS analysis of the leachate is performed using Method 8270 and a number of Appendix IX analytes are identified in the leachate. A computer search by the Environmental Mutagen, Carcinogen, and Teratogen Information Center, Oak Ridge, Tennessee, provides information on the mutagenicity of these chemicals in the Ames assay. Table 2 lists the chemical concentrations, its mutagenic activity, and references. Four chemicals are reported as mutagens: 3,3'-dichlorobenzidine, N-nitrosodiphenylamine, 2,6-dinitrotoluene, and acenaphthene. Reid et al., (1984) calculated the activity in TA98 for 3,3'-dichlorobenzidine as 68 revertants/nmole which equaled 68 revertants/0.25 μg. The concentration of the compound in the leachate is 4.4 μg/L. Therefore, the compound might produce approximately 1200 revertants/L detected (Table 1) in the leachate prepared for this project. Assuming no complicated synergistic or antagonistic reactions occurred, 3,3'-dichlorobenzidine accounts for approximately 52 percent of the activity in the leachate.

The industrial waste water treatment plant effluent (AIHL-85-0406) is the only sample that does not exhibit any emulsion or precipitation when using the original base-first protocol. Several different kinds of problems are encountered in the liquid-liquid extraction when the protocol is used without modification. The sample exhibits a strong petroleum-like odor. In the first experiment, elevated colony counts are observed but mutagenicity is not greater than twice that of background levels. Toxicity is observed at the dose of 2 mg/plate. TA98 with 10% S-9 mix produces a response close to the detection limit and an increase of the S-9 concentration is considered. Because of light precipitation and emulsion, acid-first extraction is applied. Increasing the S-9 concentration to 30% is a necessary modification

for detecting the mutagenicity. Petroleum distillate extracts have been reported to exhibit significant mutagenic response only at S-9 concentrations over 20% (Carver et al., 1985). Our results suggest the possible existence of similar petrochemicals in the industrial waste water treatment plant effluent.

The surface runoff sample (AIHL-85-0405) is extracted according to the original base-first protocol. It is active in TA98 but not in TA100. The optimum condition is without S-9. There is no toxicity in the initial screening experiment at doses up to 0.7 mg/plate. However, modification of the extraction scheme in two later experiments, to control sample precipitation, results in a response less than twice the background. Toxicity is observed in the last experiment which is performed approximately one month after receipt of the sample. The toxicity and loss of activity may be due to changes in sample composition caused by microbial degradation of mutagens, formation of antagonists, chemical interference, chemical degradation, or combinations of these factors.

The municipal effluent waste water sample (AIHL-85-0503) exhibits stable mutagenicity. It is mutagenic only in TA98 with 10% S-9 in the initial screening experiment. There is no activity in TA100. No toxicity is observed at doses up to 1 mg/plate. Similar results are obtained in two follow-up experiments. No toxicity is exhibited at 2 mg/plate. Even though there is no precipitation at pH 11, the acid-first extraction scheme is compared with the original base-first extraction because of the stability in mutagen compositions of the municipal sample. Both methods give similar mutagenic responses.

The brackish San Francisco Bay surface water (AIHL-85-0404) requires 2% S-9 mix for maximum activity. A comparison of the acid-first or base-first extraction method showes similar results. The San Francisco Bay receives local industrial effluents which may be a major source of pollutants. For comparison, a brackish surface water sample at the discharge site of an industrial waste water treatment plant is collected (AIHL-85-044A) and tested. Acid-first and base-first procedures each produces extracts which give results similar to the previous Bay water sample.

One common problem encountered by using the liquid-liquid extraction procedure is the formation of emulsions between the aqueous phase and the organic phase during extraction of most samples. In one case, a heavy emulsion forms which is independent of the extraction pH. The best separation of the aqueous-organic phases is accomplished by filtration through a funnel packed with glass wool when the organic phase is eluted. Subsequently, the glass wool filtration step is included in the liquid-liquid extraction protocol for all samples that form emulsions during extractions.

Four of the six generic types of environmental and waste water samples tested using the liquid-liquid extraction procedure form substantial precipitates when the pH is raised to 11. This presents the most difficult problem associated with this extraction technique. Since these four types of samples precipitate only when the pH is raised to

11, it is logical to perform the first extraction at pH 2. The two San Francisco Bay brackish water samples and the landfill runoff precipitate at pH 11; this interferes with the extraction procedure. When the revised acid-first protocol is followed, the residues are easily obtained, are readily dissolved in DMSO, and both samples are found to be mutagenic in the Ames assay.

Because of the persistence of the organic phase to retain trace amounts of water, it is concluded that the anhydrous sodium sulfate column used before evaporation is not adequate to completely remove all water. Residual water in the concentrated extract and in vessels results in an incomplete evaporation process. In addition, residual water greatly affect the final residue weight determination used to calculate the final mutagenic potency of the sample. To remove small quantities of residual water, this final 10 mL of extract is passed over another sodium sulfate column (5 cm x 0.5 cm) before evaporation under nitrogen. We recommend that this step be added to the protocol as a precaution against water contamination in the final residue.

In the original consensus protocol, a K-D apparatus was recommended for concentrating the extraction solvent, dichloromethane. However, dichloromethane has been reported as a Salmonella mutagen and a suspected animal and human carcinogen. When the K-D concentrator is used, the solvent is heated and is vaporized in the chemical fume hood. This practice causes release of the dichloromethane through the hood to the general environment unless an activated-charcoal filter is placed properly at the top of the venting systems above the hood to adsorb the solvent. Such control devices are not in common use. Therefore, a rotary evaporator is used as a concentrator instead of the K-D. The condenser of the evaporator cools the dichloromethane vapor, it is collected in a waste reservoir and can be disposed of properly as waste.

DRINKING WATER PROTOCOL

The protocol recommends testing in both Salmonella typhimurium strains TA98 and TA100 (ICAIR, 1985). However, because of the large amount of work involved to produce sufficient amounts of residue for testing in more than one strain, only one sample is assayed using both strains TA98 and TA100 with and without S-9. The sample used is from site AIHL-86-0301. The results of this experiment (Table 3) indicate that the condition that give the maximum number of revertants/L employes TA100 without S-9. The addition of S-9 sharply reduces the mutagenic response in TA100 and increases it in TA98. Testing in TA100 without S-9 also give the maximum mutagenic response with drinking water extracts in numerous previous studies (Cheh et al., 1980; Ringhand et al., 1987; Horth et al., in press). In addition, the two mutagenic chlorinated furanones recently identified in drinking water samples are most active in TA100 without S-9 (Hemming et al., 1986; Holmbom et al., in press; Krongberg et al., in press). Subsequently, samples are tested only in TA100 without S-9 because of the difficulties in isolating adequate amounts of organic residue for multi-condition testing.

The method background is established for the 25-cc bed volume column apparatus by processing ultrapure water blank samples (ASTM Type I water). We emphasize that cleaning of XAD resins is essential to obtain acceptable blanks. The cleaning procedure described in the protocol is strictly followed for all lots of resins used for the extraction of samples and blanks. Water blanks are processed in parallel with drinking water samples in order to compare blank with sample results. Field blanks and reagent blanks are also evaluated. Only when the mutagenicity of the water blank residue is below the detection limit (twice the response observed with DMSO, the solvent blank) are the sample results considered acceptable. Results of testing to establish the method background demonstrate near identical agreement between the means and standard deviations (SD) of the DMSO solvent (141 ± 13) and ultrapure water (141 ± 14) blanks.

Once the method background is established for the 25-cc bed volume column apparatus using ultrapure water, collection and processing of drinking water samples begins. Samples are initially extracted at ambient pH in accordance with the procedures described in EPA 600/4-85-058. After initial sample testing, the original protocol is compared with a modified method in which the drinking water sample is acidified to pH 2 with sulfuric acid prior to extraction in order to increase the mutagen yield (Krongberg et al., 1985; Ringhand et al., 1987; Van Kreijl and Slooff, 1985). An acid blank, an acid-resin blank, and an acid water blank are also prepared and tested in parallel with the acidified samples to ensure that acidification does not generate mutagenic artifacts.

A preliminary comparison of pH on mutagen yield is shown in Table 3. Using the original protocol with no pH adjustment, a sample from site AIHL-86-0301 elicits a mutagenic response of 34 revertants/L in TA100 without S-9. A second sample collected from this site on another date is acidified to pH 2 prior to extraction and elicits an approximately twelve-fold higher response of 396 revertants/L. The acidified sample also gives greatly increased mutagenic responses in TA100 with S-9 and in TA98 under both activation conditions.

To further investigate the influence of pH on mutagen yields, an experiment is performed on aliquots of the same sample comparing extraction with and without acidification. This experiment is conducted on a sample from site AIHL-86-0301. The sample is divided, and the two aliquots are extracted in parallel; one is extracted at ambient pH (designated as "XAD first extraction, ambient pH tap water") and the other is extracted at pH 2 (designated as "XAD extraction, pH 2 tap water"). Following the initial extraction, the ambient pH effluent water is acidified to pH 2 and re-processed. The results of mutagenicity testing are shown in Table 4. The pH 2 extract gives 186 revertants/L. The ambient pH extract gives 31 revertants/L. When the ambient pH effluent is re-extracted at pH 2, the residue gives 121 revertants/L, approximately four times the activity observed with the ambient pH residue. Thus most of the mutagens remaining in the ambient pH effluent could be extracted by acidifying the eluted water to pH 2 and re-processing. Combining the mutagenicity of the first and

the second extract produces approximately 82 percent of the activity of the pH 2 extract.

A similar comparison is performed on samples from two other collection sites: AIHL-86-0303 and AIHL-86-0305 (Table 5). In each sample, the mutagenic activity is best recovered when the water is acidified to pH 2 prior to extraction. The water sample from site AIHL-86-0303 gives twice as much residue weight and seven to eight times as much mutagenic activity. The water sample from site AIHL-86-0305 yields results below the detection limit when processed at ambient pH and tested at doses of up to 4 L equivalent per plate, but shows mutagenic activity when acidified prior to processing. These results confirm that acidification of water samples to pH 2 prior to column extraction leads to higher recoveries both of residue mass and sample mutagenicity. The acid modification is subsequently applied to all samples from five drinking water collection sites.

Using sample acidification, the proposed drinking water method is tested for performance using TA100 without S-9. The results of the Ames bioassay on the groups of samples from the five collection sites are shown in Table 6. The sample concentration factors from sample to plate range from 62,500 to 150,000 fold.

Seven aliquots of the sample from site AIHL-86-0301 are extracted and tested in order to provide a better estimate of the precision of the protocol based on the SD and coefficient of variation (C_v) of these replicate measurements. Such replicate measurements are not easily performed on a routine basis because of the difficulties involved in simultaneous sample collection and preparation, and the unknown effects of sample storage on mutagenic activity in water.

An estimate of the temporal variation in mutagenicity of water samples from a single collection site is derived by analyzing samples collected on different dates. Up to five samples from a single site are analyzed. Averages, SD's and C_v's are reported for multiple measurements performed on samples from the same site collected on different dates (Table 6). C_v's range from two percent to 46 percent. This variation includes not only the variations associated with the use of the protocol but also with the day-to-day changes in the levels of mutagens in the water from the faucet, water distribution systems, water sources, possible differences due to changes during storage, as well as other uncontrolled variables in the overall operation.

SOIL AND SEDIMENT PROTOCOL

Previous reports of Ames testing of soils reveal that the use of various extraction procedures and extraction solvents yielded positive results and that metabolic activation increased the yield of soil sample extracts (Brown et al., 1985; Knize et al., 1987); however, these reports did not demonstrate any consistent difference in results obtained with Salmonella strains TA98 versus TA100. In this study, a single soil sample is used to compare the effect of (1) extraction solvent, (2) the use of metabolic activation with S-9, (3) the

Salmonella strain TA98 or TA100, and (4) the use of anhydrous sodium sulfate in the extraction procedure.

The comparison of mutagenic response of extracts of the soil sample AIHL-87-0601, in two Salmonella strains with and without metabolic activation (Table 7), confirms previous findings that metabolic activation increases the number of revertants over nonactivated soil samples. These same data demonstrate that TA98 is more sensitive than TA100 in detecting mutagenic activity in this soil sample.

Table 8 presents a comparison of revertants obtained from one soil sample using different extraction solvents (dichloromethane, hexane:acetone (2:1), hexane:isopropanol (3:1), and dichloromethane: acetone (1:1)). The best extraction solvent for this sample in strain TA98 is the hexane:acetone (2:1) mixture, and for testing in TA100 is dichloromethane:acetone (1:1).

Sodium sulfate is routinely used as a drying agent absorbed residual water when mixed with soil. Past experiments have shown that when sodium sulfate is added to the soil, there was inhibition of recovery of certain phenols and analines (Hein et al., in this volume). This suggests a matrix-analyte interaction which may interfere with the extraction of soil samples for mutagenicity testing and an experiment is performed to determine if any such interaction occurred (Table 9). Strain TA98 shows an increased mutagenic activity of 17 percent with sodium sulfate while strain TA100 shows an increase of 31 percent. These data indicate that anhydrous sodium sulfate, when mixed with this soil sample in a 1:1 ratio as a drying agent, results in enhanced extraction of mutagenic materials from soil samples.

The precision of the method is established by extracting with four solvents and testing triplicate extracts in TA98 and TA100 with activation (Table 10). This is accomplished with soil from site AIHL-87-0601 that is separated into identical aliquots which are extracted and tested on the same date. The precision of the method is expressed by using the SD and C_v of these replicate measurements. In this experiment, hexane/acetone extraction with testing in TA98 with S-9 gives the best result in terms of revertants and precision.

An estimate of the temporal variation in mutagenicity of a soil sample from collection site AIHL-87-0601 is made by analyzing samples extracted/tested in series. Averages, SD's, and C_v's are reported for these serial measurements (Table 11). The C_v's range from 5 percent in TA98 with 10% S-9 mix using hexane:acetone (2:1), to 167 percent in TA100 with 10% S-9 using hexane:acetone (2:1). This variation includes differences in sample stability, as well as uncontrollable variations in both the extraction and bioassay procedures.

CONCLUSION

Three draft protocols proposed in EPA 600/4-85-058 have been tested for the preparation of environmental samples for Ames assay. These procedures are suitable for general use but improved performance was obtained in our laboratory with minor modifications. The authors are

cautious about recommending these changes for all media, since rigorous method comparisons were performed on a limited number of samples.

The methods should be used for the measurement of environmental samples and the data generated can be used to complete the refinement of these protocols. Toward this end, other laboratories may find our experience helpful. Environmental and waste waters are best extracted using a pH 2, pH 11 sequence, extract filtration through glass wool or sodium sulfate will reduce emulsions and residual water. Drinking waters should be adjusted to pH 2 before they are passed through the resin extractor system. Soil/sediment samples are best extracted using hexane/acetone (2:1, V/V) with the Soxhlet procedure. Increased revertants are obtained using TA98 with S-9 activation for soil samples and most of environmental and waste water samples and TA100 without activation for drinking water samples.

Additional studies will be required to determine the effect of storage conditions and holding times on the measurement of environmental mutagenicity using these methods.

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Table 1. Mutagenicity of Environmental and Waste Water Samples Prepared by Liquid-Liquid Extraction in Strain TA98

Sample	Sample No. (AIHL-85-)	S-9 Condition	Mutagenic Activity ± SD[1] (Revertants/L)
Contaminated Groundwater (on-site well)	(0402)	2%	3600
NBS Reference Sludge - TCLP Leachate	(0401)	2%	2300
Industrial Treatment Plant Effluent	(0406)	30%	1700 ± 160
Landfill Surface Runoff	(0405)	without	610
Municipal Treatment Plant Effluent	(0403)	10%	420 ± 80
Brackish Receiving Wastewater	(044A)	2%	240 ± 6
Brackish S.F. Bay Water	(0404)	2%	230 ± 70
Contaminated Groundwater (upgradient well)	(042A)	2%	170

1. SD representing precision measurements is obtained from results of replicate or triplicate experiments. No SD is provided where the sample is analyzed only once.

Table 2. Mutagenicity of Chemicals Identified in the EPA/NBS Reference Sludge - TCLP Leachate (AIHL-85-0401)

Chemical Name	Concentration in the Leachate (μg/L)	Mutagenicity in the Ames Assay	Reference
diethlyphthalate	7.1	-	Zeiger et al., 1985
bis-2-ethylhexylphthalate	961	-	Zeiger et al., 1985
butylbenzylphthalate	5.3	-	Zeiger et al., 1985
di-n-butylphthalate	21	-	Zeiger et al., 1985
di-n-octylphthalate	8.9	-	Zeiger et al., 1985
1,4-dichlorobenzene	2	-	Haworth et al., 1983
acenaphthene	3.6	-	Gatehouse, 1980
		+	Epler et al., 1979
acenaphthylene	0.89	-	Gatehouse, 1980
anthracene	6.2	-	McCann et al., 1975
fluorene	5.3	-	McCann et al., 1975
naphthalene	149	-	McCann et al., 1975
2-methylnaphthalene	84	-	Hermann, 1981
phenanthrene	6.2	-	McCann et al., 1975
dibenzofuran	5.3	-	Schoeny, 1982
nitrobenzene	5.3	-	Haworth et al., 1983
2,6-dinitrotoluene	16	+	Sundvall et al., 1984
3,3'-dichlorobenzidine	4.4	+	Reid et al., 1984
N-nitrosodiphenylamine	60	+	Haworth et al., 1983

Table 3. Comparison of Mutagenic Response of Extracts of Two Samples from Site AIHL-86-0301 in Two Salmonella Strains in the Presence and Absence of 10% S-9 Mix[1]

Extraction pH of the Water	Mutagenic Response (revertants/L) TA100 without S-9	TA100 10% S-9	TA98 without S-9	TA98 10% S-9
Ambient[2]	34	< 19	< 3	9
2	396	98	43	95

1. Samples are collected, prepared and tested on separate dates.

2. The ambient pH of the sample is 6.4.

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Table 4. Comparison of Mutagenic Response of Residues Following Extraction of Aliquots of a Drinking Water Sample from Site AIHL-86-0301 at Acidic and Neutral pH Levels[1]

Extraction pH of the Water	Residue Weight (mg/30 L)	Mutagenic Response TA100, without S-9 (revertants/L)
XAD extraction, pH 2 tap water	29.7	186
XAD first extraction, ambient pH tap water	18.2	31
ambient pH effluent re-extracted at pH 2	26.8	121

1. The ambient pH of the sample is 6.5.

Table 5. Mutagenic Responses of Drinking Water Samples Extracted at Ambient and Acid pH Levels

Collection Site No. (AIHL-86-)	Extraction pH of the Water	Residue Weight (mg/30 L Water)	Mutagenic Response TA100, without S-9 (revertants/L)
0303	Ambient[1]	13.2	55
0303	2	24.7	390
0305	Ambient[1]	4.5	< 38
0305	2	22.1	40

1. The ambient pH is 6.3 for the sample from site AIHL-86-0303 and 7.1 for the sample from AIHL-86-0305.

Table 6. Mutagenicity of Chlorinated Drinking Water Samples (Extracted at pH 2) from Five Collection Sites

Collection Site No. (AIHL-86-)	Source Type	Number of Samples Collected	Mutagenic Response ± SD[1] TA100, without S-9 (revertants/L)
0301	Surface Water	7	211 ± 86 (41%)
0302	Surface Water	2	153 ± 16 (11%)
0303	Surface Water	2	294 ± 136 (46%)
0304	Ground Water	1	21
0305	Combined Surface and Ground Water	2	41 ± 1 (2%)

1. C_v = (SD/average) × 100%, is given in parenthesis. The SD and C_v listed represent the variation in measurements of samples from the same collection site collected on different dates.

Table 7. Comparison of Mutagenic Response of Extracts of a Soil Sample from Site AIHL-87-0601 in Two Salmonella Strains in the Presence and Absence of 2% and 10% S-9 Mix[1]

Extraction Method and Solvent	Mutagenic Response (revertants/gram soil) TA98			TA100		
	without S-9	2% S-9	10% S-9	without S-9	2% S-9	10% S-9
Soxhlet Extraction Dichloromethane	<2780	2330	4110	<4220[2]	<2740	3650

1. This subsample is an isolated aliquot taken from the surface of the 5-gallon storage vessel.

2. Detection limit is obtained by dividing the method background value by the highest non-toxic dose tested in the experiment. The detection limit for strain TA100 is high since toxicity is reached at a low dose level.

Table 8. Comparison of Mutagenic Response of Extracts a Soil Sample from Site AIHL-87-0601 with Different Soxhlet Solvents

Extraction Solvent	Mutagenic Response (revertants/gram soil)[1] TA98 + 10% S-9	TA100 + 10% S-9[2]
Hexane:Acetone 2:1	3040	1693
Dichloromethane:Acetone 1:1	2533	2180
Hexane:Isopropanol 3:1	1903	1893
Dichloromethane	1645	1313

1. The values given are the average of soil samples tested four times on two separate dates.

2. The results are from testing the extract after storage in DMSO for 7-10 days. Initial experiment on fresh extract in TA100 gives results below the detection limit. The extracts are assayed again at higher dose levels. TA98 shows a dose response at the original low dose levels.

Table 9. Comparison of Mutagenic Response of Extracts of a Soil Sample (AIHL-87-0601) by Hexane:Acetone (2:1) Soxhlet Extraction with and without Anhydrous Sodium Sulfate

Extraction Condition	Mutagenic Response (revertants/gram soil) ± SD[1] TA98 + 10% S-9	TA100 + 10% S-9
with anhydrous sodium sulfate	2960 ± 380 (13%)	2200 ± 500 (23%)
without anhydrous sodium sulfate	2540 ± 150 (6%)	1680 ± 440 (26%)

1. The values are averages of five soil samples tested on the same date. The SD and C_v listed represent the variation in measurements of five soil samples from the same collection site extracted and tested on the same date.

Table 10. Comparison of Mutagenic Response of Replicate Soil Sample Extracts from Site AIHL-87-0601 for Precision Measurement[1]

Extraction Solvent	Mutagenic Response (revertants/gram soil) ± SD	
	TA98 + 10% S-9	TA100 + 10% S-9
Hexane:Acetone 2:1	2920 ± 230 (8%)	1340 ± 346 (26%)
Dichloromethane:Acetone 1:1	2343 ± 470 (20%)	1830 ± 181 (10%)
Hexane:Isopropanol 3:1	1970 ± 370 (19%)	1540 ± 588 (38%)
Dichloromethane	1493 ± 120 (8%)	1380 ± 75 (5%)

1. A total of twelve identical samples are extracted and tested in parallel. Four types of extraction solvents are used and triplicate samples are extracted with each solvent. SD and C_v (in parentheses) represent the variation in measurements of four samples from the same site, extracted and tested in parallel.

Table 11. Comparison of Mutagenic Response of Replicate Soil Sample Extracts from Site AIHL-87-0601 for Temporal Variation

Extraction Solvent	Mutagenic Response (revertants/gram soil) ± SD[1]	
	TA98 + 10% S-9	TA100 + 10% S-9
Hexane:Acetone 2:1	2830 ± 127 (5%)	2429 ± 1458 (167%)
Dichloromethane:Acetone 1:1	2780 ± 620 (22%)	2435 ± 940 (39%)
Hexane:Isopropanol 3:1	2450 ± 690 (28%)	1507 ± 274 (18%)
Dichloromethane	1495 ± 7 (65%)	1727 ± 530 (31%)

1. SD and C_v (in parentheses) represent the variation in measurements of samples tested on two separate dates.

EVALUATION OF AN IMMUNOASSAY FOR PENTACHLOROPHENOL

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ABSTRACT

Immunoassay technology provides a specific and cost effective mechanism for the analysis of a wide variety of chemical substances. Identification and stimulation of private sector immunoassay technology development are vital components of the immunochemistry program at the EPA's Environmental Monitoring Systems Laboratory Las Vegas (EMSL-LV).

An immunoassay specific for pentachlorophenol (PCP) was submitted to the EMSL-LV by Westinghouse Bio-Analytic Systems (WBAS) for evaluation. The assay tested was a monoclonal antibody-based competitive inhibition enzyme immunoassay (CIEIA). The study consisted of comparing the following three methods of analysis for PCP: 1) direct immunoassay; 2) immunoassay detection following solid-phase extraction; and 3) gas chromatographic (GC) detection (EPA method 604 -- Phenols).

Samples analyzed consisted of spiked ground water, surface water, and potable water. Each water type was collected from different geographical locations. Each sample was spiked with two levels of PCP. In addition, several samples were spiked with a mixture of PCP and related phenolic compounds. These additional compounds were chosen based upon the target analytes of Method 604. The inclusion of these compounds was essential to more fully evaluate the immunoassay as to cross reactivity, particularly as the immunoassay results were to be compared with those of the Agency's GC method.

The experimental protocol for one portion of the evaluation was as follows. One liter of water was spiked and split two ways. One split was analyzed by the direct immunoassay procedure; the other split was solid-phase extracted. These solid-phase extracts were, in turn, split three ways. One split was analyzed by GC, the other two splits were analyzed by immunoassay at both the EMSL-LV and WBAS. With the exception of the GC analyses, this scheme was performed at both the EMSL-LV and WBAS. Method 604 was performed solely at the EMSL-LV; however, the resultant extracts were split three ways. One split was analyzed by GC, and the other two splits were analyzed by immunoassay, again at both the EMSL-LV and WBAS. This experimental design was repeated for each water type and generated over 1300 immunoassay results for each laboratory. Precision and accuracy data between the three methods and the two laboratories were obtained for use as indications of performance.

This evaluation will form the basis for future studies using other assays. The development of standard evaluation criteria and eligibility requirements will be an outcome of this study. The results of this study will help to define the future role of immunoassay in EPA monitoring and regulatory activities.

TOXICOLOGICAL EVALUATION OF COMPLEX INDUSTRIAL WASTES BY IN VIVO AND IN VITRO BIOASSAYS

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ABSTRACT

We have evaluated a variety of short-term bioassays with different genetic endpoints to identify those that may be useful for assessing the biological effects of potentially hazardous complex industrial wastes. Included were a bacterial (Salmonella) and a mammalian cell (L5178Y/TK$^{+/-}$ mouse lymphoma) bioassay to detect gene mutations, a microbial assay to detect DNA damage as evidenced by the induction of prophage lambda in the bacterium Escherichia coli, two mammalian cell assays in Chinese hamster ovary (CHO) cells to detect cytogenetic effects in the form of sister chromatid exchanges (SCEs) and chromosomal aberrations, and the BALB/c-3T3 assay to detect morphological transformation in mammalian cells. In addition, wastes were fed by gavage to F-344 male rats for evaluation of lethality, hepatotoxicity, and mutagenic urine. Our results suggest that the Salmonella assay (either strain TA98 or TA100) and the prophage-induction assay (both in the presence of S9) were the most sensitive to the genotoxicity of these diverse wastes and would be suitable for routine screening of wastes for genotoxicity. Neither urinary mutagenicity nor any of the mammalian cell assays appear useful at this time for routine screening purposes. Hepatotoxicity was evaluated by histopathologic examination, relative liver weight, and changes in five serum enzyme and total bilirubin concentrations. Relative liver weight and a battery of serum indicators appeared useful for routine screening of complex wastes for hepatotoxicity. All of the wastes were characterized for the presence and concentration of the EPA Appendix VIII priority pollutants. The biological activities exhibited by the wastes were not readily predicted by their chemical characterizations, which were more extensive than would ordinarily be available for most wastes. These results indicate the importance of characterizing potentially hazardous complex industrial wastes by both chemical and biological means.

INTRODUCTION

In the United States, wastes are classified as hazardous if they possess one of a number of physical characteristics or if they are composed of certain industrial process wastes or contain specifically regulated chemicals (Federal Register, 1980). Guidelines for charac-

terizing the toxicity of hazardous wastes have not yet been promulgated; however, knowledge of the toxic potential of a hazardous waste can augment the available chemical characterization and provide relevant information regarding potential chronic health effects.

Because hazardous wastes may contain a wide array of chemical mutagens, and because no single bioassay will detect all chemical classes of mutagens, several short-term assays with different genetic endpoints may be advantageous for screening hazardous wastes for genotoxic potential. Consequently, combinations of short-term assays have been proposed as possible screening batteries for hazardous wastes (Barfknecht and Naismith, 1984). Nonetheless, most investigations of the genototoxicity of hazardous wastes have not used a battery of assays. Instead, most studies have used only the Salmonella assay (Nestmann et al., 1980; Houk and Claxton, 1986), although mammalian cells and other eukaryotic assays have been used to a limited extent (Hopke et al., 1982; DeMarini et al., 1984; Donnelly et al., 1985).

In addition to genotoxins, many hazardous wastes may also contain chemicals that are suspected or known hepatotoxins. Hepatomegaly and abnormal liver function, with return to normal on cessation of exposure, have been associated with human exposure to hazardous waste (Clark et al., 1982; Meyer, 1983; Harris et al., 1984). The liver was a primary target organ in mice exposed subchronically to Love Canal soil (Silkworth et al, 1984). Thus, we evaluated the acute hepatotoxicity of 10 of the chemically characterized wastes and assessed whether the chemical analysis was predictive of the biological results.

This report reviews the efforts of our laboratory to determine the usefulness of a variety of short-term bioassays for assessing the genotoxic and hepatotoxic potential of hazardous industrial wastes. Most of the wastes we evaluated had been partially chemically characterized, permitting comparisons of biological activity and chemical composition. The chemical analysis indicated that many of the wastes contained high concentrations of carcinogenic metals, chlorinated compounds, and solvents that are detected poorly by the Salmonella assay (Kier et al., 1986; Zeiger and Tennant, 1986; Claxton et al., 1988). Thus, in addition to the Salmonella assay, we included a prophage-induction assay in Escherichia coli that may be more sensitive than Salmonella for these classes of compounds (Rossman et al., 1984; Houk and DeMarini, 1987). Because in vivo mammalian metabolism may be a critical factor in the generation of mutagenic metabolites from complex hazardous wastes, we also studied the urinary mutagenicity of rats fed hazardous wastes by gavage. Because most batteries include mammalian cell assays, we evaluated a series of such assays and compared the results to those obtained with Salmonella for a set of four diverse hazardous wastes. The advantages and disadvantages of testing crude versus extracted waste samples are discussed along with some of the problems encountered when trying to select appropriate test methodologies for a wide variety of hazardous waste types.

MATERIALS AND METHODS

Wastes and waste extracts

Two sets of hazardous wastes were used for these studies (Table 1). Set 1 was obtained from Edward L. Katz, Hazardous Waste Engineering Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. Three of the waste samples (A, B, and C) were from three different manufacturing processes (petrochemical, pharmaceutical, and plastics). The remaining 12 samples (D through O) were from four commercial hazardous waste incineration facilities that burn a mixture of hazardous wastes from a variety of industrial sources. Each waste was analyzed for the presence of a limited number of priority organics and/or metals identified in the U.S. EPA Appendix VIII list of priority pollutants (U.S. EPA, 1984). Dichloromethane (DCM) extracts were solvent exchanged into dimethyl sulfoxide (DMSO), and crude wastes and waste extracts were tested in the Salmonella assay. Crude wastes were evaluated in the phage-induction assay and fed by gavage to rats to determine hepatotoxicity and to detect mutagenic urine (DeMarini et al., 1987b).

The second set of hazardous wastes (Table 1) consisted of four wastes from four different industries: coke plant, herbicide manufacturing, pulp and paper mill, and oil refining. These wastes were obtained from Dr. M. McKown, Battelle Columbus Laboratories, Columbus, Ohio. DCM extracts were prepared as described (DeMarini et al., 1987a) and solvent exchanged into DMSO for bioassay. These four waste extracts were tested in Salmonella and in the mammalian cell assays.

Urine and urine extracts

F-344 male rats were dosed with 4 different doses of waste C 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 nine other wastes, a single dose of the crude waste was administered by gavage to 70-day-old male F-344 rats. All urines were collected on dry ice for 24 h, centrifuged, filter sterilized, and frozen at -20°C. All 10 raw urines were tested for mutagenicity in Salmonella TA98 with S9 and β-glucuronidase. In addition, six of these raw urines were also extracted and tested as follows.

1 ml of β-glucuronidase (Sigma Type VII from *E. coli*) at a 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. Then, each mixture was poured through two serially connected Sep-Pak® C_{18} cartridges (Waters Associates, Milford, MA) followed by water. Concentrates were then eluted with methanol, the methanol was evaporated, and the residue was solvent exchanged into a volume of DMSO to produce 5-X concentrates, which were stored at -20°C.

Table 1

Physical descriptions of hazardous wastes

Wastes	Description
Waste Set 1	
A	Black, thin oil from a petrochemical manufacturing plant
B	Black, oily liquid from pharmaceutical manufacturing plant
C	Black tar produced from a plastic manufacturing plant
D and E	Composite of aqueous wastes; watery liquid with oil drops
F and G	Composite of organic wastes; biphasic gray sludge with reddish-brown liquid
H and I	Composite of aqueous wastes; thin, gray slurry
J	Composite of organic wastes; gray, thick liquid with suspended solids
K	Similar to J, but lighter in color and thinner
L and M	Composite of organic wastes; black, thin, pourable tar
N and O	Composite of aqueous wastes; clear, watery liquid
Waste Set 2	
P	Light-brown liquid with suspended solids from coke plant
Q	Brown clear liquid from herbicide manufacturing plant
R	Brown semi-solid with wood chips from pulp and paper mill
S	Dark liquid with brown flocculant and oil drops from oil refining plant

Salmonella assay

The Salmonella plate-incorporation assay was performed as described (Maron and Ames, 1983). Wastes and waste extracts were assayed at least twice, each in either duplicate or triplicate, in strains TA98 and TA100 in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9 (~1 mg of protein/plate) prepared as described (Maron and Ames, 1983). Urines and urine extracts were assayed in strain TA98 in the presence of S9 and β-glucuronidase (1000 units/plate). Raw urine and urine extracts were tested twice, each in singlet. A dose-related increase in the number of revertants/plate was considered a positive response.

Phage-induction assay

The Microscreen phage-induction assay developed by Rossman et al.

(1984) was performed using modifications described previously (Houk and DeMarini, 1988). The two bacterial strains used for this assay are derived from E. coli B/r. $WP2_s(\lambda)$ is a lambda lysogen of $WP2_s$ (trpE, uvrA); SR714 (trpE; $uvrD_3$) is the indicator strain. The lysogenic strain was exposed overnight to various dilutions of the crude wastes both in the presence and absence of S9. Following exposure, each suspension was sampled for the presence of lambda particles by plating onto the indicator strain. The criterion for a positive response was an increase in the number of induced plaque-forming units per plate that reached or exceeded the upper limit of the 99% confidence interval based on the negative controls.

Mammalian cell assays

Because of the limited amount of DCM extract of each of the four wastes used in the mammalian cell assays, and because of the cost of performing a set of such assays with each extract, it was not possible to perform the assays according to currently accepted protocols or established guidelines. Instead, limited protocols were used that required a minimum number of steps and amount of sample to permit an extract to be identified as a presumptive positive or negative. In order to conserve sample, the dose range for each extract was estimated for all of the assays by performing a preliminary cytotoxicity study in Chinese hamster ovary (CHO) cells. The assays were then performed only once with only a few doses of extract and in the presence of S9. Otherwise, the assays were performed essentially as described below.

The L5178Y/$TK^{+/-}$ -3.7.2C mouse lymphoma assay was performed in the presence of S9 as described (Clive et al., 1979). Cytogenetic effects induced by the four waste extracts in the presence of S9 were determined by scoring for chromosomal aberrations and sister chromatid exchanges (SCEs) in CHO-WBL cells as described (Galloway et al., 1985). The ability of the four waste extracts to induce morphological transformation in BALB/c-3T3 cells was determined as described (Matthews et al., 1985). Metabolic activation was provided by X-irradiated rat liver cells, and 12-O-tetradecanoyl-13-phorbol acetate (TPA) was used to promote the formation of the transformed phenotype.

Hepatotoxicity assays

Ten wastes were evaluated for hepatotoxicity as described (Simmons et al., 1988). Briefly, male F-344 rats were exposed by gavage to a single dose of waste that ranged from 0.5 to 5 ml/kg. 24 h after dosing, the rats were weighed, anesthetized with 50 mg/kg of sodium pentobarbital i.p., and exsanguinated from the abdominal aorta. Serum chemistry profiles were obtained for concentrations of total bilirubin (BILI) and activities of aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphatase (ALKPH), and lactate dehydrogenase (LDH). The activity of ornithine carbamyl transferase (OCT) was determined as described by Simmons et al. (1988).

The liver was excised quickly, rinsed in saline, blotted, and weighed, and then relative liver weight (liver-to-body-weight ratio) was determined. Liver samples were dried to a constant weight (no change in 24 h) in a 100°C oven; the wet-to-dry-weight ratio was used as an index of hepatic water content. Samples from the left lobe were taken for open histopathological examination; lesions were evaluated on hematoxylin- and eosin-stained tissue sections. Data were analyzed as described by Simmons et al. (1988).

RESULTS AND DISCUSSION

The quantitative results of our genotoxicity studies of these wastes and waste extracts have been published elsewhere (DeMarini et al., 1987 a,b; Houk and DeMarini, 1988); the qualitative responses are summarized here. Table 2 summarizes the mutagenic responses in Salmonella of the first set of wastes and waste extracts, the mutagenic responses in Salmonella of the urine or urine extracts from rats fed these wastes, and the responses of these wastes in the phage-induction assay. Table 3 summarizes the genotoxic responses of the second set of wastes in Salmonella and in the mammalian cell assays.

Table 2

Genotoxic responses of crude wastes/extracts and raw urines/extracts

	Mutagenic responses in Salmonella										Phage-induction responses	
	Crude wastes				Waste extracts							
	TA98		TA100		TA98		TA100		Urines[a]			
Wastes	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	Raw	Ext.	+S9	-S9
C	-	-	+	+	+	-	+	-	+	+	+	+
L	+	-	-	-	-	-	-	-	+	+	+	+
M	+	-	-	-	-	-	-	-	+	+	+	+
G	+	+	-	-	-	-	-	-	-	-	+	+
O	+	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	NT[b]	NT
H	-	-	-	-	+	+	-	-	-	NT	+	+
J	-	-	-	-	-	+	-	-	-	NT	+	-
K	-	-	-	-	-	-	-	-	-	NT	-	-
B	-	-	-	-	NT	NT	NT	NT	-	NT	+	+
A	-	-	+	+	-	-	+	+	NT	NT	+	+
F	-	-	+	+	-	-	-	-	NT	NT	+	+
D	-	-	-	-	-	-	-	-	NT	NT	+	-
I	-	-	-	-	-	-	-	-	NT	NT	+	+
N	-	-	-	-	-	-	-	-	NT	NT	-	-

[a]Urines tested in strain TA98 in the presence of S9 and β-glucuronidase.
[b]Not tested.

Table 3

Genotoxic responses of waste extracts in Salmonella and mammalian cells

Waste extracts	Mutagenicity in Salmonella TA98 +S9	TA98 -S9	TA100 +S9	TA100 -S9	Genotoxicity in mammalian cells: Mutagenicity in L5178Y/TK$^{+/-}$	Cytogenetic effects: SCEs	Cytogenetic effects: Chromosomal aberrations	Transformation
P	+	-	+	-	+	+	+	-
Q	+	-	-	-	+	-	-	-
R	+	-	-	-	+	+	-	-
S	-	-	-	-	+	+	-	-

Liquid extraction, as opposed to solid-phase extraction, was the only type that was suitable for these diverse wastes. However, as discussed previously (DeMarini et al., 1987b), DCM may not be a suitable solvent for all types of wastes. Table 2 shows that DCM failed to extract mutagenic activity from five wastes (L, M, G, F, O) that were mutagenic in their crude, unextracted form. Nearly 80% of these wastes would have been detected as mutagenic if only the crude wastes had been tested. The additional time and expense required to prepare organic extracts of these wastes did not produce extracts that yielded much additional information that was not obtainable from the crude wastes. However, not 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. An innovative approach involving fractionation by thin-layer chromatography coupled with the Salmonella assay has been shown to be useful with some wastes (Houk and Claxton, 1986).

Judicious selection of a test matrix (and battery) is required in order to screen hazardous wastes in a cost-effective manner without an unacceptable loss of detection capability. For example, all of the wastes and waste extracts that were mutagenic in Salmonella in Table 2 would have been detected if they had been tested only in the presence of S9 (Table 2), reducing the testing matrix in half. Considering both the crude wastes and waste extracts, only two wastes (A and F) were mutagenic solely in strain TA100. Thus, if the crude wastes and extracts had been studied only in TA98 in the presence of S9, nearly 80% of the mutagenic wastes identified with the present matrix would have been identified.

Based on the wastes tested here, the urinary mutagenesis assay did not provide any additional information beyond that obtained from testing

the wastes or waste extracts directly in the Salmonella assay. Only three of the 10 wastes tested produced mutagenic raw urine, and all three of these wastes were mutagenic when tested directly in Salmonella (Table 2). Extracting the urines by means of C_{18}/methanol elution did not identify a urine as mutagenic that was not identified as mutagenic from studies with raw urine (Table 2). Considering the time and expense of performing the urinary mutagenesis assay, this assay was not a useful adjunct to testing the wastes or waste extracts directly for mutagenicity.

The phage-induction assay in E. coli detected five crude waste samples that were not mutagenic in Salmonella (Table 2). As described in the INTRODUCTION, the Microscreen phage-induction assay has been shown to detect some carcinogenic metals and chlorinated organics and solvents that are not mutagenic in Salmonella. Metals and compounds of these types are present in most of the waste samples studied here (U.S. EPA, 1984), and the ability of some of these compounds to induce prophage may account for the detection by the phage-induction assay of the five additional waste samples. Accumulating evidence indicates that prophage induction (and the SOS response in general) is a broader genetic endpoint than reverse mutation in bacteria (Elespuru, 1984, 1987; Hofnung and Quillardet, 1986), making it especially useful for screening chemically diverse wastes.

A comparison of the genotoxic responses of DCM extracts of four wastes in the Salmonella assay to their responses in a set of mammalian cell assays indicates that the inclusion of mammalian cell assays may not have improved significantly the ability to detect the genotoxicity of the wastes beyond that afforded by the Salmonella assay alone (Table 3). The oil refining waste was the only waste detected by the mammalian cell assays that was not detected by the Salmonella assay. The BALB/c-3T3 transformation assay did not detect any of the extracts as positive, even though all four were genotoxic in one or more assays. Based on these limited results, it appears that this assay may not be useful for screening hazardous wastes.

Currently, there is only a small data base on the use of mammalian cell assays with complex mixtures, let alone with hazardous wastes. Perhaps some of the reasons for this are that mammalian cell assays are difficult to use with toxic complex mixtures and are more costly and time consuming to perform than microbial assays. Recently, several studies (Ashby et al., 1985; Tennant et al., 1987) have shown that mammalian cell assays may not provide much additional detection capability than that afforded by the Salmonella assay for pure compounds. Our results with these hazardous wastes suggest that the same may be true for complex mixtures.

The results of the hepatotoxicity study have been published (Simmons et al., 1988) and are summarized in Table 4. Based on histopathological evaluation of the liver, eight of the 10 wastes were hepatotoxic (Table 4). Under the experimental conditions, wastes H and O were nonhepatotoxic. Nine of the 10 wastes caused an increase in relative liver weight, and various wastes increased the serum concentrations of different combinations of the serum enzymes and bilirubin (Table 4).

Table 4

Summary of hepatotoxic effects of wastes

Waste	N[a]	Dose (ml/kg)	Histo-patho-logy	Relative liver weight	Serum indicators of hepatic injury					
					AST	ALT	LDH	ALKPH	OCT	BILI
A	5	1	+[b]	+	o[c]	o	+	+	+	+
B	6	1	+	+	+	o	o	o	o	o
E	4	2.5	+	+	o	o	o	+	o	o
	2	5	+	+	+	+	o	+	o	+
G	5	5	+	+	o	o	o	o	o	+
H	6	5	o	+	o	o	o	o	o	o
J	6	0.5	+	+	+	+	o	o	+	o
K	6	0.5	+	+	+	+	o	+	+	o
L	4	2.5	+	+	+	o	+	o	o	o
M	5	5	+	+	+	+	+	+	o	o
O	6	5	o	o	o	o	o	o	o	o

[a]N = number of rats used for evaluation of hepatotoxicity.
[b]+ = a significant increase compared to concurrent controls.
[c]o = no change compared to concurrent controls.

With histopathology as the criterion of hepatotoxicity, the best single predictor of hepatotoxicity was relative liver weight (Simmons et al., 1988). Assessed individually, single serum indicators could not distinguish hepatotoxic from nonhepatotoxic wastes. However, as a battery, the serum indicators correctly identified the eight hepatotoxic wastes and the two nonhepatotoxic wastes (Simmons et al., 1988).

The chemical characterization available for these wastes (U.S. EPA, 1984) is more extensive than would ordinarily be available for most complex wastes and allowed for a limited assessment of the relationship between chemical characterization and biological effects. Comparing observed toxicity to that expected based on a limited understanding of the chemical composition of the waste is important because the U.S. EPA identifies wastes as hazardous based primarily on partial chemical characterization (Greer, 1984). As discussed extensively elsewhere

(DeMarini et al., 1987b; Houk and DeMarini, 1988; Simmons et al., 1988), the observed biological effects (genotoxicity, cytotoxicity, and hepatotoxicity) were not readily predicted from the chemical characterization data.

Our main purpose in these studies has been to determine which individual bioassays or groups of bioassays could serve as inexpensive, rapid screening tools to assess the toxicity of a large number of chemically different industrial wastes. Although diverse, the hazardous wastes we have studied here cannot be considered to represent the "universe" of wastes. In addition, there are many other bioassays that we have not yet examined that may be useful for screening hazardous wastes. Given these limitations, however, our studies suggest that the Salmonella assay using either strain TA98 or TA100 in the presence of S9 or the phage-induction assay in the presence of S9 may be useful in screening wastes for genotoxicity. A 1-day gavage of rats followed by determination of relative liver weight appears to be highly predictive of hepatotoxicity. The combination of this hepatotoxicity assay with one of the genotoxicity assays might provide an inexpensive, rapid, and simple battery of bioassays that could be used routinely to characterize large numbers of waste samples. This battery could prove to be a useful adjunct to chemical analysis for identifying hazardous wastes.

Both government (Federal Register, 1983, 1984a,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. We have reported here on the use of only genotoxicity and hepatotoxicity bioassays to evaluate hazardous wastes; however, wastes may induce other biological effects, such as neurotoxicity. Additional investigations are needed to explore the effects of hazardous wastes at these and other toxicological endpoints. The results presented here suggest that short-term bioassays may be useful adjuncts to chemical analysis in identifying wastes as hazardous.

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ENFORCEMENT

ANALYSIS OF RCRA GROUND-WATER SAMPLES

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INTRODUCTION

Ground-water is a very important resource. Contamination of this resource has occurred across the country and is being detected with an ever increasing frequency. One of the goals of the Resource Conservation and Recovery Act (RCRA) program is to protect human health and the environment from releases of hazardous waste and their constituents from facilities which manage these materials. This goal is put into practice by monitoring and assessing the risks associated with chemicals entering the environment. The foundation for the assessment of the risks associated with chemicals impacting the environment on a facility-specific basis is accurate analytical work. It is essential that environmental problems be identified and characterized quickly and accurately so that the appropriate actions can be taken in a technically efficient manner.

In the fall of 1984, the Administrator of the U.S. Environmental Protection Agency established the Hazardous Waste Ground-Water Task Force to evaluate the level of compliance and address the cause of poor compliance with ground-water monitoring regulations at hazardous waste disposal facilities. The basic regulations or requirements have been in-place since 1980; however, EPA surveys had shown that the regulated community had not achieved compliance. Some of the information collected by the Task Force during facility inspections is presented in examples in this paper.

REGULATORY REQUIREMENTS

The RCRA regulations establish requirements for monitoring the ground-water quality beneath facilities which store, treat and dispose of hazardous waste in landfills, surface impoundments, and land treatment areas. The success of a ground-water monitoring program is predicated upon the assumption that the measured and reported concentrations of chemicals in ground-water

samples extracted from wells at a facility are representative of the insitu ground-water quality. For this reason the regulations require owner/operators of hazardous waste disposal units to implement procedures to generate high quality analytical data from ground-water samples. Table 1 lists those regulations which address ground-water analytical programs. The requirements are two phased: first, a comprehensive written sampling an analysis plan must be developed, and second, the field team and laboratory must implement the plan. Although problems may arise in all phases of the sampling and analysis process, the focus of this paper will be on problems and concerns which occur after samples have been procured.

TABLE 1

RCRA REGULATORY REQUIREMENTS FOR THE GENERATION OF RELIABLE GROUND-WATER MONITORING DATA*

- Interim Status Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities.
  - 40 C.F.R. 265 Subpart F--Ground-Water Monitoring Section 265.92 Sampling and analysis.
- Administered Permit Programs: The Hazardous Waste Permit Program.
  - 40 C.F.R. 270 Subpart B--Permit Application, Section 270.14(c) Contents of Part B: Additional information requirements.
- Standards For Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities.
  - 40 C.F.R. 264 Subpart F--Releases from Solid Waste Management Units, Section 264.97 general ground-water monitoring requirements.

* Note: This table does not reference EPA's corrective action permitting or enforcement authorities which may be used to require analysis of ground-water samples.

GROUND-WATER SAMPLING AND ANALYSIS PLANS

The RCRA regulations require an owner or operator to develop procedures and techniques for: sample collection; sample preservation and shipment; analytical methodologies; and chain-of-custody control. A generic technical framework which can be modified on a facility-specific basis to develop a comprehensive ground-water sampling and analysis program is included as an Appendix to this paper. This framework has been used to develop facility specific sampling and analysis plans for RCRA corrective action.

CONSTITUENTS/PARAMETERS TO BE MEASURED

The RCRA regulations dictate the parameters which must be determined for ground-water samples. The Interim Status regulations focus on parameters which: characterize the suitability of the ground-water as a drinking water supply, establish ground-water quality, and indicate ground-water contamination. The standards for a permitted facility in a detection monitoring program requires monitoring for indicator parameters (e.g. specific conductance, total organic carbon, or total organic halogen), waste constituents (Appendix IX), or reaction products which provide a reliable indication of the presence of hazardous constituents in ground-water. Once contamination has been detected it must be completely characterized. A listing of some of the analytes which were found in recently completed investigations of RCRA facilities by the Hazardous Waste Ground-Water Task Force are illustrated in Table 2. Good communication back and forth between field and laboratory personnel is essential to ensure that the samples are being analyzed for the proper parameters, that the appropriate method and detection limits are being used, and that the resultant data is appropriate for its intended use.

TABLE 2

RCRA Hazardous Waste Ground-Water Task Force
Analyte Occurrence at
31 Commercial Facilities*

COMPOUND	PERCENT OF SAMPLES WITH COMPOUND HITS
CHROMIUM	58.5
ARSENIC	39.1
LEAD	37.3
CADMIUM	35.0
PHENOL	34.2
METHYLENE CHLORIDE	21.0
ACETONE	19.5
MERCURY	18.9
SILVER	13.4
BIS(2-ETHYHEXYL)PHTHALATE	12.8
METHYL ETHYL KETONE (MEK)	9.6
TOLUENE	5.9
CHLOROFORM	5.3
BENZENE	5.3
TRICHLOROTHYLENE	5.1
1,1-DICHLOROETHANE	4.6
CYANIDE	4.6
TETRACHLOROTHYLENE	4.6
TRANS-1,2-DICHLOROETHYLENE	3.9
1,2-DICHLOROETHANE	3.9
XYLENE (TOTAL)	3.7
1,1,1-TRICHLOROETHANE	3.5
P-CRESOL	3.2
BENZOIC ACID	2.9
4-METHYL-2-PENTANONE	2.8
TETRAHYDROFURAN	2.8
ETHYLBENZENE	2.7
CHLOROBENZENE	2.7
DI-N-BUTYLPHTHALATE	2.6
VINYL CHLORIDE	2.4

* Note: This is not a complete Listing of the Analytes Identified.

LABORATORY ANALYSIS

Once the sample has been received in the laboratory, the laboratory personnel should clearly

document the processing steps that are applied to the sample. All sample preparation techniques (e.g., extraction) and instrumental methods must be identified in the logbook. Experimental conditions, such as the use of specific reagents (e.g., solvents, acids), temperatures, reaction times, and instrument settings, should be noted. The results of the analysis of all quality control samples should be identified specific to each batch of ground-water samples analyzed. The laboratory logbook should include the time, date, and name of the person who performed each processing and quality assurance step.

ANALYTICAL PROCEDURES

The sampling and analysis plan should describe in detail the analytical procedures that will be used to determine the concentrations of constituents or parameters of interest. These procedures should include suitable analytical methods as well as proper quality assurance and quality control protocols. The required precision, accuracy, detection limits and percent recovery (if applicable) specifications should be clearly identified in the plan.

The sampling and analysis plan should identify one method that will be used for each specific parameter or constituent. The plan should specify a method in SW-846 or an EPA-approved method, and clearly indicate if there are going to be any deviations from the stated method and the reasons for these deviations.

Records of ground-water analysis should include the methods used, extraction date, and date of actual analysis. Data from samples that are not analyzed within recommended holding times should be considered suspect. Any deviation from an EPA-approved method (SW-846) should be adequately tested to ensure that the quality of the results meets the performance specifications (e.g., detection limit, sensitivity, precision, accuracy) of the reference method. When the analysis is not performed in a laboratory at the facility site, the laboratory should be furnished with a copy of the facility plan. Additionally, each batch of samples should be accompanied by a copy of the sampling field log.

LABORATORY QUALITY ASSURANCE CONTROL (QA/QC)

The owner/operator's sampling and analysis plan should describe explicitly the QA/QC program that will be used in the laboratory. Many owner/operators use commercial laboratories to conduct analyses of ground-water samples. In these cases, it is the owner/operator's responsibility to ensure that the laboratory of choice is exercising a proper QA/QC program. The QA/QC program described in the owner-operator's sampling and analysis plan should be used by the laboratory analyzing samples for the owner/operator.

The owner/operator's sampling and analysis plan should provide for the use of standards, laboratory blanks, duplicates, and spiked samples for calibration and identification of potential matrix interferences. The owner/operator should use adequate statistical procedures (e.g., QC charts) to monitor and document performance and implement an effective program to resolve testing problems (e.g., instrument maintenance, operator training). Data from QC samples (e.g., blanks, spiked samples) should be used as a measure of performance or as an indicator of potential source of cross-contamination, but should not be used to alter or correct analytical data. These data should be submitted to the Agency with the ground-water monitoring sample results.

LABORATORY EVALUATIONS/AUDITS

In most cases, individual ground-water sampling events will take place over a long period of time. Periodic laboratory evaluations should be performed to ensure that ground-water samples are managed and analyzed according to the sampling and analysis plan and that data of high quality is being generated.

The laboratory evaluation should cover the following general areas:

- QA/QC - This includes reviewing the facility's QA/QC program or plan checking to see that holding time criteria are met, ensuring that proper chain-of-custody procedures are used, and determining whether U.S. EPA-approved analytical methods are used.

- Equipment - This includes reviewing the adequacy and effectiveness of the laboratory equipment.

- Recordkeeping - This includes reviewing data sheets for accuracy and detail. The frequency that QA/QC analysis are reported should be noted.

- General Maintenance - This includes cleanliness of the instruments and glassware.

- Laboratory Employee Qualifications - This includes reviewing the training of chemists and laboratory technicians.

Some of the specific laboratory evaluation questions that one should ask when evaluating a laboratory follow below.

A. Laboratory Evaluation

1) Have laboratories been changed since analysis began? Which?
2) Is the lab quality assurance plan available?
3) Is there documentation of U.S. EPA acceptable methods?
4) Are instrument calibration records available?
   - Are analytical instruments routinely calibrated and in good repair?
   - Are calibration records and other record keeping logs kept at the laboratory?
5) Is there an adequate QA/QC program for all analyses?
6) Are detection limits for each parameter identified?
7) Is glassware cleaned and baked according to proper laboratory procedure and in accordance with standard methods?

B. Sample Receipt

1) Was the condition of samples checked?
   - Were containers intact?
   - Was the preservative present?
   - Was the custody seal intact?
2) Was the sample information checked?
3) Are chain-of-custody records present?
4) Does sample and custody seal information match chain-of-custody record?
5) Are chain-of-custody records signed?

6) Is the request for analysis present?
7) Was receipt of sample entered in laboratory logbook?
8) Was laboratory sample number assigned?
9) Are samples refrigerated after collection?
   - Are samples stored in a secure area?
10) What is the average holding time before samples are analyzed?
   - Sample holdling times not exceeded? (See Table 1)
11) Are time-sensitive parameters measured in the field or in the lab?

C. Analytical Procedures

1) Have samples been filtered, where appropriate, prior to analysis?
2) Are the required drinking water suitability parameters tested for?
3) Are the required ground-water quality parameters tested for?

CONCLUSION

A quality RCRA sampling and analysis program for ground-water monitoring can only be developed and implemented with a thorough understanding of the facility specific characteristics and regulatory requirements and standards. The ground-water sampling and analysis program should be re-evaluated and modified on a continuing basis to assure that the data generated accurately reflects the chemical quality of the ground-water beneath the facility.

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APPENDIX

[The components of this appendix are a Data Collection Quality Assurance Plan and a Data Management Plan. This appendix should be modified to take into account site-specific technical detail.]

A. Data Collection Quality Assurance Plan

The Respondent shall prepare a plan to document all monitoring procedures: sampling, field measurements and sample analysis performed during the investigations to characterize the source and contamination, so as to ensure that all information, data and resulting decisions are technically sound, and properly documented.

1. Data Collection Strategy

The strategy section of the Data Collection Quality Assurance Plan shall include but not be limited to the following:

a. Description of the intended uses for the data, and the necessary level of precision and accuracy for these intended uses;
b. Description of methods and procedures to be used to assess the precision, accuracy and completeness of the measurement data;
c. Description of the rationale used to assure that the data accurately and precisely represent parameter variations at a sampling point, a process condition or an environmental condition. Examples of factors which shall be considered and discussed include:
    i) Environmental conditions at the time of sampling;
    ii) Number of sampling points;
    iii) Representativeness of selected media; and
    iv) Representativeness of selected analytical parameters.

2. Sampling and Field Measurements

The Sampling and Field Measurements section of the Data Collection Quality Assurance Plan shall discuss:

a. Selecting appropriate sampling and field measurement locations, depths, etc.;
b. Providing a sufficient number of sampling and field measurement sites;
c. Measuring all necessary ancillary data;
d. Determining which media are to be sampled (e.g., ground water, air, soil, sediment, etc.);
e. Determining which parameters are to be measured and where;

f. Selecting the frequency of sampling and field measurement and length of sampling period;
g. Selecting the types of sample (e.g., composites vs. grabs) and number of samples to be collected;
h. Documenting field sampling and field measurement operations and procedures, including;
    i) Documentation of procedures for preparation of reagents or supplies which become an integral part of the sample (e.g., filters, and adsorbing reagents);
    ii) Procedures and forms for recording the exact location and specific considerations associated with sample and field measurement data acquisition;
    iii) Documentation of specific sample preservation method;
    iv) Calibration of field devices;
    v) Collection of replicate samples;
    vi) Submission of field-biased blanks, where appropriate;
    vii) Potential interferences present at the facility;
    viii) Construction materials and techniques, associated with monitoring wells and piezometers;
    ix) Field equipment listing and sample containers;
    x) Sampling and field measurement order; and
    xi) Decontamination procedures.
i. Selecting appropriate sample containers;
j. Sample preservation; and
k. Chain-of-custody, including:
    i) Standardized field tracking reporting forms to establish sample custody in the field prior to shipment; and
    ii) Pre-prepared sample labels containing all information necessary for effective sample tracking.

3. Sample Analysis

The Sample Analysis section of the Data Collection Quality Assurance Plan shall specify the following:

a. Chain-of-custody procedures, including:
    i) Identification of a responsible party to act as sample custodian at the laboratory facility authorized to sign for incoming field samples, obtain documents of shipment, and verify the data entered onto the sample custody records;
    ii) Provision for a laboratory sample custody log consisting of serially numbered standard lab-tracking report sheets; and
    iii) Specification of laboratory sample custody procedures for sample handling, storage, and dispersement for analysis.

b. Sample storage and holding times;
c. Sample preparation methods;
d. Analytical procedures, including:
    i) Scope and application of the procedure;
    ii) Sample matrix;
    iii) Potential interferences;
    iv) Precision and accuracy of the methodology; and
    v) Method detection limits.
e. Calibration procedures and frequency;
f. Data reduction, validation and reporting;
g. Internal quality control checks, laboratory performance and systems audits and frequency, including:
    i) Method blank(s);
    ii) Laboratory control sample(s);
    iii) Calibration check sample(s);
    iv) Replicate sample(s);
    v) Matrix-spiked sample(s);
    vi) "Blind" quality control sample(s);
    vii) Control charts;
    viii) Surrogate samples;
    ix) Zero and span gases; and
    x) Reagent quality control checks.
h. Preventive maintenance procedures and schedules;
i. Corrective action (for laboratory problems); and
j. Turnaround time.

GROUND-WATER SAMPLING AT RCRA FACILITIES

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INTRODUCTION

Ground-water sampling is implemented at facilities subject to RCRA Subtitle C through numerous authorities. A routine ground- water sampling schedule is required at these facilities pursuant to 40 CFR 264 and 265 Subpart F. The sampling program initiated under these federal regulations must be capable of determining the facilities impact on the quality of the ground water in the upper most aquifer beneath the facility.

Federal regulations 40 CFR Part 265, and 264 Subpart F, require owners/operators of facilities subject to RCRA Subtitle C to obtain and analyze samples from their ground-water monitoring network. Pursuant to these regulations, a sampling and analysis plan must be developed and kept at the facility. The sampling and analysis plans must delineate the techniques and procedures for; sample collection, sample preservation and shipment, analytical procedures, and chain of custody control.

The regulations set forth in 40 CFR 265.92 specify indicator parameters for which concentrations in the ground water must be determined. The required sampling parameters are divided into two categories; those that determine ground-water quality, and those used as indicators of ground-water contamination. For each indicator parameter, the owner/operator must determine the initial back-ground arithmetic mean and variance by comparing replicate measurements for the respective parameter concentrations against those obtained from the hydraulically up-gradient wells in the first year.

Once a treatment, storage or disposal facility recieves a final RCRA permit, the facility's ground-water monitoring permit requirements should be based on the regulations set forth in section 264.97. Where appropriate, these facilities must establish the quality

of background ground water for each of the monitoring parameters specified in the permit. While in detection monitoring, the concentration of the monitoring parameters at the point of compliance are compared to the background values. The Behrens-Fisher Student's t-test (or an equivalent statistical procedure) is used to determine any statistical deviations between background ground-water quality and the ground-water quality down-gradient at the point of compliance.

Under the compliance monitoring program, the owner/operator must determine the concentration of all hazardous constituents contained in Appendix VIII of 40 CFR 261 in the ground water at each monitoring well at the point of compliance. If Appendix VIII constituents are detected in the ground water that are not specified in the permit, the owner/operator is required to report these additional constituents to the Regional Administrator.

In addition to the federal regulations and permit conditions which pertain to the daily operation of RCRA hazardous waste management units, numerous enforcement authorities (e.g. sections 3008(h), 3013, 7003, 3004(u)(v)) can require the initiation of a ground-water sampling program as an integral part of corrective action activities. Such activities can include the characterization of ground-water contaminant plumes, and the designing and implementation of remedial programs. In any case, the ultimate goal of all ground-water sampling programs is to collect samples for analysis that are representative of the insitu ground-water quality.

HAZARDOUS WASTE GROUND-WATER TASK FORCE

In the Fall of 1984, EPA established the Hazardous Waste Ground-Water Task Force to evaluate the level of compliance and identify the causes of poor compliance with ground-water monitoring regulations at hazardous waste disposal facilities. As a part of this effort a special joint regional, state and Headquarters facility evaluation team was formed to determine the status of ground-water monitoring at existing hazardous waste treatment, storage and land disposal facilities and to decide on actions to be taken to resolve any problems found. The inspection team evaluated 58 commercial and private land disposal facilities. Some of the problems with facility sampling and analysis plans and

sampling procedures or techniques identified during the Task Force inspections are used as examples and discussed in the following narrative.

Many components of the sampling process can bias the sample analysis. The problems with the sampling procedures and techniques identified by the Task Force present several cases in which the field activities can have an adverse effect on the data quality.

It is important for laboratory personnel to have a good understanding of the field sample collection procedures implemented, and how these field activities can affect the sample analysis. The sample and analysis plan and the field notes recorded by the sampling team should be reviewed by the laboratory personnel as an integral part of the sample analysis interpretation.

SAMPLING AND ANALYSIS PLANS

As noted above, sampling and analysis plans must identify specifically procedures and techniques to be used at a facility for the collection and analysis of samples. Several recurring problems with sampling and analysis plans were noted during the Task Force inspections. Many sampling and analysis plans were found to be non-site specific, and/or lacked narrative describing the equipment and procedures to be used throughout sample collection and analysis. Vague or non-specific sampling and analysis plans can not be used as stand alone field guides, and leave room for error in both the field and laboratory. Sampling and analysis plans should ensure the consistency of sampling procedures and sample analysis even with a change in field or laboratory personnel.

In addition, the data obtained pursuant to non-site specific sampling and analysis plans are often inconsistent, and thus may be inadequate for future statistical comparisons. Owner/operators must create site-specific, detailed sampling and analysis plans to provide accurate and consistent field and laboratory data.

MEASUREMENT OF STATIC WATER LEVEL ELEVATIONS

The measurement of static water levels is pertinent in determining ground-water flow directions (down gradient and up gradient well locations) and the

hydraulic gradient. Pursuant to sections 264.97(f) and 265.92(e) of the federal regulations, ground-water surface elevations must be determined each time the ground water is sampled. It is recommended in the RCRA Ground-Water Monitoring Technical Enforcement Guidance Document that an electronic device be used to determine the static water level or the depth to light and dense phase immiscibles. If an electronic device is not available, a weighted steel tape will normally suffice. In any case, the device used to measure the static water level must be sufficiently sensitive so that a measurement accurate to $\pm$0.01 of a foot can be determined.

The Task Force found unweighted and/or twisted tapes, and "chalk and tape" devices are often used in the field to measure static water levels. These devices are inadequate, as they will often lead to erroneous static water levels. The measurement of static water levels accurate to $\pm$0.01 of a foot is especially important when the hydraulic gradient is know to be very gradual. In this case, small deviations from the true static water level can lead to incorrect ground-water flow interpretations. Misinterpretations of the static water level are also observed due to the use of devices that are not regularly calibrated. The calibration dates of all field equipment should be recorded and checked prior to use.

All static water levels plus depth to light and dense phases should be measured from a permanent reference point which is usually located at the top of the well casing and established by a licensed surveyor. The reference point should be instituted in relation to an established bench mark.

The Technical Enforcement Guidance Document recommends that the following procedures be used to detect the presence of immiscible phases prior to well evacuation:

1. Remove the locking and protective caps

2. Sample the air in the well head for organic vapors using either a photoionization analyzer or an organic vapor analyzer, and record measurements.

3. Determine the static liquid level using a manometer and record the depth

4. Lower an interface probe into the well to determine the existence of any immiscible layer(s), light or dense.

It has been observed that facilities do not always obtain the static water level for all monitoring wells during each sampling event. Thus, subtle changes in ground-water flow paths can remain undetected for extended periods of time. In addition, the total depths of monitoring wells are not always measured prior to well purging. The measurement of the total depth of all monitoring wells prior to well evacuation assists in calculating accurate well volumes to purge, and in identifying potential problems with improper well development and silt accumulation within the monitoring well.

The process of identifying light and dense layers of immiscible organics is also frequently over-looked in both sampling and analysis plans and actual practice. The determination of the presence of immiscible phases facilitates identification of the ground-water contamination problem.

Sloppy practices, such as allowing static water level measuring tapes to lie on the bare ground around the well head, and neglecting to decontaminate the down-hole equipment before and after its use should be eliminated. Such practices can lead to the introduction of particulates or other contaminants into the ground water, and cause cross-contamination between wells.

WELL EVACUATION

Prior to sampling ground water, it is necessary to evacuate all water in the well and filter pack, thus allowing the formation water to replace the stagnant water. The owner/operator's approach should allow for drawing the water down from above the screen in the uppermost part of the water column in high yield formations to ensure that fresh water from the formation will move upward in the screen.

Methods used to purge stagnant water from monitoring wells will depend on the site-specifics and the individual hydraulic yield characteristics of the monitoring well. It is recommended that a minimum of three well volumes be evacuated from the monitoring wells or until it has been determined that the water

has stabilized through the measurement of consistent pH values. Wells that are unable to yield three well volumes should be purged to dryness once. The rate at which the water is purged from the well should not cause the formation water to cascade down the sides of the screen, thus allowing this water to become agitated and induce a loss of volatile constituents/components.

The use of positive-gas-displacement, fluorocarbon resin bladder pumps, or fluorocarbon resin or stainless steel bailers to be used in the purging of monitoring-wells. If these purging devices are unavailable, peristaltic pumps, centrifugal pumps, and venturi pumps may be used.

All non-dedicated purging equipment must be decontaminated after each use. In addition, all purged water should be collected and screened for the presence of possible contaminants. If contaminants are detected, the purge water must be disposed of properly.

Through the observation of purging techniques used at RCRA facilities, recurring problems were noted. Several of the errors in purging techniques resulted from vague sampling and analysis plans. A description of the purging equipment was often missing from the sampling and analysis plan, thus, leaving room for unacceptable or different equipment to be used in the field. In addition, several sampling and analysis plans did not specify procedures to be used for low yield-wells. In this case, different well evacuation procedures and purge volumes were used during various sampling events possibly resulting in inconsistent sampling results.

Purge volumes were often miscalculated in the field. Erroneous purge volumes resulted from incorrect calculation procedures, or the use of an inaccurate total well depth. The measurement of the total well depth prior to purging was frequently neglected, and inaccurate total depths obtained from well logs were used. Furthermore, it was noted that the purge water was not always analyzed in the field for pH and specific conductance. These parameters should be measured throughout the purge process to verify the purging efficiency. The use of miscalculated purge volumes leads to inconsistent volumes of water purged between sampling events, thus creating potential problems with statistical comparisons of the analytical data.

It was observed that several facilities used dedicated bailers with glued joints as their purge equipment. While not recommended, these devices may be acceptable. However, a problem does arise when this equipment is allowed to hang in the well below the static water level between sampling events. In this case the solvents/organic within the glue can leach out into the sand-pack and formation water, and possibly affect future sample analyses. In addition, the integrity of the well material may be compromised due to the presence of the leached solvents.

Another common error observed involved the placement of the pump within the well during well evacuation. The device is often allowed to rest at the bottom of the well through out the purging process. In high-yield wells, this procedure may not ensure the total evacuation of the stagnant water, especially the water with in the upper portion of the well casing.

As mentioned above, the decontamination of all non-dedicated equipment is essential. It was noted during several Task Force inspections, that purge equipment was not decontaminated prior to or after use, thus allowing for cross-contamination between wells. It was also observed that purge-water is often discharged to the ground, and in close proximity to the well head. In areas with very permeable substrata, and a shallow water table , the discharged purge water can effect recharge rates. Furthermore, the purge water is often contaminated, and should be collected and disposed of in a safe manner so as not to compound the contamination problem at the facility.

SAMPLE COLLECTION

Techniques used to collect ground-water samples should be selected to minimize physical or chemical alteration of the samples during the withdrawal process. In order to reduce the likelihood of sample contamination, owner/operators should use only fluorocarbon resin or stainless steel sampling devices, and use dedicated samplers for each well. If dedicated equipment is not available, thorough decontamination procedures (as specified in the sampling and analysis plans) should be used to clean the sampler between sampling events, and blanks should be collected and analyzed to detect any cross-contamination. In any case, all sampling equipment should be constructed of inert material.

The sampling and analysis plans should include a preferred sample collection order, starting with those parameters that are most sensitive to volatilization. For referency the Technical Enforcement Guidance Document includes a preferred order of sample collection. Field parameters (temperature, pH, and specific conductance) should be measured and recorded immediately after sample collection.

Sample preservation is required for many chemical constituents and physicochemical parameters to be evaluated that are not chemically stable. The sample preservation techniques are intended to (1) retard biological action, (2) retard hydrolysis, and (3) reduce sorption effects. A discussion of appropriate sample preservation methods is included in Test Methods for Evaluating Solid Waste - Physical/Chemical Methods (SW-846), (Table II).

Ground-water samples collected for organic analysis should never be filtered. Samples that will be analyzed for metals should be split into two portions. One portion should be field filtered through a 0.45-micron membrane filter immediately after sample collection. This sample portion should then be preserved and analyzed for disolved metals. The other sample aliquot should be preserved and analyzed for total metals. The difference between the disolved and total metal fractions in the ground water may then be credited to the original metallic ion content of the particles and sorption of ions onto the aquifer materials.

Sample containers that are selected for the collection of ground water should be specified according to the analytical methodology to be used. Organic samples should be collected in glass bottles with polytetrafluoroehylene (PTFE)-lined screw caps. Samples to be analyzed for volatile compounds should be collected in 40 ml glass vials with PTFE-backed silicon septum screw caps. Extractable organic compounds should be collected in four 1-liter or one 1-gallon glass bottles with PTFE -lined caps.

High density 1-liter polyethylene bottles with solid polyethylene or polyethylene-line caps should be used to collect samples for metal analyses. Samples collected for ammonia and TOC analysis should be collected in 500-ml polyethylene bottles. All bottles and liners, new or used, should be decontaminated prior

to use. Sample containers that are recommended for each sample parameter are identified in SW-846 and illustrated in Table III.

All samples collected must be clearly labeled by the collector with proper identification for easy tracking in the laboratory and for use in verification or enforcement actions. Each sample should be assigned a unique number that is identifiable with the facility point of collection, type of analysis to be performed and reference to the sampling and analysis plan. Once samples are properly labeled, they should be packaged for shipment and sent to the appropriate laboratory for analysis. Metal or plastic coolers should be used as the outside shipping container for environmental samples. In addition to the sample bottles, each chest should be accompanied with ice packs or an equivalent to keep samples cooled to approximately 4 C while on route to the laboratory. All samples should be shipped according to applicable Department of Transportation regulations (49 CFR Parts 171-177).

The Task Force observed sampling techniques utilized at many RCRA facilities. Sampling and analysis plans often lack a sampling schedule, which is a necessary component to ensure sample consistency. Different sampling techniques used at different wells located at the same facility also contributes to inconsistent analysis data. As discussed above statistical comparisons of analyses require data collection to be consistent at the facility and between sampling events.

Sampling and analysis plans often neglect to specify a set order for sampling wells. It is recommended that monitoring wells suspected to be least contaminated (back-ground wells) be sampled first, and those wells more contaminated be sampled last. This sampling order minimizes the potential for cross-contamination, and is especially important when non-dedicated sampling equipment is being used. A specific order of sample collection was also absent from sampling and analysis plans. Volatile organic (VOA) should be the first samples collected, thus minimizing the volitization of the organics. Sample parameters least susceptible to volitization should be sampled last.

Samples collected prior to the evacuation of the stagnant water within the well casing can create

variation in the analytical results even if a specific sampling order for the monitoring wells was followed. The stagnant water will have been exposed to the well casing/screen materials as well as the air for varied time periods. Leaching of constituents from the well construction material and/or the absorption of constituents from the ground water onto the well components can alter the ground water chemistry from that of the actual insitu ground water. In addition, exposure of the stagnant ground water to the air within the well casing may increase oxidation reactions with the ground water and the existing contaminants/constituents.

Equipment used to collect ground-water samples can often impact the laboratory analysis. It was observed that facilities often use impeller driven pumps to withdraw ground water from the monitoring wells. These pumps have the capability to increase volitization of the sample and produce a pressure differential resulting in variability in the analysis of pH, specific conductivity, metals and volatile organics (Bacelona, et al., 1987).

It was observed that at some facilities vehicles were allowed to run in close proximity to the well during sampling procedures. The exhaust from the vehicle and debris thrown-up by the tires could become a source of contamination thus influencing the sample analysis. Incorrect sample preservation techniques may also effect the sample analysis. The measurement of the pH of the preserved sample is often overlooked in the field, and sample preservation is often incomplete.

In addition, sample equipment was not decontaminated between wells at all facilities. The reuse of such equipment without decontamination will allow for cross-contamination between wells, thus invalidating sample data.

QUALITY ASSURANCE/QUALITY CONTROL SAMPLES

Owners/operators must include in their sampling and analysis plans a scheme for qaulity assurance/quality control which accounts for the variations which were discussed above. Sampling and analysis plans should include a section on the preparation and analysis of several sets of quality control samples. There are two main categories of quality control samples: field blanks and duplicate samples.

Three types of quality control field blanks should be collected for every sampling event. These samples include trip blanks, field blanks, and equipment blanks. Trip blanks are prepared in the laboratory and are used to determine whether the sample bottles are introduced to contamination while on route to the facility. Trip blanks should be prepared by using distilled, deionized water of known high purity and sent with the other sample bottles to the site. One set of trip blanks should be prepared for each parameter to be sampled.

Field blanks are prepared to establish whether contamination is introduced to the ground-water samples from sample collection activities or the surrounding environment. These blanks should be prepared in the field by pouring distilled deionized water into the appropriate sample bottles for each parameter to be analyzed. The number of field blanks that should be collected will depend on the site conditions.

Equipment blanks are used to determine if the sampling equipment introduced any contaminants to the samples. Again the number of these blanks will depend on the sampling equipment used and field conditions. f contamination is found in the blanks, the source of the contamination should be identified and corrective action, including resampling, should be initiated.

Field duplicate samples are collected to evaluate the precision of the laboratory analyses of the ground-water samples. At least one full set of duplicate samples should be collected from a well at all sampling events. The Protocol for Ground-Water Evaluations prepared for the Hazardous Waste Ground-Water Task Force recommends that two wells be selected for duplicate samples when more than ten wells are sampled.

The collection of quality assurance/quality control samples was neglected at several facilities. Thus, the quality of the sampling procedures and laboratory analysis could not be determined at these facilities.

CHAIN-OF-CUSTODY RECORD

An adequate chain-of-custody program capable tracking the possession and handling of all samples collected from a facility from the time of collection to laboratory analysis must be described in the sampling and analysis plan. A chain-of-custody program should

include provisions for; sample labels, sample seals, field logbooks, chain-of-custody record, sample analysis request sheets, and laboratory logbook. Each sample shipment should be accompanied by a chain-of-custody record.

Upon review of sampling and analysis plans, it was observed that procedures for a chain-of-custody were frequently absent. The lack of a paper-trail of sample documentation often resulted in this situation. Possible litigation down the road would be greatly hindered with out such documentation.

Several "easy to fix" problems were observed with regard to the chain-of-custody procedures. Chain-of-custody's were not always signed by the field sampling team, and it was noted that laboratories do not consistently retain copies of the chain-of-custodies.

SUMMARY

Agency guidance should be reviewed prior to selecting a technique(s). Once techniques are selected, the method/equipment should be documented in detail in the facility sampling and analysis plan and the procedure layed out in a step by step fashion to ensure that it is performed correctly and consistently the field.
Extreme caution must be used in the choosing the sampling techniques that will produce a ground water sample representative of the aquifer system, thus ensuring compliance with the RCRA requirements.

As illustrated, many components of the sampling process can have an adverse effect on the data quality. It is important for laboratory personnel to compare the sampling and analysis plan with the field notes to obtain an understanding of the techniques used throughout the sample collection process. Any samples collected with suspect techniques that could bias the analytical results should be identified.

REFERENCES

Ground-Water Technical Enforcement Guidance Document. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, OSWER Dir. 9950.1, September, 1986.

Handbook: Ground Water. Office of Research and Development, U. S. Environmental Protection Agency, EPA/625/6-87/016, March 1987.

Protocol for Ground-Water Evaluations. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, OSWER Dir. 9080.0-1, September, 1986.

TABLE I

PREFERRED COLLECTION ORDER FOR
SOME COMMON GROUND-WATER PARAMETERS

- Volatile organics (VOA)
- Purgeable organic carbon (POC)
- Purgeable organic halogens (POX)
- Total organic halogens (TOX)
- Total organic carbon (TOC)
- Extractable organics
- Total metals
- Dissolved metals
- Phenols
- Cyanide
- Sulfate and chloride
- Turbidity
- Nitrate and ammonia
- Radionuclides

Reference: Ground-Water Technical Enforcement Guidance Document. OSWER Dir. 9950.1.

TABLE II

SAMPLE PRESERVATION METHODS

Parameter	Preservative
Indicators of Ground Water Contamination[a]	
pH	Field determined
Specific conductance	Field determined
TOC	Cool 4°C, HCl to pH <2
TOX	Cool 4°C, add 1 mL of 1.1M sodium sulfite
Ground Water Quality Characteristics	
Chloride	4°C
Iron Manganese Sodium	Field acidified to pH <2 with HNO_3
Phenols	4°C/H_2SO_4 to pH <2
Sulfate	Cool, 4°C
EPA Interim Drinking Water characteristics	
Arsenic Barium Cadmium Chromium Lead Mercury Selenium Silver	Total Metals Field acidified to pH <2 with HNO_3 Dissolved Metals 1. Field filtration (0.45 micron) 2. Acidify to pH <2 with HNO_3
Fluoride	Field acidified to pH <2 with HNO_3
Nitrate	4°C/H_2SO_4 to pH <2

(Continued)

TABLE II (Continued)

SAMPLE PRESERVATION METHODS

Parameter	Preservative
Endrin Lindane Methoxychlor Toxaphene 2,4 D 2,4,5 TP Silvex	Cool, 4°C
Radium Gross Alpha Gross Beta	Field acidified to pH <2 with HNO_3
Coliform bacteria	Cool, 4°C
Other Ground Water Characteristics of Interest	
Cyanide	Cool, 4°C, NaOH to pH >12
Oil and Grease	Cool, 4°C H_2SO_4 to pH <2
Semivolatile, volatile organics	Cool, 4°C

Reference: Test Methods for Evaluating Solid Waste - Physical/Chemical Methods. SW-846 (3rd edition, 1986).

TABLE III

RECOMMENDED SAMPLE CONTAINERS

Parameter	Recommended Container[a]
Indicators of Ground Water Contamination	
pH	T, P, G
Specific conductance	T, P, G
TOC	G, Teflon-lined cap
TOX	G, amber, Teflon-lined cap
Ground Water Quality Characteristics	
Chloride	T, P, G
Iron	T, P
Manganese	
Sodium	
Phenols	G
Sulfate	T, P, G
EPA Interim Drinking Water characteristics	
Arsenic	T, P
Barium	
Cadmium	
Chromium	
Lead	
Mercury	
Selenium	
Silver	Dark Bottle
Fluoride	T, P
Nitrate	T, P, G

TABLE III (Continued)

RECOMMENDED SAMPLE CONTAINERS

Parameter	Recommended Container[a]
Endrin	T, G
Lindane	
Methoxychlor	
Toxaphene	
2,4 D	
2,4,5 TP Silvex	
Radium	P, G
Gross Alpha	
Gross Beta	
Coliform bacteria	PP, G (sterilized)
Other Ground Water Characteristics of Interest	
Cyanide	P, G
Oil and Grease	G only
Semivolatile, volatile organics	T, G

[a] Container Types:
P = Plastic (polyethylene)
G = Glass
T = Teflon
PP = Polypropylene

Reference: Test Methods for Evaluating Solid Waste - Physical/Chemical Methods. SW-846 (3rd edition, 1986).

INORGANICS

VALIDATION OF METHODS FOR DETERMINING ELEMENTS IN SOLID WASTE BY MICROWAVE DIGESTION

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ABSTRACT

The techniques which are typically used to prepare RCRA wastes for analysis for metals and other elements are generally 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 decisionmaking. Given these concerns, the OSW Methods Section 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 Evaluation of Solid Waste SW-846" and made available to the user community.

This paper reports on the evaluation of a microwave assisted sample preparation method for determining elements in solid waste. The Method was evaluated for microwave digestion of sediments, sludges, soils, and oils.

INTRODUCTION

One particularly attractive sample preparation technique that is now receiving considerable attention is microwave assisted sample dissolution. A typical example of this application involves placing a sample in an acid solution in a closed vessel equipped with a pressure relief valve. The vessel is then subjected to microwave energy in a modified microwave oven. The conditions of high pressure gener-

ated in the container, coupled with the rapid heating of the sample via direct microwave energization of the acid molecules can result in significantly reduced preparation time; from several hours in a conventional convection oven, hot plate, or steam bath to several minutes in the microwave oven.

Previous work has reported on the evaluation of a commercially available microwave oven sample preparation system.[1] 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.

Based on in vessel temperature and pressure profile studies conducted by NBS, microwave oven preparation conditions for oils and soils have been determined and written as a draft method. These involve the use of concentrated nitric acid as the digestion medium. The intent is not to completely solubilize all elements in the sample. Rather, it is to solubilize those most likely to be made environmentally available.

This paper reports on the evaluation in a single laboratory of the draft method using NBS Standard Reference Material representative of oils and soils. Analyses were carried out by Inductively Coupled Plasma Spectrometry and Graphite Furnace Atomic Absorption.

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 MΩ/cm specific resistivity was used.

Standard Reference Materials

The microwave method evaluation was carried out using the following materials:

- NBS SRM 4355—Peruvian Soil
- NBS SRM 2704—Buffalo River Sediment
- NBS SRM 1085—Wear Metals in Oil
- NBS SRM 1634b—Trace Elements in Fuel Oil.

In addition, to simulate a contaminated soil a 1:1 mixture of 1634b and 2704 was prepared and analyzed.

Microwave Preparation Method

The method described below was developed for two vessels in the microwave oven and is optimized for temperatures and pressures that would produce efficient chemical decomposition of the sample. Essentially the same conditions could be utilized for six vessels.

A 0.25 g sample was heated in the microwave oven with 10 mL concentrated HNO_3 for ten minutes at a power setting of 344 watts. Two sample vessels at a time were placed in the microwave oven carousel with accompanying vapor trap vessels.

To reduce the likelihood of analyte loss when decomposing samples producing significant gas on decomposition, a configuration was employed using a second vessel to trap the hot acid vapor and any aerosol expelled when the pressure valve opens. A PFA Teflon tube connects the digestion vessel to a second vessel with a double-ported cap. The second port on the catch vessel remains open to the atmosphere preventing pressure buildup in the second vessel. The acid and any sample condensed in the second vessel is washed back into the

sample vessel at the end of the microwave procedure. The contents of the sample vessel are then analyzed. Ten replicates of each of the four NBS SRMS and the mixture were digested and analyzed.

Samples were analyzed for 19 elements by ICP. As and Se were determined by graphite furnace AA.

An initial weight of 0.5 g of Peruvian Soil was used for digestion. This was found to cause undue pressure buildup and venting within the sample and overflow vessel. For subsequent runs the sample weight was reduced to approximately 0.25 g. In addition, a tube was introduced, connecting the overflow vessel with the center well on the carousel to capture potential venting from the overflow vessel.

An additional study was done examining the overflow/capture solutions for any appreciable recoveries. Four replicates each of Peruvian soil and wear metals in oil were digested with the condensate collected and analyzed separately.

RESULTS

Microwave results for the two soil SRMs and the mixture were compared with results obtained using SW-346 method 3050 since the microwave method is a non-rigorous acid-leach digestion (Tables 1, 2, and 3) like 3050.

In general, good agreement was obtained between the two methods. For most elements comparative values were within 25 percent. Exceptions were Al and V in Peruvian Soil; Al, Ba, Be, and V in Buffalo River Sediment; and As and Be in the 1:1 mixture of Buffalo River Sediment and Fuel Oil. As predicted, using a non-rigorous digestion, the soil values were below the certified levels.

Graphite furnace analyses for As and Se were hindered by an apparent interference due to the high acid concentration of the digestate (approximately 20 percent). This was the apparent cause of a large erratic background signal. Buffalo River Sediment certification is not yet available.

Results for digestion of the two oil SRMs, along with analysis of spiked samples, are shown in Tables 4 through 7. In the case of

SRM 1085, excellent agreement was obtained with the NBS Certified Values (Table 4). All certified elemental concentrations were within 14 percent of NBS values. SRM 1634b, Trace Elements in Fuel Oil, is a more difficult material because of increased viscosity, a larger amount of aromatic compounds, and lower elemental levels. Certain of the metals, notably Al and Ca, were considerably higher than the Certified Values (Table 5). For this type matrix microwave digestion time might need to be increased to achieve complete decomposition. Spike recoveries for both oil SRMs were excellent (Tables 6 and 7) with the exception of As and Se (see above).

An examination of the overflow/capture vessels for any appreciable elemental recoveries produced no measurable concentrations. With the exception of Zn in Peruvian soil with a mean condensate concentration of 6.4 μg/g of soil, no recoveries above background level were observed for four replicates each of Peruvian Soil and Wear Metals in Oil. A small quantity of condensate was observed in four of the ten vessels.

CONCLUSIONS

Evaluation of a draft microwave digestion method for determining elements in solid waste indicates that this method should prove a suitable alternative for SW-846 method 3050 with a substanital time/cost savings and will provide satisfactory results for microwave digestions of oils.

In addition, using this method there should be no appreciable loss of volatile elements through pressure related venting.

Based on these results, a collaborative study will be conducted for final method validation.

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TABLE 1. ICP ANALYSIS OF NBS SRM 4355 PERUVIAN SOIL[a]

Element	Mean ± S.D. (n=10)	3050 (n=3)	% Bias	IAEA values
Al	2.12 ± 0.20 %	3.34 ± 0.33	-36	8%
As	46.4 ± 1.5[b]	51.6 ± 3.5[b]	-10	90
Ba	140 ± 7	182 ± 17	-23	600
Be	0.758 ± 0.039	0.959 ± 0.059	-21	2
Cd	1.91 ± 1.01	ND[c]		2
Ca	10,500 ± 700	11,000 ± 900	-4.5	2%
Cr	13.7 ± 3.0	13.0 ± 1.6	+5.4	30
Co	10.6 ± 0.8	10.4 ± 1.2	+1.9	10
Cu	64.5 ± 2.0	60.3 ± 3.9	+7.0	80
Fe	2.40 ± 0.16 %	2.80 ± 0.24 %	-17	4%
Pb	131 ± 10	149 ± 9	-12	100
Mg	7,250 ± 300	7,480 ± 640	-3.1	2%
Mn	531 ± 20	565 ± 39	-6.0	900
Mo	ND[d]	ND[d]		2
Ni	10.1 ± 2.6	10.3 ± 2.0	-1.9	10
Se	ND[b,e]	ND[b,e]		1
Ag	ND[f]	ND[f]		2
Sr	85.3 ± 4.9	112 ± 9	-24	300
V	65.3 ± 4.3	93.0 ± 8.3	-30	20
Zn	396 ± 23	356 ± 30	+11	400

[a]Results in μg/g.

[b]Determined by GFAA.

[c]D.L. 1.0 μg/g.

[d]D.L. 2.75 μg/g.

[e]D.L. 0.2 μg/g.

[f]D.L. 3.0 μg/g.

TABLE 2. ICP ANALYSIS OF NBS 2704 BUFFALO RIVER SEDIMENT[a]

Element	Mean ± S.D. (n=10)	3050 (n=3)	% Bias
Al	1.25 ± 0.08 %	2.50 ± 0.19	-50
As	11.6 ± 0.5[b]	12.8 ± 1.1[b]	-9.4
Ba	79.3 ± 3.4	132 ± 10	-40
Be	0.689 ± 0.110	1.05 ± 0.05	-34
Cd	ND[c]	ND[c]	
Ca	187 ± 1.04 %	1.88 ± 0.01 %	-0.5
Cr	69.4 ± 3.4	78.9 ± 2.9	-9.5
Co	9.42 ± 1.26	10.8 ± 0.5	-13
Cu	89.1 ± 3.6	88.5 ± 1.7	+0.7
Fe	2.91 ± 0.11 %	3.29 ± 0.07 %	-12
Pb	153 ± 19	169 ± 8	-9.5
Mg	7,990 ± 240	9,080 ± 150	-12
Mn	465 ± 15	486 ± 4	-4.3
Mo	ND[d]	ND[d]	
Ni	37.8 ± 3.2	41.8 ± 0.6	-9.6
Se	ND[b,e]	ND[b,e]	
Ag	ND[f]	ND[f]	
Sr	30.9 ± 2.8	41.4 ± 1.0	-25
V	25.1 ± 1.5	49.4 ± 2.8	-49
Zn	392 ± 19	403 ± 4	-2.7

[a]Results in μg/g.

[b]Determined by GFAA.

[c]D.L. 1.0 μg/g.

[d]D.L. 2.75 μg/g.

[e]D.L. 0.2 μg/g.

[f]D.L. 3.0 μg/g.

TABLE 3. ICP ANALYSIS OF 1:1 MIXTURE: NBS 2704--BUFFALO RIVER SEDIMENT NBS 1634b--TRACE ELEMENTS IN FUEL OIL[a]

Element	Mean ± S.D. (n=10)	3050 (n=3)	% Bias
Al	6,550 ± 680	8,720 ± 1,760	-25
As	5.52 ± 0.32[b]	3.84 ± 0.99[b]	+44
Ba	45.7 ± 4.0	58.2 ± 8.0	-21
Be	0.741 ± 0.110	0.559 ± 0.053	+32.6
Cd	ND[c]	ND[c]	
Ca	1.04 ± 0.08 %	1.18 ± 0.04 %	-12
Cr	41.1 ± 13.8	45.1 ± 2.8	-8.9
Co	ND[c]	5.99 ± 0.29	
Cu	42.1 ± 5.6	51.6 ± 2.9	-18
Fe	1.40 ± 0.08 %	1.66 ± 0.09 %	-16
Pb	72.1 ± 13.4	83.4 ± 5.4	-14
Mg	4,000 ± 310	4,650 ± 250	-14
Mn	225 ± 16	252 ± 11	-11
Mo	ND[d]	ND[d]	
Ni	26.6 ± 5.6	35.4 ± 1.4	-25
Se	ND[b,e]	ND[b,e]	
Ag	ND[f]	ND[f]	
Sr	ND	15.1 ± 1.6	
V	41.5 ± 1.9	47.1 ± 5.0	-12
Zn	211 ± 18	231 ± 8	-8.6

[a]Results in µg/g.

[b]Determined by GFAA.

[c]D.L. 1.0 µg/g.

[d]D.L. 2.75 µg/g.

[e]D.L. 0.2 µg/g.

[f]D.L. 3.0 µg/g.

TABLE 4. ICP ANALYSIS OF NBS SRM 1085 WEAR METALS IN LUBRICATING OIL[a]

Element	Mean ± S.D. (n=10)	NBS values	% Bias
Al	337 ± 22	296	+14
As	b	---	
Ba	0.928 ± 0.890	---	
Be	ND[c]	---	
Cd	2.07 ± 0.92	---	
Ca	67.3 ± 91.6	---	
Cr	310 ± 10	298	+4
Co	ND[d]	---	
Cu	316 ± 11	295	+7
Fe	320 ± 11	300	+7
Pb	305 ± 19	(305)	0
Mg	300 ± 15	297	+1
Mn	0.837 ± 0.325	---	
Mo	265 ± 9	292	-9
Ni	310 ± 11	303	+2
Se	b	---	
Ag	309 ± 27	(291)	+6
Sr	ND[d]	---	
V	ND[e]	(<0.3)	
Zn	8.12 ± 4.89	---	

( ) Not certified

[a]Results in μg/g.

[b]To be determined by GFAA.

[c]D.L. 0.6 μg/g.

[d]D.L. 2.4 μg/g.

[e]D.L. 1.6 μg/g.

TABLE 5. ICP ANALYSIS OF NBS SRM 1634b TRACE ELEMENTS IN FUEL OIL[a]

Element	Mean ± S.D. (n=10)	NBS values
Al	29.7 ± 8.1	16
As	ND[b,c]	0.12
Ba	3.68 ± 0.36	(1.3)
Be	0.220 ± 0.023	---
Cd	ND[d]	---
Ca	84.8 ± 10.3	(15)
Cr	2.54 ± 0.84	(0.7)
Co	ND[e]	0.32
Cu	ND[f]	---
Fe	38.7 ± 4.4	31.6
Pb	ND[g]	(2.8)
Mg	13.7 ± 1.6	---
Mn	0.492 ± 0.224	0.23
Mo	ND[h]	---
Ni	29.3 ± 3.0	28
Se	ND[b,c]	0.18
Ag	ND[i]	---
Sr	ND[j]	---
V	57.7 ± 2.2	54.4
Zn	3.28 ± 3.69	3.0

( ) Not certified

[a]Results in μg/g.

[b]Determined by GFAA.

[c]D.L. 0.4 μg/g.

[d]D.L. 0.9 μg/g.

[e]D.L. 2.4 μg/g.

[f]D.L. 3.6 μg/g.

[g]D.L. 9.2 μg/g.

[h]D.L. 10.0 μg/g.

[i]D.L. 4.8 μg/g.

[j]D.L. 3.0 μg/g.

TABLE 6. ICP ANALYSIS OF SPIKED NBS OILS SRM 1085--WEAR METALS IN LUBRICATING OIL[a]

Element	Expec.	Found	% Rec.	Unspiked concentration
Al	2.00	1.91	96	1.47
As	0.200	0.121[b]	60	ND
Ba	2.00	1.90	95	0.004
Be	2.00	1.93	96	ND
Cd	2.00	1.85	92	0.009
Ca	2.00	1.74	87	0.293
Cr	2.00	1.84	92	1.35
Co	2.00	1.93	96	ND
Cu	2.00	1.86	93	1.38
Fe	2.00	2.00	100	1.40
Pb	2.00	1.85	92	1.33
Mg	2.00	1.83	92	1.31
Mn	2.00	1.97	98	0.004
Ni	2.00	1.80	90	1.35
Se	0.200	0.120[b]	60	ND
Ag		c		
Sr	2.00	1.97	98	ND
V	2.00	1.94	97	ND
Zn	2.00	1.86	93	0.005

[a]Results in μg/mL.

[b]Determined by GFAA.

[c]Spike unsuccessful.

TABLE 7. ICP ANALYSIS OF SPIKED NBS OILS SRM 1634b--FUEL OIL[a]

Element	Expec.	Found	% Rec.	Unspiked concentration
Al	2.00	2.06	103	0.127
As		b		
Ba	2.00	1.84	92	0.016
Be	2.00	1.87	94	0.001
Cd	2.00	1.82	91	ND
Ca	2.00	1.77	88	0.364
Cr	2.00	1.89	94	0.011
Co	2.00	1.92	96	ND
Cu	2.00	1.88	94	ND
Fe	2.00	2.05	102	0.166
Pb	2.00	1.87	94	ND
Mg	2.00	1.90	95	0.059
Mn	2.00	1.94	97	0.002
Mo	2.00	1.77	88	ND
Ni	2.00	1.92	96	0.126
Se		b		
Ag		c		
Sr	2.00	2.02	101	ND
V	2.00	1.94	97	0.247
Zn	2.00	1.84	92	ND

[a]Results in μg/mL.

[b]Not spiked.

[c]Spike unsuccessful.

DEVELOPMENT OF A TOXIC GAS GENERATION REACTIVITY METHOD

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ABSTRACT

A reactivity method for cyanide and sulfide was designed, developed, and tested. Ideally, the method was to mimic a worse-case mismanagement scenario where a truckload of cyanide or sulfide containing waste is discharged into an acid pit. The experimental design for method development and testing was based on (a) discussions and recommendations of the OSW Task Manager and an industrially-sponsored Reactivity Discussion Group; (b) results of a literature review, (c) preliminary results from chamber experiments; and (d) practical constraints associated with a laboratory test method.

In the method, a 20 g aliquot of the waste sample is added to a buffered (pH 4), stirred, aqueous solution. The gas generated is swept with nitrogen into an impinger. The emission rate and total amount of hydrogen cyanide and hydrogen sulfide emitted are quantified by analysis of the impinger solution using Methods 9010 and 9030, respectively.

The method was tested for ruggedness using deionized water samples fortified with cyanide ion. The flow rate and the buffer strength were found to have no significant effect on the emission rate, but stirring rate has a profound effect.

VALIDATION OF ANALYTICAL METHODS FOR DETERMINING TOTAL CHLORINE IN USED OILS AND OIL FUELS

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ABSTRACT

A current EPA regulation prohibits the sale for burning in nonindustrial boilers of used oils and oil fuels contaminated above specified levels with certain metals and total chlorine (the "Burn Ban" Regulation). 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 the 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. One means of establishing the chlorine content of the oil is to test it for total chlorine.

To provide regulatory agencies and the regulated community with accurate, cost effective methods for determining chlorine in used oil, EPA/OSW has evaluated several analytical techniques, prepared written test methods for the most promising and subjected these to a collaborative study to generate precision and bias statements for each method.

The methods evaluated were based on microcoulometric titration, X-ray fluorescence spectrometry, two test kits, and five analytical finishes to oxygen bomb combustion.

The results of this collaborative study are reported here. More than 70 laboratories representing generators, collectors, recyclers, burners, and regulatory agencies participated. The study was a cooperative effort with ASTM Committee D2 on Petroleum Products and Lubricants. Based on this study, final versions of the test methods will be proposed for inclusion in the ASTM Book of Standards and the Federal Register as acceptable for compliance with the "Burn Ban" Regulation.

DEVELOPMENT OF ICP-MS METHOD 6020

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ABSTRACT

Inductively coupled plasma mass spectrometry (ICP-MS) offers multi-elemental analysis with simplified spectra and with most detection limits below 1 ppb. The simplified spectra of ICP-MS in comparison to inductively coupled plasma atomic emission spectrometry reduces the potential for interferences. The low detection limits in combination with the multi-elemental capacity provide an especially attractive alternative to the slow, single-element procedure involved in conventional furnace atomic absorption spectroscopy. The method development has involved preparation of a draft version, reviews by users and manufacturers of both types of commercial instruments, criteria development, method revision, and performance testing. Although the ICP-MS technique also offers a more convenient way than thermal ionization mass spectrometry to determine stable isotope ratios and to quantify some elements by the isotope-dilution procedure, these features are not considered here.

INTRODUCTION

In preparing the first version of ICP-MS Method 6020, there were two major concerns: (1) the need to obtain data of known quality, and (2) a desire to avoid excessive quality-control requirements. The latter can be considered a costbenefit issue. The quality-control requirements were made conditional on some specific needs. In ICP-MS there is a physical extraction of ions from the argon plasma into the mass spectrometer. Consequently, the mass spectrometer receives ions that exist in the plasma plus any ion clusters that occur during the extraction process. At any given instant during an analysis, the mass spectrometer is only measuring ions with a single mass-charge (m/z) ratio value. Since there are five potential sources for ions at any m/z value, more information than the ion-count rate at a single m/z value is needed to obtain data of known quality. The five potential sources for ions at any m/z value are (1) singly charged ions of an analyte element, (2) singly charged ions of another element, (3) doubly charged ions of an element with twice the mass of the analyte element, (4) polyatomic ions such as chloride, hydroxide and oxide species, and (5) ions from a huge signal at an adjacent m/z value. For example, measuring for cadmium at m/z 112, 114, or 116 without any measurement for the tin level in a sample results in cadmium data of unknown quality. Measuring for cadmium using an isotope that is not affected by tin ions avoids this problem, but not that of molybdenum oxide ions on seven of the eight cadmium isotopes. Since the cadmium isotope that is not affected by a molybdenum oxide only has an abundance near 0.9%, it is not present in sufficient quantity to be useful for trace analyses. While the pattern of

isotope proportions for an element can provide support for the presence of an element, this type of information is not definitive evidence as illustrated by the fact that the molybdenum oxides mimic the cadmium isotope pattern.

When the molybdenum level in a sample is low, the contributions of molybdenum oxide ions to the cadmium signals are not of measurable consequence and there is no need for a correction on the cadmium data. While a 100-ppm Mo concentration can cause an actual Cd concentration of 20 ppb appear to be 80 ppb (400% high), a 1-ppm Mo concentration will only have a 3% effect on 20 ppb Cd. In the case of an oxide interference on cadmium by molybdenum, the amount of quality control is conditional on evidence that the molybdenum level is sufficient to justify a correction (such as 10% or 15%) for the oxide effect with the associated verification that the correction is accurate. Fred Lichte et al. at the U.S. Geological Survey found the oxide level to vary during an analysis session and recommend use of the ThO/Th ratio as an oxide internal standard when an oxide correction is applied (1).

RESULTS AND DISCUSSION

The attractive detection limits offered by ICP-MS for the elements determined in the Contract Laboratory Program (CLP) are shown in Table 1. While ICP-AES detection limits do not meet the required limits for 5 elements, ICP-MS meets the limits for all the elements listed. However, the mercury detection limit by ICP-MS is near the CRDL, and mercury exhibits severe instrumental memory in ICP-MS and in other nebulizer systems.

Elemental internal standards are not commonly used for ICP-AES, but the signal variation in ICP-MS is such that an appropriate internal standard for each analyte is more of a necessity than a luxury. The benefit in precision when internal standards are used to normalize analyte signals is shown in Figures 1 and 2. Internal standards are useful for both instrumental variations and for physical interferences. Physical interferences are effects associated with the sample nebulization and transport processes as well as ion-transmission efficiencies. Changes in matrix composition can cause significant suppressions and enhancements (2). Dissolved-solid levels can contribute deposits on the nebulizer tip and on the interface cones (reducing the orifice sizes and the instrument performance). To minimize solid deposition, total solid levels below 0.2 percent (2,000 ppm) have been recommended (3). Physical interferences are mitigated when the internal standard and the analyte signals are affected equally. Acceptable internal standards in order of increasing mass are ^{6}Li, Sc, Y, Rh, In, Tb, Ho, Au and Bi. The predominant isotope of Li in nature is ^{7}Li. Consequently, the use of ^{6}Li as an internal standard minimizes the influence of any endogeneous Li in samples. When an appropriate element is used as the internal standard for an analyte, the use of the method of additions will not improve the data quality because the internal standard signal and the analyte signal are altered similarly.

As mentioned above, more than one element may form singly charged ions with the same mass/charge ratio. Since the natural abundances of the isotopes are well known, corrections can be readily applied by the instrument software, which involves the system determining the signal for another isotope of the interfering element and subtracting the appropriate fraction from the signal that

contains the isobaric interference. In some instances, a secondary correction is applied to compensate for interference on the primary correction peak. The coauthors of this report have extended Method 6020 by adding equations for the elemental corrections and by listing the potential molecular-ion interferents.

Among the quality control objectives was development of one or more interference check solutions, which would demonstrate the magnitude of interferences and provide an adequate test of any corrections for interferences such as the chloride species ClO^+ on $^{51}V^+$ and $ArCl^+$ on $^{75}As^+$, the contribution of an ion on an adjacent m/z value, and metal oxide and carbon contributions. The coauthors have developed two solutions that include 1000 mg/l chloride and carbon, 500 mg/l Fe (for any contribution on adjacent m/z for Mn) and 10 mg/l Mo (for oxide contributions on cadmium isotopes) with and without analytes. To provide some perspective on these interferences, 600 mg/L chloride contributes only about 4 ppb apparent ^{51}V and about 1 ppb apparent ^{75}As.

Table 1. Estimated ICP-MS and ICP-AES Detection Limits for CLP Elements

Element	CRDL*	Estimated Detection Limit (ug/L) ICP-MS	ICP-AES
Aluminum	200	0.1	45
Antimony	60	0.02	32
Arsenic	10	0.4	53†
Barium	200	0.02	2
Beryllium	5	0.1	0.3
Cadmium	5	0.07	4
Calcium	5000	10.0	10
Chromium	10	0.02	7
Cobalt	50	0.01	7
Copper	25	0.03	6
Iron	100	0.2	7
Lead	5	0.02	42†
Magnesium	5000	0.10	30
Manganese	15	0.04	2
Mercury	0.2	0.08	17†
Nickel	40	0.03	15
Potassium	5000	1000.0	Variable
Selenium	5	1.0	75†
Silver	10	0.04	7
Sodium	5000	0.06	29
Thallium	10	0.05	40†
Vanadium	50	0.03	8
Zinc	20	0.08	2

*Contract Required Detection Limit
†Detection limit does not meet CRDL

The coauthors have observed that the plasma interface in ICP-MS can contribute to instrumental memory between samples by providing a surface for deposition and evaporation of analytes. When a low-concentration sample follows a high-

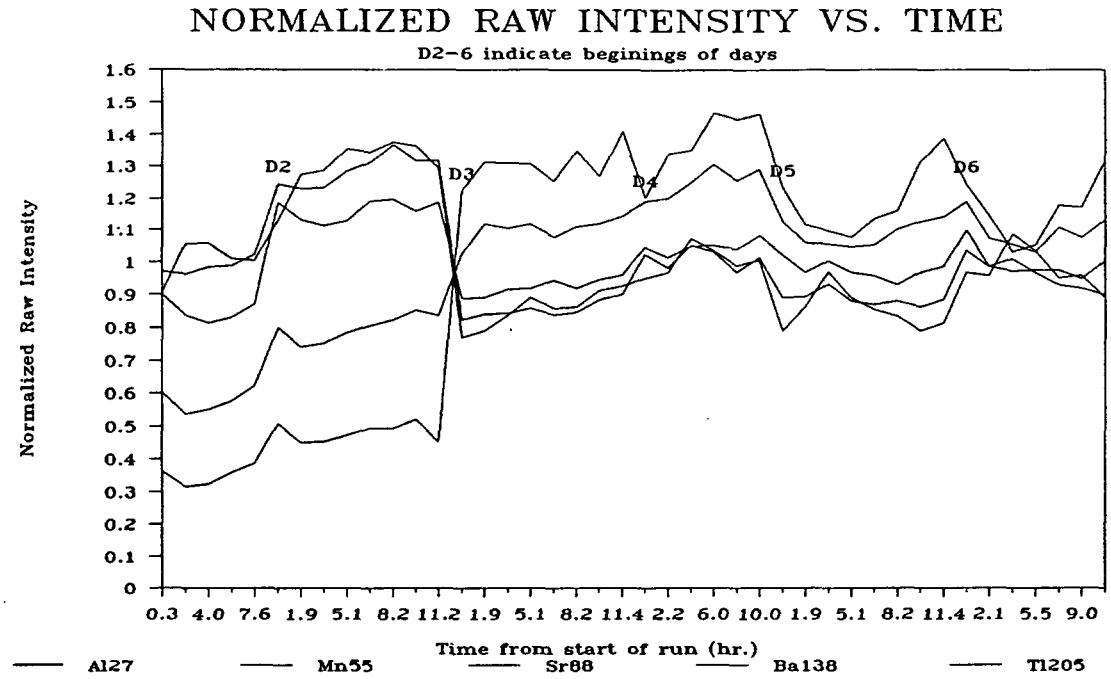

Figure 1. ICP-MS signal variation for 5 ions without internal standards.

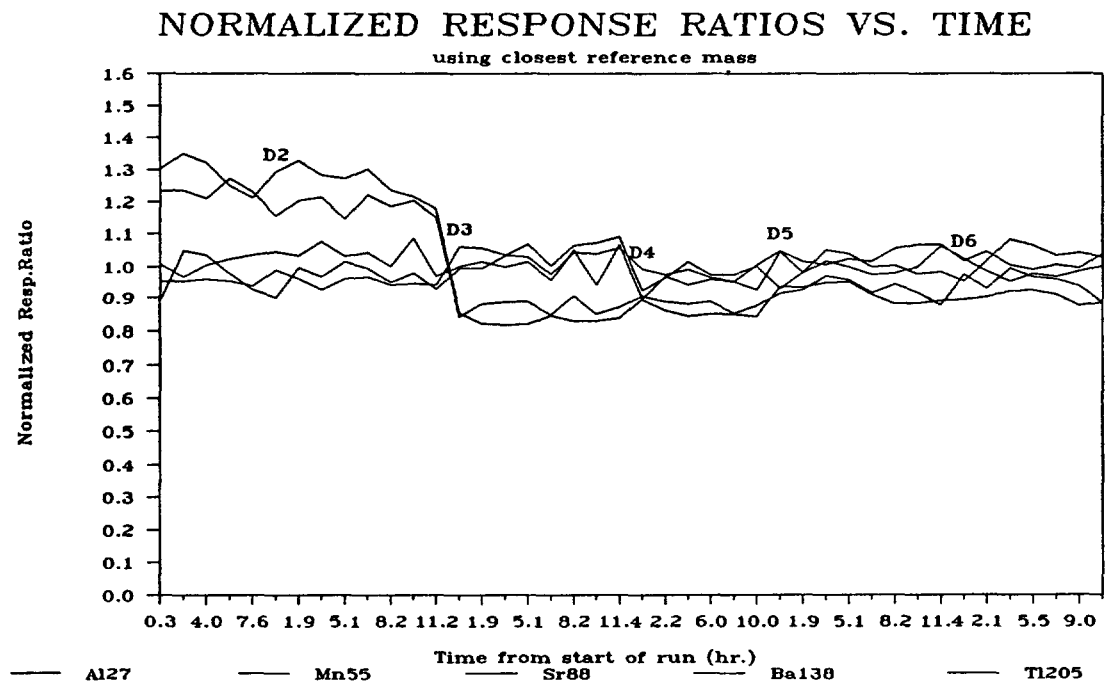

Figure 2. ICP-MS signal variation for 5 ions with internal standards.

concentration sample, the data quality can be affected by memory from the previous sample. This problem can be avoided by using a prolonged rinsing period between samples and verified as absent by measuring the rinse solution, but these procedures decrease the productivity of the method to address what could be a rare situation. An alternative that could impact productivity less while still providing data of known quality would be to develop a macro program that allows the computer to inspect the data (obtained with a typical rinse period between samples) using memory equations to determine (and flag) any analyte data affected more than 5 or 10 percent by memory. The flagged samples could be corrected for the memory (if reliable) or reanalyzed for the affected analytes when not preceded by a high-concentration sample.

Sample preparation procedures for this ICP-MS method have received considerable attention by members of the ICP-MS work group, especially Ted Martin at EMSL-CIN. While chloride ions at sufficient concentration aid the solubility of elements such as Ag and Sb, chloride ions contribute to interferences in ICP-MS, as noted above. The latest version of Method 6020 contains alternate preparation procedures for liquids and solids that involve hydrochloric and nitric acids when Ag and Sb are of special concern and involve nitric acid and hydrogen peroxide when the other elements are of more concern. When the Ag or Sb concentrations appear to be limited by the chloride level in a sample, the hydrochloric acid digestion procedures can be used. Since bromide ions could provide the solubility benefit provided by chloride ions while not contributing to chloride interferences in ICP-MS, use of hydrobromic acid instead of hydrochloric acid could be preferred. Any BrO^+ ions formed could affect two Mo isotopes, but Mo is not on the CLP list. Any $ArBr^+$ ions formed could affect one isotope of Sn and one of Sb. Hydroiodic acid is another alternative to hydrochloric acid.

Besides the typical quality control of initial and continuing calibration verification, reagent blanks, serial dilution and spike tests, duplicates, and laboratory control samples, an instrumental tuning solution consisting of 100 ug/L Be, Co, Sb and Tl has been developed for verifying instrument stability (10% criterion), for checking and adjusting mass calibration (0.1-amu criterion), and for checking and adjusting resolution (criterion of less than 1.0-amu full width at 10 percent of peak height maximum).

An interlaboratory study involving 10 instruments (2 brands) and 33 samples is underway, and a report is expected to be available by October 1988.

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DETERMINATION OF BERYLLIUM IN LIQUID AND SOLID WASTE SAMPLES BY USEPA METHODS 7090/7091, FLAME AND FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY

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ABSTRACT

The application of flame and furnace atomic absorption spectrophotometry (AAS) to the determination of beryllium in digests of liquid and solid samples demonstrates that the USEPA Methods 7090/7091 are sensitive, precise, and accurate for the quantification of this element. The instrument detection limits for flame and furnace AAS were found to be 0.003 mg/kg and 0.08 µg/L respectively. Aluminum was found to be the only element, of five investigated, that interfered with the determination of beryllium by flame AAS. The addition of 0.1 percent hydrofluoric acid eliminated this interference for aluminum concentrations up to 1000 mg/L. Only very high concentrations of molybdenum (1000 mg/L) interfered with the determination of beryllium by furnace AAS when peak area was used to calculate the concentration data.

INTRODUCTION

Beryllium, a possible component of hazardous chemical wastes, is one of the 23 inorganic elements targeted by the United States Environmental Protection Agency's (USEPA) Superfund program and one of the 27 metals of concern to the USEPA's Resource Conservation and Recovery Act (RCRA) program. While the Superfund's analytical methodology utilizes inductively coupled plasma instrumentation for the determination of beryllium (1), the RCRA analytical procedures include the determination of beryllium by flame and furnace atomic absorption spectrophotometry (AAS). These methods are included in the USEPA publication SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, as "Method 7090 (Beryllium, Atomic Absorption, Direct Aspiration)" and "Method 7091 (Beryllium, Atomic Absorption, Furnace Technique)" (2).

The objective of this study was to examine the application of these two beryllium detection methods to the analysis of selected liquid and solid samples after digestion by appropriate USEPA methods (2). Data were collected following the procedures outlined in Guidelines for Selection and Validation of U.S. EPA's Measurement Methods (3) and Test Methods for Evaluating Solid Waste, SW-846 (2) for the following parameters: detection limits, optimum concentration ranges, spike recoveries, interferences, precision, accuracy.

Instrument detection limits and the optimum concentration ranges were determined in aqueous samples that were free from interferences [2 percent (v/v) HNO_3]. Method detection limits, spike recoveries, precision, and accuracy were determined in digests of each of 12 target samples.

The twelve target samples that were chosen for this study are listed in Table I. Five of these were liquid samples, of which two were U.S. National Bureau of Standards - Standard Reference Materials (NBS-SRMs). Seven of the target samples

were solids, including two NBS-SRMs, two Canadian Certified Reference Materials, and one USEPA-Contract Laboratory Program Reference Material.

A literature survey revealed a number of reports of elements whose presence enhanced or suppressed the absorbance of beryllium in solution (4-15). Therefore, the individual effects of Al, Mg, and Si on the determination of beryllium by flame AAS (Method 7090) and of Al, Ca, Ce, Cr, La, Mg, Mn, Mo, Si, Sr, H_2SO_4, W, and Fe on beryllium determination by furnace AAS (Method 7091) were investigated. Hydrofluoric acid and 8-hydroxyquinoline have been reported to eliminate some of the interferences (5-7). The usefulness of these chemicals as matrix modifiers was also investigated.

EXPERIMENTAL

Reagents

A 1000 mg/L beryllium plasma standard, Spex lot 0860d, was used to prepare the beryllium standards and spiking solutions. The desired concentrations of interfering elements were obtained by using appropriate dilutions of 10,000 mg/L Spex standards. Ultrapure Reagent grade (Ultrex, J.T. Baker Chemical Co.) HNO_3, HCl, and Analytical Reagent grade (Mallinckrodt) H_2O_2 were used in digesting the samples and in preparing the analytical solutions. Reagent grade hydrofluoric acid (Mallinckrodt) and 8-hydroxyquinoline (Eastman Kodak) were used as matrix modifiers to combat the interference from aluminum in flame AAS analysis.

Sample Preparation

Since the analytical methods are for the analysis of aqueous samples, all samples were digested prior to analysis according to EPA procedures specified in SW-846 (2). Liquid samples were digested, using acid treatment, by Method 3010 for analysis by flame AAS or by Method 3020 for analysis by furnace AAS. All solid samples were digested with HNO_3 and HCl by Method 3050 (2).

Flame AAS Analysis (Method 7090)

A Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer, fitted with a nitrous oxide-acetylene burner head, AS-50 Auto Sampler and a hollow-cathode beryllium lamp was used to obtain data for Method 7090. The operating conditions are listed below:

wavelength:	234.9 nm
slit:	high, 0.7 nm
flame:	nitrous oxide-acetylene (red, reducing)
flame red zone:	ca. 3 cm
lamp current:	30 ma
background correction:	D_2 lamp

Graphite Furnace AAS Analysis (Method 7091)

A Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer, fitted with a Model HGA-500 Graphite Furnace/Programmer, AS-50 Auto Sampler, and a Data System 10, was used to obtain data for Method 7091. Pyrolytically coated platforms were

used for all furnace AAS analyses. The temperature program for the furnace is listed below:

Step	Temperature (deg. C)	Ramp (s)	Hold (s)
Dry	200	5	25
Ash	900	5	25
Atomize	2700	0	4
Clean	2700	1	1
Cool	50	1	30

During the atomization step, the argon carrier gas flow rate was reduced to 0 mL/min (stopped-flow conditions).

RESULTS AND DISCUSSION

Detection Limits

The instrument detection limit (IDL) is defined in SW-846 (2) as three times the standard deviation of seven consecutive measurements of the signal from a reagent blank, or

$$IDL = KS_B$$

where: $K = 3$

S_B = the standard deviation of the average instrument noise

For this study, a two percent (v/v) HNO_3 solution, free from interferences, was used as the reagent blank. When absorbance readings were used to calculate S_B for the flame AAS method, the IDL was found to be 9.0 µg/L. However, when concentration readings were obtained in the scale expansion mode and used to calculate S_B, the IDL was found to be 3.3 µg/L. The lower IDL results merely from the greater number of significant figures in the scale expansion mode. Only concentration readings were used to calculate S_B for the furnace AAS method. The IDL for the furnace method, using a platform, was found to be 0.08 µg/L.

The method detection limit (MDL) is defined as the minimum concentration of analyte that can be measured in a sample which has been processed through the preparative procedure (2). The MDL for this study was derived from the 12 target sample digests by the following relationship:

$$MDL = KS_B/m$$

where: $KS_B = IDL$

m = the slope of the calibration line in the sample digests

The calibration lines were obtained from determinations of beryllium in the unspiked sample digests and three portions of the digests to which standard additions of beryllium were added. The MDL for beryllium by flame AAS using the scale expansion mode averaged 5.2 µg/L. The MDL of 5.2 µg/L is somewhat higher than the IDL, 3.3 µg/L, possibly due to a small amount of signal

attenuation from the sample matrices. The MDL for beryllium by furnace AAS averaged 0.08 μg/L. MDL data is summarized in Table II for Method 7090 (flame AAS) and in Table III for Method 7091 (furnace AAS).

Optimum Concentration Ranges

The range over which the relationship between absorbance and concentration is linear is termed the optimum concentration range. Two sets of standard solutions of beryllium (one for flame AAS analysis and one for furnace AAS analysis) were prepared in two percent (v/v) HNO_3. After each set of standards was analyzed, a simple linear regression of the measured absorbance versus the known concentrations was performed, using values obtained from a reagent blank and two standards known to be within the linear range of the instrument. The estimators for y-intercept ($\hat{B}_o$) and slope ($\hat{B}_1$) obtained from each regression were used to calculate the projected absorbance values ($\hat{Y}_i$), where $\hat{Y}_i = \hat{B}_o + \hat{B}_1 X_i$. The point at which the measured absorbance differed from the predicted absorbance by more than five percent for a particular concentration was chosen to be the upper limit of the optimum concentration range.

The linear range for beryllium determination by flame AAS was found to extend from 0 mg/L up to 2.6 mg/L. The linear range for beryllium determination by furnace AAS was found to extend from 0 μg/L to 8.5 μg/L if peak height is used, and from 0 μg/L to 16 μg/L using peak area.

Interferences

Aluminum, magnesium, and silicon have been reported to interfere with beryllium determination by flame AAS (4-8). However, these reports were published before background correction was in general use. The effects of these three elements on the absorbance of a 0.5 mg/L beryllium solution were measured separately for each element at concentrations of 100 mg/L, 500 mg/L, 1000 mg/L, and 5000 mg/L with deuterium background correction. Calcium and sodium were also tested since they are present in most environmental samples at significant levels. Aluminum was observed to have a severe suppressive effect on beryllium absorbance. A concentration of only 100 mg/L caused a greater than 10% reduction of the signal, and 5000 mg/L decreased beryllium absorbance by more than 50%. Magnesium, sodium, and calcium caused a slight enhancement of the signal (less than 10% at 5000 mg/L). Silicon suppressed beryllium absorbance by 10%, but only at a concentration of 5000 mg/L. Interference data for the flame AAS method are presented in Table IV and Figure 1.

Efforts to correct for the suppression of the beryllium signal due to aluminum have included the use of 0.15% sodium fluoride (5), 0.3 M hydrofluoric acid (6), and 2.5% 8-hydroxyquinoline (7). The effectiveness of a 0.1% addition of hydrofluoric acid as recommended in Method 7090 (2) to correct for aluminum interference was tested. As shown in Table V, 0.1% HF is effective for aluminum concentrations up to 1000 mg/L. Increasing the HF addition to 0.3 % was necessary to correct the suppression at aluminum concentrations above 1000 mg/L (up to 5000 mg/L). The use of 2.5% 8-hydroxyquinoline was investigated using a 0.5 mg/L beryllium solution containing 1000 mg/L aluminum. Both 0.1% HF and 2.5% 8-hydroxyquinoline were found to be equally effective at aluminum

concentrations up to 1000 mg/L. Data are compared in Table V. The use of HF in particular was found to be convenient and inexpensive.

Some of the environmental samples that may be analyzed by flame AAS may contain high levels of calcium as well as aluminum. There was concern that the addition of HF to sample digests would result in the formation of CaF_2, with possible coprecipitation of beryllium (9). In a 0.5 mg/L solution of beryllium containing 5000 mg/L calcium, the addition of 0.1% HF resulted in the formation of a visible precipitate and an apparent 33% decrease in the beryllium concentration; increasing the HF addition to 0.3% increased the amount of precipitate and reduced the concentration reading by 90%. A series of solutions was prepared, each containing 0.5 mg/L beryllium and varying amounts of aluminum and calcium. After HF was added, it was noted whether a visible precipitate formed in any of the solutions and the beryllium concentration was measured. The data and observations are presented in Table VI. In general, where the calcium concentration exceeded the aluminum concentration by a factor of ten or more, the addition of HF resulted in formation of a precipitate and a significant reduction in the apparent beryllium concentration. This can most likely be attributed to coprecipitation of beryllium by CaF_2.

Many elements are reported to interfere with the determination of beryllium by furnace AAS (10-15). Therefore, the effects of the following elements on beryllium determination by furnace AAS were investigated: Al, Ca, Ce, Cr, La, Mg, Mn, Mo, Si, Sr, H_2SO_4, and W. Each of these elements was tested individually at concentrations of 100 mg/L, 500 mg/L, 1000 mg/L, and 5000 mg/L in solution with beryllium at a concentration of 5 μg/L. Iron and copper were also tested; iron is a major component of soils, and copper is the major element in one of the reference materials used for this study (NBS-SRM No. C1123, the copper-beryllium alloy).

When peak height was used, the interferences from many of the elements were pronounced (Table VII, Figures 2 and 3). For example, at 1000 mg/L, the following effects on the beryllium signal were observed: Al (+11%), Ca (+77%), Ce (+54%), Cr (+38%), La (+111%), Mg (+97%), Mn (+6%), Mo (+18%), Si (+5%), Sr (+61%), H_2SO_4 (+6%), W (-12%), and Fe (+27%). These large effects are in sharp contrast to the results where peak area was used to calculate the beryllium concentration. In the case of magnesium (97% enhancement of the signal), it is possible that MgO interacts with the sites on the platform where heats of adsorption are highest, causing more efficient release of beryllium during atomization from sites that have lower heats of adsorption (16). The enhancement due to calcium (77%) may result from a similar action.

The results of the furnace AAS interference studies are presented in Table VII for data based on peak area, and on peak height. When the data were collected using peak area, only the presence of Mo or W interfered with the determination of beryllium, as shown in Figures 4 and 5. When molybdenum is present at concentrations greater than 1000 mg/L, the absorbance of beryllium was greatly enhanced, approximately 20 percent at 1000 mg/L and 90 percent at 5000 mg/L. While the effect of tungsten was small, there was a 13 percent decrease in the beryllium absorbance when the tungsten concentration was increased from 0 mg/L to 100 mg/L, and another 11 percent drop when the tungsten concentration is increased from 100 mg/L to 5000 mg/L. This may be due to the formation of tungsten carbide.

The interferences that result when peak height is used are apparently due to a change in peak shape relative to the calibration standard that was made up in two percent (v/v) HNO_3. The effect of calcium on the shape of the beryllium peak is illustrated in Figure 6. To avoid positive bias, the use of peak area is highly recommended.

Spike Recoveries

Prior to digestion, two portions of each of the 12 target samples to be analyzed by flame AAS were spiked with enough beryllium to produce concentrations of 0.25 mg/L and 0.50 mg/L above the levels leached from each sample into the final digests. Spike recovery data are listed in Table VIII. Spike recoveries below 100 percent may be due to physical loss of beryllium during the digestion procedure or the presence of interfering elements, such as aluminum, in the sample matrix. Spike recoveries above 100 percent may be caused by the presence of elements which enhance the beryllium signal. The addition of 0.1 percent HF increased spike recovery to nearly 100 percent for most of the samples with the exception of the Mineral Sample and the Municipal Digested Sludge (Table VIII). Increasing the HF concentration to 0.3 percent increased the spike recoveries in these samples to 95 percent and 100 percent, respectively. The effect of HF on improvement of the spike recoveries may be related to the concentration of aluminum in the samples.

Spikes equivalent to one and two times the endogenous levels of beryllium were added to portions of the samples to be analyzed by furnace AAS prior to digestion. Spike recovery data are listed in Table IX. Overall, the spike recoveries are very good, which indicates that the determination of beryllium using peak area is not affected by the presence of the other elements in the sample matrices.

Precision

Precision was determined using three replicate concentration measurements for each sample. The percent relative standard deviation (%RSD) was used as an estimate of precision. Precision as a function of increasing beryllium concentration was determined in each of the 12 sample digests for flame AAS and furnace AAS. Portions of the digests were spiked with standard additions of beryllium within the optimum concentration ranges established for the two methods. The %RSDs for the flame method ranged from about four percent for concentrations as low as 0.016 mg/L to one percent at concentrations above 0.2 mg/L. For the furnace method, the %RSDs were approximately 10 percent for beryllium concentrations below 3 μg/L and less than one percent for concentrations above 3 μg/L.

Accuracy

The accuracy for Method 7090 and Method 7091 was determined by analyzing three NBS-SRMs, both before and after digestion, and comparing the measured beryllium concentration with the certified values. As shown in Table X, both the flame method and the furnace method for beryllium determination yield very accurate results.

CONCLUSION

In summary, USEPA Methods 7090 (flame AAS) and 7091 (graphite furnace AAS) are sensitive, precise, and accurate for the determination of beryllium in a variety of sample matrices. In addition, they are subject to few interferences. Suppression of beryllium absorbance in the nitrous oxide-acetylene flame due to the presence of aluminum can be eliminated by the addition of 0.1-0.3% hydrofluoric acid; 2.5% 8-hydroxyquinoline may be used as an alternative. When peak area is used to calculate the beryllium concentration of samples analyzed by the furnace method, only molybdenum and tungsten interfere.

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TABLE 1. TARGET SAMPLES

LIQUIDS

1. NBS-SRM No. 1643b, Trace Elements in Water
2. NBS-SRM No. 2127-2, Beryllium Spectrometric soln.
3. A synthetic interference solution, containing beryllium and 500 mg/L concentrations of Al, Ca, Fe, and Mg
4. A "natural waste water" prepared by contacting double-deionized water (adjusted to pH 5) with French geostandard mineral # MA-N
5. A TCLP extract of soil from a U.S. EPA hazardous waste site

SOLIDS

1. NBS-SRM No. 1633a, Coal Fly Ash
2. U.S. EPA Municipal Digested Sludge # 0319
3. UNLV-QAL/EPA - CLP Reference Materials -Three Kids Mine
4. Certified Reference Material No. MESS-1, Marine Sediment (Canada)
5. Certified Reference Material No. SY-2, Syenite Rock (Canada)
6. Soil from a U.S. EPA hazardous waste site
7. NBS-SRM No. C1123, Copper-Beryllium Alloy

TABLE II. METHOD DETECTION LIMIT FOR THE DETERMINATION OF BERYLLIUM BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY, METHOD 7090

Matrix	slope [a]	MDL[a] (μg/L)
Trace Elements in Water	0.92	4.4
Beryllium Spectrometric Solution	0.90	4.4
Synthetic Interference Solution	0.54	7.4
Natural Waste Water	0.90	4.5
TCLP Extract of Hazardous Waste	0.94	4.0
Coal Fly Ash	0.77	5.2
Municipal Digested Sludge	0.73	5.4
Three Kids Mine Material	0.64	6.3
Marine Sediment	0.74	5.4
Mineral Sample	0.68	5.9
Hazardous Waste Site Soil	0.73	5.5
Copper-Beryllium Alloy	0.90	4.4
Average	0.78	5.2

[a] The method detection limit is defined by MDL = 3 S_B/m, where S_B is the standard deviation of the instrument background signal and m is the slope of the calibration line in the sample matrix.

TABLE III. METHOD DETECTION LIMIT FOR THE DETERMINATION OF BERYLLIUM BY FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY, METHOD 7091

Matrix	Slope	MDL(μg/L)[a]
Trace Elements in Water	0.99	0.08
Beryllium Spectrometric Solution	0.95	0.08
Synethetic Interference Solution	1.01	0.08
Natural Waste Water	1.06	0.08
TCLP Extract of Hazardous Waste	1.08	0.07
Coal Fly Ash	0.99	0.08
Municipal Digested Sludge	0.93	0.09
Three Kids Mine Material	0.96	0.08
Marine Sediment	0.94	0.08
Mineral Sample	0.97	0.08
Hazardous Waste Site Soil	0.94	0.09
Copper-Beryllium Alloy	0.85	0.09
Average	0.97	0.08

[a] The method detection limit is defined by MDL = 3 S_B/m where S_B is the standard deviation of the instrument background signal and m is the slope of the calibration line in the sample matrix.

TABLE IV. EFFECT OF ALUMINUM, CALCIUM, MAGNESIUM, SILICON, AND SODIUM ON THE OBSERVED ABSORBANCE OF BERYLLIUM BY FLAME ATOMIC ABSORPTION SPECTROPHOMETRY, METHOD 7090

	Observed Be Absorbance[a]				
Concentration Element (mg/L)	Al	Ca	Mg	Si	Na
0[b]	0.089	0.089	0.089	0.089	0.089
100	0.079	0.090	0.092	0.090	0.088
500	0.062	0.093	0.094	0.089	0.090
1000	0.053	0.093	0.095	0.089	0.092
5000	0.041	0.096	0.097	0.080	0.093

[a] Be concentration = 0.500 mg/L
[b] The absorbance of 0.500 mg/L of Be at 0 mg/L of added element is normalized to 0.89.

TABLE V. EFFECT OF HYDROFLUORIC ACID AND 8-HYDROXYQUINOLINE ON THE ABSORBANCE OF BERYLLIUM WITH ADDED ALUMINUM BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY, METHOD 7090

Added Al (mg/L)	Be + Al	Beryllium absorbance in the presence of Al with HF (0.1%)	with 8-hydroxyquinoline (2.5%)
0	0.084	0.084	0.084
100	0.075	0.084	--
500	0.058	0.084	--
1000	0.050	0.085	0.086
5000	0.039	(0.073) (0.084)[a]	--

[a] 0.3 percent HF added.

TABLE VI. Combined Effects of Al, Ca, and HF on Measurable Be Concentration

Be Conc. Actual (ppm)	Be Conc. Measured (ppm)	Al Conc. (ppm)	Ca Conc. (ppm)	HF Conc. (%)	Al-to-Ca Ratio	Suppr./ Enhan.	ppt.
0.500	0.497	0	0	0	n/a	n/a	no
0.500	0.518	0	0	0.1	n/a	+ 4.2%	no
0.500	0.517	0	0	0.3	n/a	+ 4.0%	no
0.500	0.475	100	0	0	n/a	- 4.4%	no
0.500	0.513	100	0	0.1	n/a	+ 3.2%	no
0.500	0.508	100	100	0.1	1:1	+ 2.2%	no
0.500	0.489	100	500	0.1	1:5	- 1.6%	sl.
0.500	0.448	100	1000	0.1	1:10	- 9.9%	sl.
0.500	0.455	100	5000	0.1	1:50	- 8.5%	yes
0.500	0.403	500	0	0	n/a	- 18.9%	no
0.500	0.513	500	500	0.1	n/a	+ 3.2%	no
0.500	0.519	500	1000	0.1	1:2	+ 4.4%	no
0.500	0.443	500	5000	0.1	1:10	- 10.9%	no
0.500	0.355	1000	0	0	n/a	- 28.6%	no
0.500	0.532	1000	1000	0.1	1:1	+ 7.0%	no
0.500	0.400	1000	5000	0.1	1:5	- 19.5%	no
0.500	0.285	5000	0	0	n/a	- 42.7%	no
0.500	0.481	5000	5000	0.3	1:1	- 3.2%	no
0.500	0.454	1000	5000	0.3	1:5	- 8.7%	yes
0.500	0.354	500	5000	0.3	1:10	- 28.8%	yes
0.500	0.231	100	5000	0.3	1:50	- 53.5%	yes
0.500	0.045	0	5000	0.3	n/a	- 90.9%	yes
0.500	0.332	0	5000	0.1	n/a	- 33.2%	yes

TABLE VII. EFFECT OF ALUMINUM, CALCIUM, CERIUM, CHROMIUM, LANTHANUM, MAGNESIUM, MANGANESE, MOLYBDENUM, SILICON, STRONTIUM, SULFURIC ACID, TUNGSTEN, AND IRON ON THE OBSERVED ABSORBANCE OF BERYLLIUM IN FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY, METHOD 7091

Interfering Element (mg/L)	Observed Be Absorbance in Presence of these Chemicals [a]												
	Al	Ca	Ce	Cr	La	Mg	Mn	Mo	Si	Sr	H_2SO_4	W	Fe
Peak Area													
0	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427
100	0.424	0.452	0.430	0.419	0.457	0.470	0.458	0.431	0.434	0.460	0.446	0.370	0.435
500	0.444	0.430	0.430	0.414	0.449	0.475	0.464	0.467	0.437	0.450	0.428	0.363	0.427
1000	0.421	0.423	0.406	0.408	0.443	0.468	0.466	0.513	0.435	0.445	0.432	0.343	0.433
5000	0.400	0.452	0.388	0.387	0.438	0.484	0.467	0.805	0.408	0.448	0.427	0.330	0.430
Peak Height													
0	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427
100	0.398	0.524	0.432	0.445	0.476	0.620	0.465	0.431	0.433	0.494	0.473	0.378	0.558
500	0.498	0.719	0.517	0.578	0.722	0.846	0.452	0.465	0.442	0.638	0.458	0.387	0.520
1000	0.474	0.755	0.657	0.587	0.901	0.841	0.455	0.504	0.449	0.688	0.451	0.372	0.544
5000	0.479	1.074	0.551	0.495	0.621	0.715	0.703	0.777	0.437	0.978	0.431	0.349	0.491

[a] Data has been normalized.

TABLE VIII. PREDIGESTION SPIKE RECOVERIES OF BERYLLIUM DETERMINED BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY, METHOD 7090, AFTER DIGESTION OF LIQUIDS BY METHOD 3010 AND SOLIDS BY METHOD 3050

	Be Added (mg/L)	Percent Recovery Without HF	Percent Recovery With HF[a]
Trace Elements in Water	0.250	112	109
	0.500	106	103
Beryllium Spectrometric Solution	0.250	104	106
	0.500	106	105
Synthetic Interference Solution	0.250	78	104
	0.500	87	104
Natural Waste Water	0.250	67	105
	0.500	106	106
TCLP Extract of Hazardous Waste	0.250	101	--
	0.500	95	--
Coal Fly Ash	0.250	97	98
	0.500	87	96
Municipal Digested Sludge	0.250	86	85
	0.500	83	82
Three Kids Mine Material	0.250	78	101
	0.500	74	97
Marine Sediment	0.250	85	98
	0.500	82	97
Mineral Sample	0.250	78	89
	0.500	79	87
Hazardous Waste Soil	0.250	85	103
	0.500	83	101
Copper-Beryllium Alloy	0.500	98	99
	1.000	96	96

[a] With the addition of 0.1 percent HF.

TABLE IX. PREDIGESTION SPIKE RECOVERIES OF BERYLLIUM DETERMINED BY FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY, METHOD 7091, AFTER DIGESTION OF LIQUIDS BY METHOD 3020 AND SOLIDS BY METHOD 3050

Matrix	Be Added (µg/L)	Percent Recovery
Trace Elements in Water	2.5	97
	5.0	100
Beryllium Spectrometric Solution	2.5	103
	5.0	95
Synthetic Interference Solution	5.0	107
Natural Waste Water	2.5	96
	5.0	98
Coal Fly Ash	20	83
	40	94
Municipal Digested Sludge	5.0	89
	10.0	89
Three Kids Mine Material	10.0	97
Marine Sediment	10	105
	20	91
Mineral Sample	80	111
	160	98
Hazardous Waste Soil	5.0	108
Copper-Beryllium Alloy	2.5	88
	5.0	81
TCLP Extract of Hazardous Soil	2.5	92
	5.0	92

TABLE X. ACCURACY FOR THE DETERMINATION OF BERYLLIUM IN NATIONAL BUREAU OF STANDARDS - STANDARD REFERENCE MATERIALS BY FLAME AND FURNACE AAS (METHOD 7091 AND METHOD 7091) - BEFORE AND AFTER DIGESTION

Sample	Beryllium concentration			
	Before Digestion	After Digestion	NBS Value	Percent of NBS Value (Before Digestion)
Furnace AAS				
Trace Elements in Water-1643b µg/L	19.82±0.04	18.96±0.20	19±2	104
Be Spectrometric Solution-2127-2 µg/L	4.96±0.15	4.97±0.22	5.00±0.10	99
Cu-Be Alloy-C1123 mg/kg	4400±300	4200±100	4600±100	96
Flame AAS				
Trace Elements in Water-1643b µg/L	22±1	21±2	19±	116[a]
Be Spectrometric Solution-2127-2 µg/L	0.500±0.011	0.525±0.003	0.500	100
Cu-Be Alloy-C1123 mg/kg	4600±100	4600±100	4600±100	100

[a] The values for NBS-SRM 1643b, while reproducible, are only 10 times the IDL. However, the observed values are within the uncertainties of the "NBS Value" and measured values.

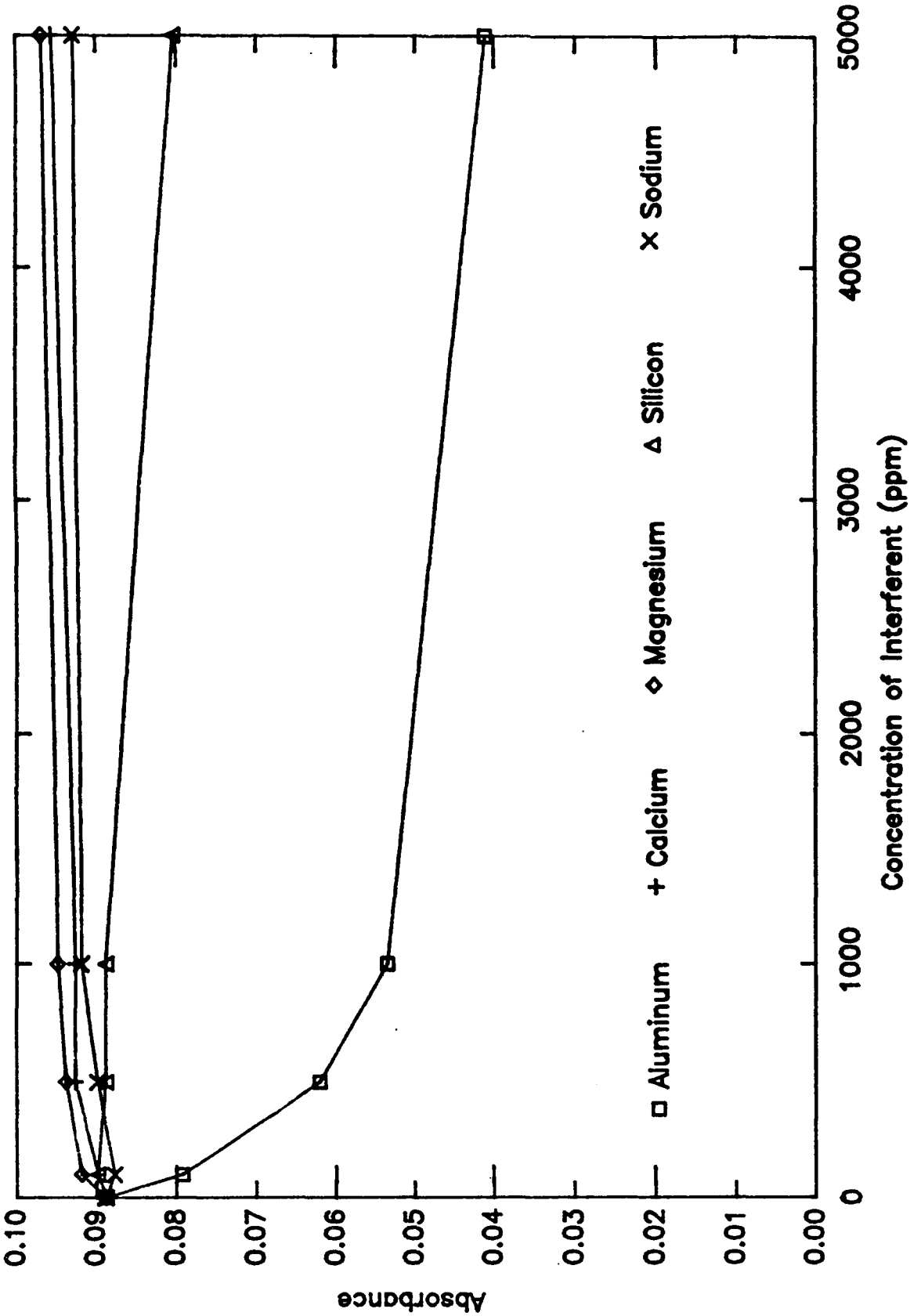


FIGURE 1

Effect of Al, Ca, Mg, Si, and Na on the absorbance of beryllium by flame AAS.

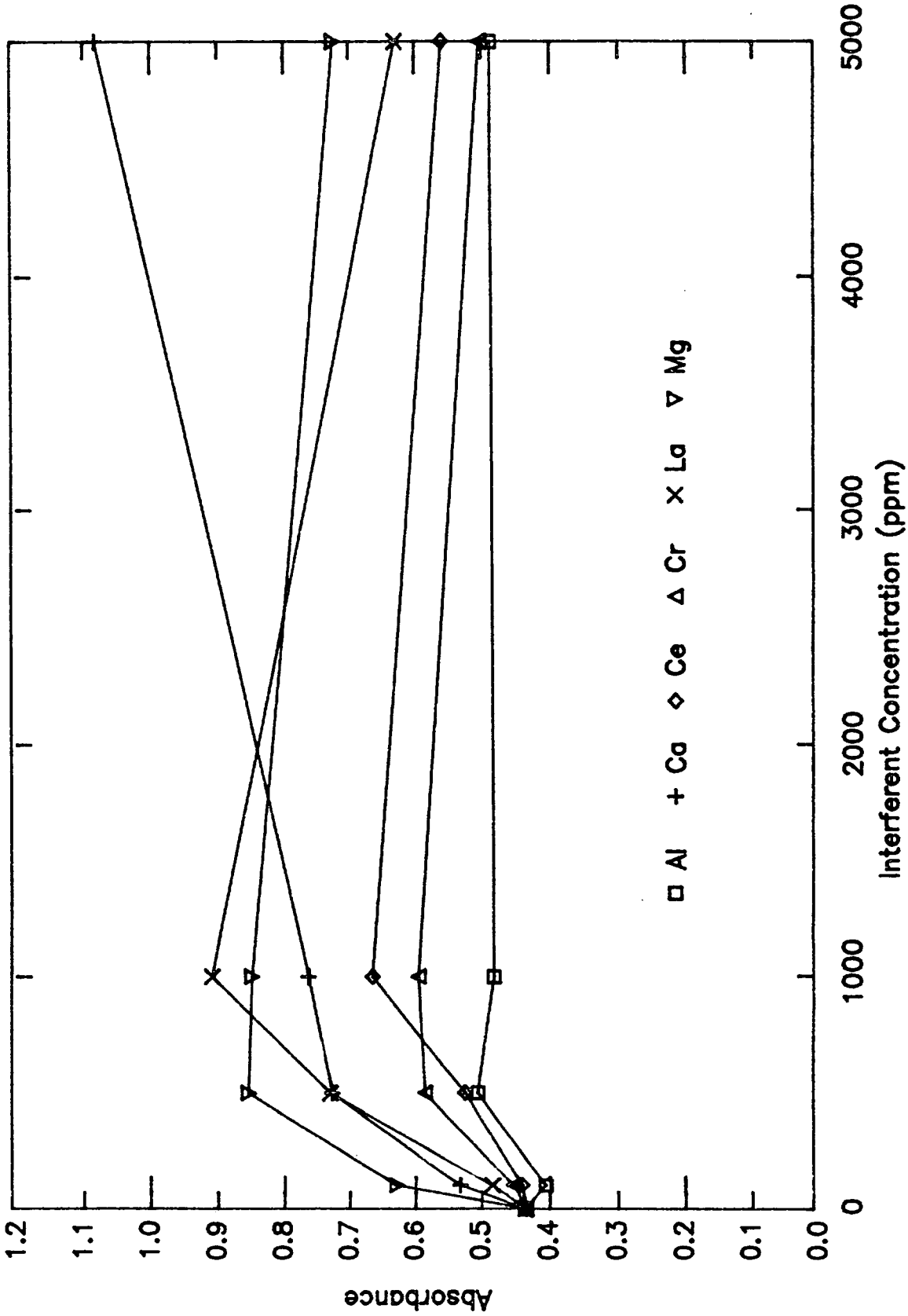


FIGURE 2

Effect of Al, Ca, Ce, Cr, La, and Mg on the absorbance of beryllium by furnace AAS, calculated from peak height.

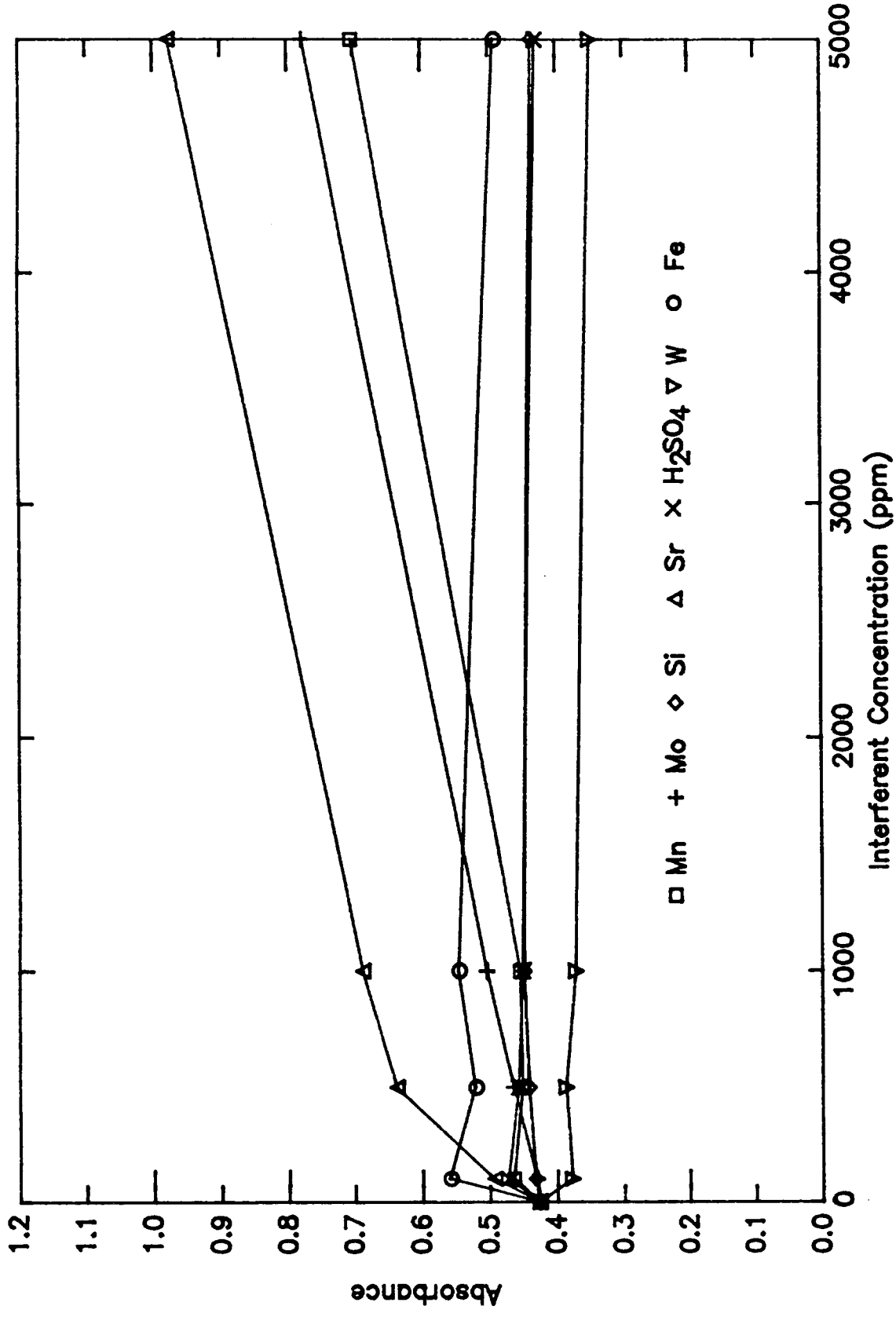


FIGURE 3

Effect of Mn, Mo, Si, Sr, H_2SO_4, W, and Fe on the absorbance of beryllium by furnace AAS, calculated from peak height.

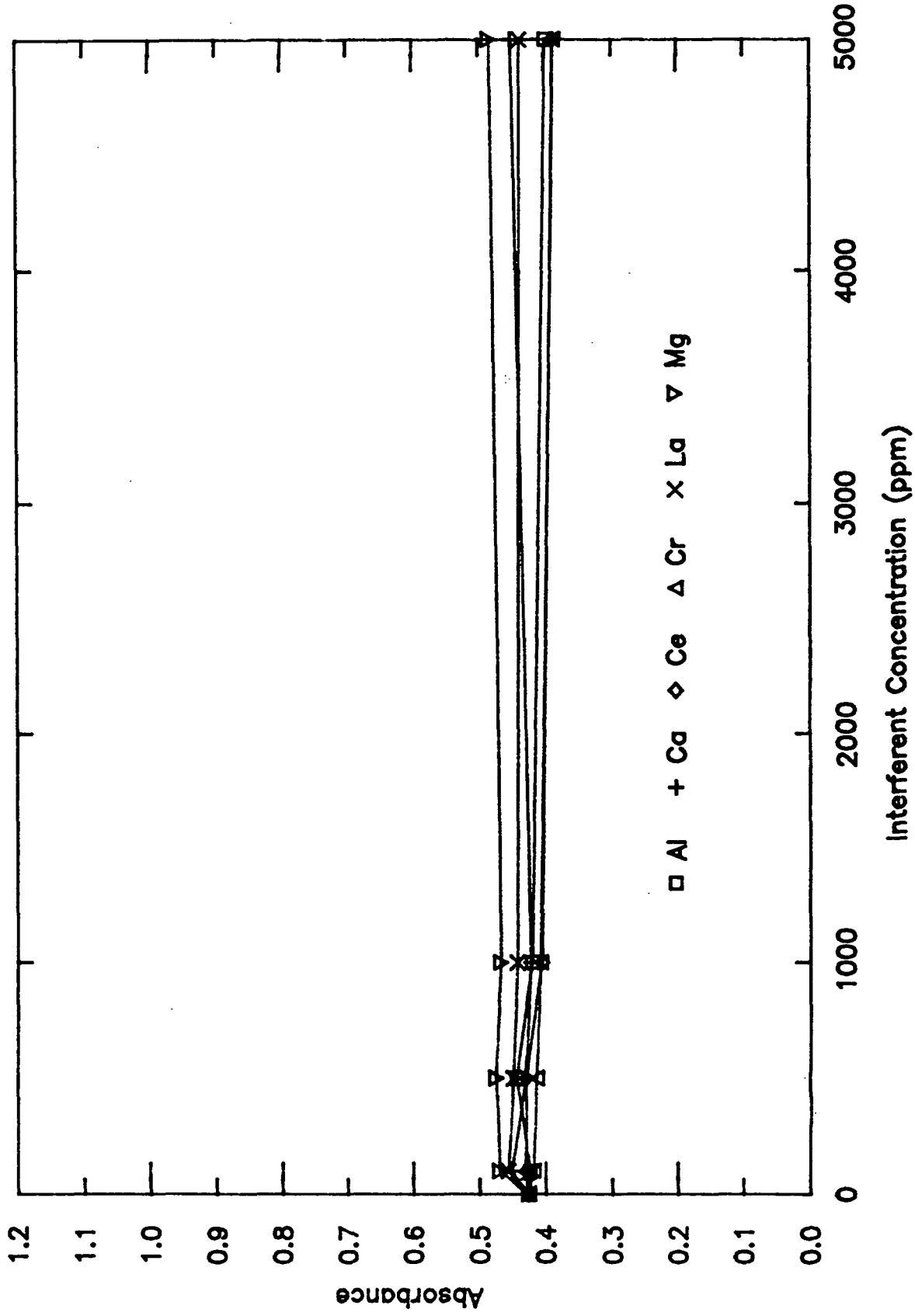


FIGURE 4

Effect of Al, Ca, Ce, Cr, La, and Mg on the absorbance of beryllium by furnace AAS, calculated from peak area.

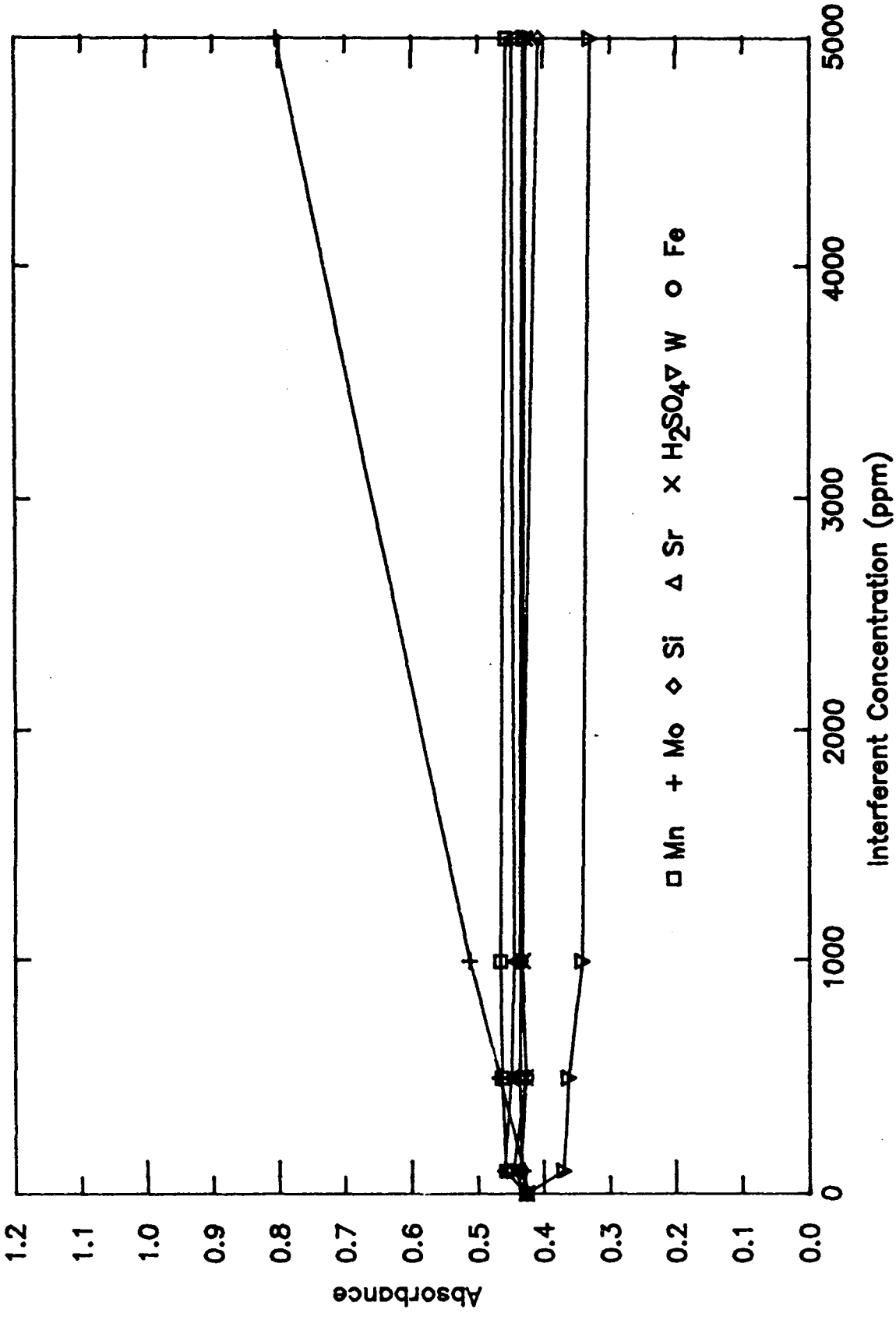
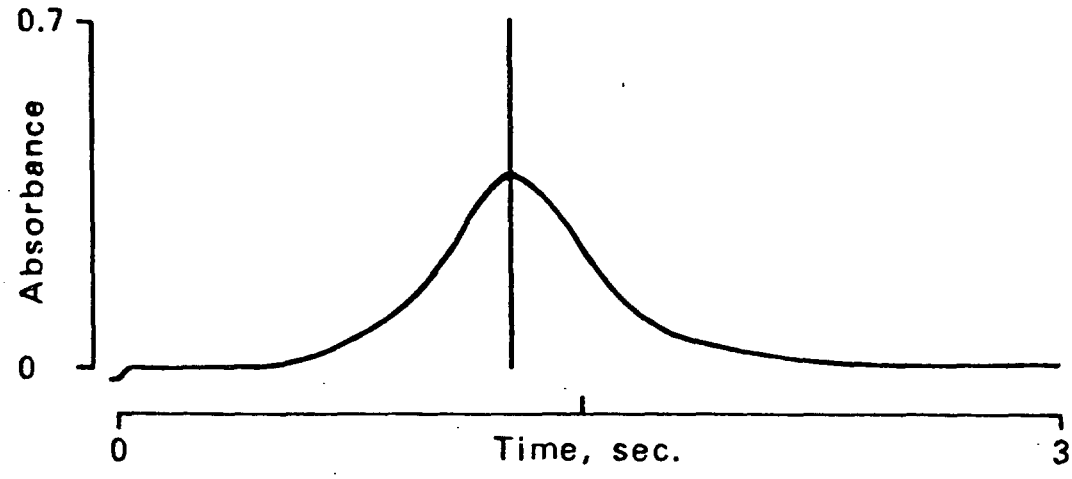
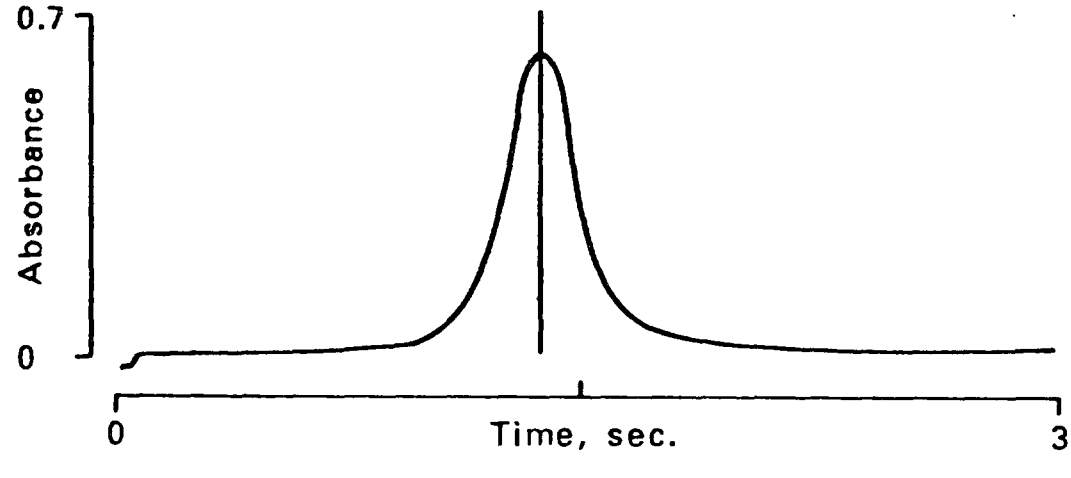


FIGURE 5

Effect of Mn, Mo, Si, Sr, H_2SO_4, W, and Fe on the absorbance of beryllium by furnace AAS, calculated from peak area.



5.0 ppb standard
Peak Height – 5.00 ppb Peak Area – 5.01 ppb



4 ppb Be in 5000 ppm Ca
Peak Height – 8.30 ppb Peak Area – 3.91 ppb

FIGURE 6
Effect of calcium on the peak shape of beryllium by furnace AAS.

FUNDAMENTAL RELATIONSHIPS IN ACID DECOMPOSITION OF ENVIRONMENTAL SAMPLES FOR ELEMENTAL ANALYSIS USING MICROWAVE ENERGY

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Introduction

Appropriate sample preparation is essential to achieve both accuracy and precision in analysis of materials. This preliminary step is one of the most time-consuming parts of many analyses and has become the rate limiting step for such multi-element techniques as ICP, AA, GFAAS and ICP-MS. Acid dissolution of biological and botanical samples can take from 4 to 48 hours using classical digestion techniques. Standard reflux methods used in EPA procedures may take from 24-96 hours. Many of these same samples require only 10 to 20 minutes with microwave digestions, dramatically reducing preparation time. Volatile elements such as selenium and phosphorus can be quantitatively retained in a sealed vessel using microwave decomposition prior to instrumental analysis (1). The 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. The development of microwave procedures for each of these sample types is currently an intense area of research.

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. It is necessary, however, to have an understanding of the fundamental concepts controlling interactions between microwave energy and the acid solution containing the sample.

Research has been conducted to identify these fundamental relationships and to develop methods that allow the analyst to predict, before programming and running the equipment, the conditions that will be generated during microwave digestion. This has been accomplished by measuring many of the parameters required to calculate the microwave power absorption by the mineral acids. Once the amount of energy that will be absorbed by a quantity of acid is determined, the equation is solved for the final temperature that will be reached by the sample at specific power settings. This method of predicting the temperature, or the time it takes to reach a particular temperature, is useful in estimating the decomposition conditions (2).

Thermodynamic Basis of Measurement and Predictive Calculations. Power measurements in a microwave system are derived from elementary thermal concepts based on the heat capacity of a mass at constant pressure. Heat capacity, C_p, is that quantity of heating experienced by a given mass per degree of temperature from energy absorbed to produce a rise in temperature, ΔT. If a quantity of energy is delivered for a unit of time, then P, the power absorbed by a substance (power density) in the microwave cavity may be expressed in the following relationship

$$P = \frac{(K)(C_p)(m)(\Delta T)}{t} \qquad (1)$$

P is the apparent power absorbed by the sample in watts (W). 2 ($W = joule.sec^{-1}$)
K is the conversion factor for thermochemical calories.sec^{-1} to W (4.184),
C_p is the heat capacity, thermal capacity, or specific heat ($cal \cdot g^{-1} \cdot °C^{-1}$),
m is the mass of the sample in grams (g),
ΔT is T_f, the final temperature minus T_i, the initial temperature (°C), and
t is the time in seconds (s).

Calibration of Microwave Equipment. This basic equation has been used with minor modifications to establish quantitatively the significance of local variations in tissue temperature related to changes in the heat content of the body as a whole (3) as well as to express the absorbed power density of tissue exposed to electromagnetic radiation (4-6). The equation may also be used to evaluate the power available for heating in a microwave cavity. This is accomplished by measuring the temperature rise in 1 kg of water exposed to electromagnetic radiation for a fixed period of time. Reliable measurements are made on replicates of weighed, 1 kg samples of room-temperature distilled water in thick-walled microwave-transparent vessels. The container should be circulated continuously through the field for at least 2 min at full power. Although 2 min represent a compromise between short and long exposures to produce a small change in temperature, 1 kg samples can be exposed for 90-240 seconds at full power without observing significant differences in apparent power absorption. Problems that can cause errors in this determination are heat loss from the vessel, not stirring the water before measuring the temperature and using microwave absorptive or reflective containers. Teflon containers are the most microwave transparent and have been used frequently in these calibrations. One kg of water, in one container or equally divided between 2 to 5 containers, absorbs the same amount of power in a homogeneous microwave field.

Such a measurement is critical to the calibration of microwave equipment. In order that absolute power settings may be interchanged from one microwave unit to another, the actual delivered power must be determined. A variety of microwave equipment is available that delivers from 500 to nearly 800 W of power to the cavity. To transfer power settings from one instrument to another, it is necessary to calculate the power delivered to the cavity. When the number of watts of power available to the samples in both systems is known, previously designed digestion schemes can be adapted to different systems. A microwave unit that has its magnetron protected from reflected microwave energy can be expected to continue producing constant power over many years of operation. The unit used for this investigation has maintained a constant output of 574 ± 7 W for over 4 years of frequent operation. Equation 1 can be adapted to measure the power uptake of any quantity of material for which the heat capacity is known and for which initial and final temperatures can be measured. Like water, the power absorbed by any mass of acid exposed to microwave radiation can be calculated from Equation 1 (2). Heat capacity values in the literature are frequently given as the apparent molal heat capacities (Φ_c, cal $°C^{-1}$ $mole^{-1}$). From values given in Parker's monograph (7) and the equations therein, the heat capacities (cal g^{-1} $°C^{-1}$) have been calculated for commonly used acids and are summarized in Table I. The heat capacity of an acid varies inversely with its concentration. For example, the more dilute the acid the greater its heat capacity and the more nearly its value approaches that of water.

TABLE I. HEAT CAPACITY OF (AQUEOUS, MINERAL) ACIDS

Acid	Concentration, $mol \cdot L^{-1}$	Heat Capacity, $cal \cdot g^{-1} \cdot °C^{-1}$*
Hydrochloric(37.2%)	12	.5885
Hydrochloric	6	.7168
Nitric(70.4%)	15.9	.5771
Nitric	8	.7177

*Parker, V. B. Nat'l Stand. Ref. Data Ser. (U.S. Nat'l Bur. Stand.) 1965, NSRDS-NBS 2.

Power absorption by small volumes of acids decreases proportionally as the mass in the cavity decreases (Table II) because some portion of the incoming radiation never travels through the sample as the waves traverse the cavity and leave through the waveguide.

TABLE II. POWER ABSORBED BY SMALL VOLUMES OF CONCENTRATED MINERAL ACIDS COMPARED WITH DISTILLED WATER (n = 5)

	Power Absorbed, W ± 1SD		
Reagent	50 mL	100 mL	200 mL
Water	344 ± 9	408 ± 3	468 ± 5
HNO_3 (16M)	184 ± 2	234 ± 5	313 ± 4
HNO_3 (1M)	212 ± 3	269 ± 6	332 ± 3
HCl (12M)	148 ± 3	173 ± 6	251 ± 2
HCl (1M)	227 ± 4	287 ± 7	340 ± 5

When microwave power absorptions are compared in Table II, it becomes apparent that dilute acids absorb power more strongly than concentrated acids. This is attributable to the larger fraction of water present and is revealed in the larger heat capacity value for the more dilute acid. In this case, water is a better absorber of the 2,450 MHz radiation. Values for the absorbed power are derived from measurements made at full power for different time intervals, so that the net temperature change is between 5 and 50 °C. Large temperature increases are accompanied by heat losses and changes in heat capacity. Neither of these conditions is considered in the original equation. Empirically, one observes that small samples get hotter than large samples for the same exposure in the cavity at full power. This seems logical, since there is less mass to heat. A 200 mL sample of 6 M HCl exhibits a net increase of 50 °C, whereas 500 mL of 6 M HCl increases only 35 °C in temperature when both are exposed to 574 W of power for 2 min. Small samples actually absorb proportionally less power than larger samples. A portion of the magnetron power sent to the cavity is not available for absorption by the sample.

A nonlinear relationship appears to exist between quantity and power for water and for all the acids. In this system, power is not limited for any but the largest cavity loads. However, there is a critical volume for each acid and water as well, above which very little additional power appears to be absorbed. For the

materials studied to date, that volume is near 500 mL. The nominal 600 W microwave systems are essentially power limited beyond this quantity of sample.

These techniques can be used to establish the absorbed microwave power for other dielectrics at specific volumes. The power absorptions are necessary for calculations to predict final temperature conditions using a rearrangement of the fundamental thermodynamic relationship.

Using Calculations to Predict Conditions. With the large number of sample dissolutions required in most laboratories, it is not efficient to digest one sample at a time, although measuring the conditions in a single sample is practical for testing new materials. Multiple dissolutions usually bring the total acid volumes in the cavity to between 25 and 500 mL, depending on the individual sample sizes. The value of P for any mass of acid in the cavity can be calculated using the empirical equations 2 through 4 (8).

Distilled, deionized water

$$y = 170.3133 \times X^{0.1757} \quad (2)$$

Nitric Acid

$$y = 64.5182 \times X^{0.2655} \quad (3)$$

Hydrochloric Acid

$$y = 41.7545 \times X^{0.3172} \quad (4)$$

The value of "y" is the power absorbed (P, in watts), by a given mass (m, in g) "X" of acid or water at full magnetron power. This transformation enables the power absorption to be calculated for any mass of acid from these few previously measured volumes. Using this transformation, one may expect to maintain approximately ± 13% accuracy at the 95% confidence limit for nitric acid. If greater accuracy is necessary for a particular volume of acid or reagent, then P can be specifically measured at that volume.

It should be noted that there is an upper power limit that is dependent on the output of the magnetron and tuning of each particular microwave system. The power absorption for very large samples is constrained by the actual power delivered to the microwave cavity and by the unique conductance and dielectric relaxation time of that particular acid. This upper limit will be slightly different for each acid and for microwave equipment delivering different amounts of power to the microwave cavity. The power absorption appears to reach its maximum between 500 and 1,000 mL of acid. Some slight increase beyond 1,000 mL has been observed for sulfuric and nitric acids. Analysts will find that these equations are useful for acid volumes between 20 mL and 1,000 mL. This will prevent extending equations 2-4 beyond their appropriate limits.

Each microwave system is a unique device. The power delivered to the sample depends not only on the power output of the magnetron but also on the tuning of the waveguide and cavity dimensions. The apparent power absorbed by water at full power should be used to calibrate all microwave equipment using 2450 mHz at a power output of between 500 W and 800 W. The P calculated for that unit should be divided by 574 which is the maximum power (100%) or the unit which was used to measure these acids. When this ratio is multiplied times a calculated P it will correct the data giving the calculated apparent power absorbed by that particular

unit. This correction is for 100% of full power and must be further adjusted if partial power is to be used.

Once a value of P has been calculated for a specific amount of acid, both the target temperature and the time that microwave power is applied can be estimated by a simple transformation of Equation 1.

The final temperature can be estimated from

$$T_f = T_i + \frac{(P)(t)}{(K)(C_p)(m)} \quad (5)$$

and the time it will take to reach some final temperature from

$$t = \frac{(K)(C_p)(m)(\Delta T)}{P} \quad (6)$$

Trial and error evaluation can be minimized by using these equations to predict the conditions that will result from specific power and time exposures of a sample(s), or the analyst can decide on appropriate conditions of temperature for a specific sample and use these equations to establish the approximate power and time settings.

These thermodynamic relationships can reliably predict temperature and time within a few percent, for the mineral acids tested, despite small changes in their dielectric constants, when the parameters are < 150 °C and 2 min. It is at higher temperatures (150-250 °C) and longer exposure times (5-20 min) that the actual conditions deviate from their predicted values. The major error consideration becomes heat loss through the walls of the container. Table III demonstrates the deviation from predicted values for each of eight samples containing 5 mL of concentrated nitric acid and evaporated Human Urine (SRM 2670, originally 10 g each wet weight). Only the nitric acid significantly couples with the microwave power; the sample is neglected in the calculations. This table of predicted times at given temperatures was calculated by using the heat capacity from Table I, the power absorbed for eight samples (7.2 g each or 57.6 total g) of acid using the equation relating power consumption for nitric acid (equation 3), and the thermodynamic equation solved for time (equation 6). Various final temperatures (T_f) were used to calculate the theoretical time to reach a particular temperature at 574 W; these times are compared with the actual elapsed time in Table III.

The agreement is within experimental error for up to 108 sec. when a negative bias in the predicted value becomes apparent. This bias is caused by heat loss through the container walls. Such losses in Teflon PFA containers may reduce the effective power provided to the acid by as much as 50% in 6 min. At a given microwave power setting, the heat loss from the container results in higher predicted temperatures than are actually measured. Conversely, heat loss results in under prediction of time to a given temperature. Both biases will increase with longer time or higher temperatures. For this type of Teflon PFA container the reproducibility of this heat loss under exactly the same conditions is within several percent when measured between different container lots.

TABLE III. COMPARISON OF THE PREDICTED AND ACTUAL TIME FOR SAMPLE TO REACH A TARGET TEMPERATURE (2)

	Time, s	
Temperature, °C	Predicted	Actual
110	64 ± 13	54
130	79 ± 16	72
150	94 ± 19	81
160	101 ± 21	102
170	108 ± 23	113
180	116 ± 24	144

These deviations will vary with the heat loss of the vessel and become less with greater thermal insulation of the vessel. Accurate use of these transformed equations is limited to the initial increase in temperature when uninsulated Teflon vessels are used. No predictive information can be obtained about the amount of power required to sustain the temperature. Such power requirements are dependent on the equilibrium which is established between the heat input and the heat loss at a given temperature. This relationship is dependent on the specific vessel used and will be unique for each type.

Actual temperature conditions in the thick-walled Parr bomb probably closely follow the theoretical predictions over a much wider range of temperatures and for longer times, because it is better insulated and has only a fraction of the heat loss of the Teflon PFA vessels.

These predictions also have application in the safe use of the closed-vessel microwave technique. Unsafe conditions can be prevented by using calculations to predict a maximum temperature or minimum time to reach given conditions. If these calculations are made first then unsafe conditions can be avoided.

Partial Power and Power Programming. Microwave systems can deliver variable power to the sample cavity. This is accomplished by time-chopping the power to the magnetron at full power. It is often necessary to deliver a specific amount of reduced power per unit time to the cavity to reach or hold specific temperatures. Full-power settings are not normally used to digest 250 to 500 mg biological and botanical samples in 5-10 mL of nitric acid. Instead, fractional power provides a more controlled method of heating. When using a partial power setting, one must know the number of watts absorbed at full power, assuming that the linearity of proportional power has been maintained. The equipment used to acquire the data presented here has been determined to be linear within 1% of ideal over the entire power range. With all other parameters the same, a proportion of P equivalent to the partial power desired is used. For example, at 20% power equation 6 becomes

$$t = \frac{(K)(C_p)(m)(\Delta T)}{(0.20)P} \qquad (7)$$

The delivered power linearity and accuracy of partial output power of each microwave unit should be checked before using partial powers. This is accomplished by doing a calibration with water at several partial power settings.

The partial power output is not always linear and for some microwave units may not be a simple fraction of the full power calculated from calibrations done at 100% power. The calibration which is done at full power can be repeated at fractional powers such as 25%, 50% and 75%, and graphed to observe any deviation from linearity. If the partial power is not linear then the setting should be corrected for bias between the fractional power setting and the actual power delivered to the cavity.

Nitric Acid. Decompositions with nitric acid are among the most common. It is a strong oxidizing agent and is widely used for liberating trace elements in biological and botanical matrices and is one of the few acids that can be obtained in ultrahigh purity for very low-level analytical analyses. Also, most nitrate salts are highly soluble. As a consequence of its relatively low boiling point (120 °C), open-vessel nitric acid decompositions are traditionally time consuming. They often require higher temperatures or the addition of other strong oxidizing agents, such as peroxide or perchloric acid, to completely destroy a complex organic matrix.

Nitric acid behaves ideally under microwave energy excitation. In a closed container, nitric acid can attain 176 °C, while the pressure inside the Teflon PFA vessel remains at about 5 atm. This temperature is more than 50 °C above its boiling point. Substantial increases in oxidation power are achieved as a result, and these reactions proceed more rapidly and more thoroughly than at the lower temperatures (9).

Hydrochloric Acid. Concentrated HCl is an excellent solvent for certain metal oxides as well as for metals oxidized more easily than hydrogen (10). Under high pressure and at elevated temperature (11) many silicates and numerous other refractory oxides, sulfates, and fluorides are attacked by hydrochloric acid to produce generally soluble chloride salts. Hydrochloric acid may decompose under high pressure or in the presence of strong oxidants to produce chlorine gas (11).

Acid Mixtures. Acid combinations for microwave decomposition are practical for many of the same reasons they are used in other wet-ashing procedures. Additional benefits accrue from the closed-vessel microwave dissolution technique when the acids are heated in combination with one another. Acid combinations are frequently chosen for the effectiveness of one of the acids as a digestion agent and for the resultant aqueous solubility of the complexed elemental salts formed with a second acid during dissolution. This is true for all decompositions, both open and closed vessel.

Attempts to describe or calculate the temperature profile of acid mixtures require knowledge of the heat capacities of the mixed acids. Most of the data are for very dilute aqueous electrolyte solutions of a single mineral acid or salt. These constants are not available for acid mixtures. At present, the development of microwave applications using mixed acids is restricted to actual measured results. Previous high pressure acid dissolution techniques have included inaccessible systems like Carius tubes and steel-jacketed bombs so that mixed acid systems have not been well characterized for temperature and pressure.

Microwave Vessels. New microwave-transparent vessels made of PFA Teflon and specifically engineered for this purpose permit the use of high temperatures (180-250 °C) and pressures (1000 kPa or 10 atm). These vessels can be obtained with safety relief valves that prevent vessel over-pressurization. Vessels of other

materials which have not been engineered for this purpose and tested under these conditions should not be used without being thoroughly evaluated. Multiple layer vessels have also recently become available. These vessels resemble steel jacketed digestion bombs but they are made of plastic materials. These vessels (available from Parr) have ratings of 1,200 psi (81 atm). However, they only hold small sample quantities.

Reproducibility of Conditions. Because microwave energy is transferred directly to the acid, the reproducibility of decomposition conditions is better than can be achieved by traditional hotplate heating. Figure 1 shows the excellent reproducibility of sample conditions; it compares the temperature profile of two sets of six rice flour samples digested separately. The maximum difference between the sample temperatures at any point on the curve is 1.7%. Not only do the samples reach the same end point, but they achieve the same conditions at every point within this uncertainty.

Specific temperatures were identified for the rapid decomposition of the three basic components of biological and botanical matrices in nitric acid. Carbohydrate matrices decomposed rapidly at a temperature of 140 °C, protein molecules are rapidly decomposed at 150 °C, and lipid molecules are decomposed at approximately 160 °C. These temperatures were determined by observing the nitric acid decomposition of each of these biological components separately. The oxidation by nitric acid was determined by measuring a rapid rise in pressure inside the closed container during a small change in solution temperature.

Biological materials decompose rapidly at temperatures that are closely related to their major components. Because the biological matrix is converted to CO_2 and nitric acid is converted to NO_2, these gaseous decomposition products produce a sharp rise in pressure with barely perceptible temperature changes. This rise is a good indication of the occurrence of decomposition. This sharp rise in pressure occurs in any sample that produces gaseous digestion products. Other examples are oils containing carbon and soils containing carbonates.

New Configuration of the Decomposition Vessel Proposed. There is data to support the concern for analyte loss when decomposing gas-producing samples. To reduce or eliminate this problem, a new configuration of the currently used PFA vessel value combination was recommended for testing. This configuration uses a second vessel in conjunction with the digestion vessel and valve system to trap the hot acid vapor and some aerosol expelled when the pressure valve opens. A PFA tube connects the digestion vessel to a second vessel with a double ported cap. The second port on the catch vessel remains open to the atmosphere, thus preventing pressure build-up in the second vessel. The acid and any sample condensed in the second vessel can be combined in the sample vessel at the end of the microwave procedure.

Recommended Nitric Acid Decomposition/Leach for Soil Samples. Using the fundamental concepts obtained from temperature and pressure profile studies, specific conditions can be recommended for the limited decomposition of soil samples. The object of these procedures was to optimize the efficiency of the decomposition and to simplify the test procedure. The conditions chosen were based on both a mathematical model (1) and on empirical data. The final conditions recommended are shown in Figure 2. A temperature exceeding 160 °C was maintained for 7 minutes of a 10 minute decomposition procedure.

For soils these conditions should be considered a hot acid leach since the only acid used in these EPA procedures is nitric. Under these conditions, elements held in the silicate fraction will not be solubilized.

The temperature profile obtained using these conditions is optimum for several types of sample decompositions. It was tested on the soil samples and produced a reasonable profile which may be optimal for hot nitric acid leach of this type of inorganic sample. Figure 2 shows the profile of temperature and pres-sure conditions produced for a 1 g sample of soil in 10 mL of nitric acid. When this data is transformed, as in Figure 3, the pressure vs temperature profile shows that no excess pressure build-up occurs with nitric acid during this procedure. This profile is similar to that obtained for nitric acid alone. There is no pressure build-up because no gaseous decomposition products are formed. Because the sample is not totally decomposed it is necessary to filter or centrifuge the sample prior to instrumental analysis.

If this procedure could be used for oils and soils there would be an advantage of using the same microwave conditions for both soil and oil samples since only a single decomposition procedure would be necessary. This is important to EPA since many samples have components of both oils and soils.

This work has led to many new applications. Because microwave digestions occur in a well-defined, precisely controlled system, they are suitable for integration into automated applications. Acid digestion conditions have previously been too arbitrary for automation. With direct control of the power, the acid temperature, and the time for digestion, the microwave technique has become sufficiently structured that it is possible to automate sample decomposition prior to instrumental analysis.

Because of the precise reproducibility of the conditions and decomposition, microwave dissolution has great potential as a tool in analytical laboratories. This new technique effectively addresses the problems of precision, accuracy and efficiency.

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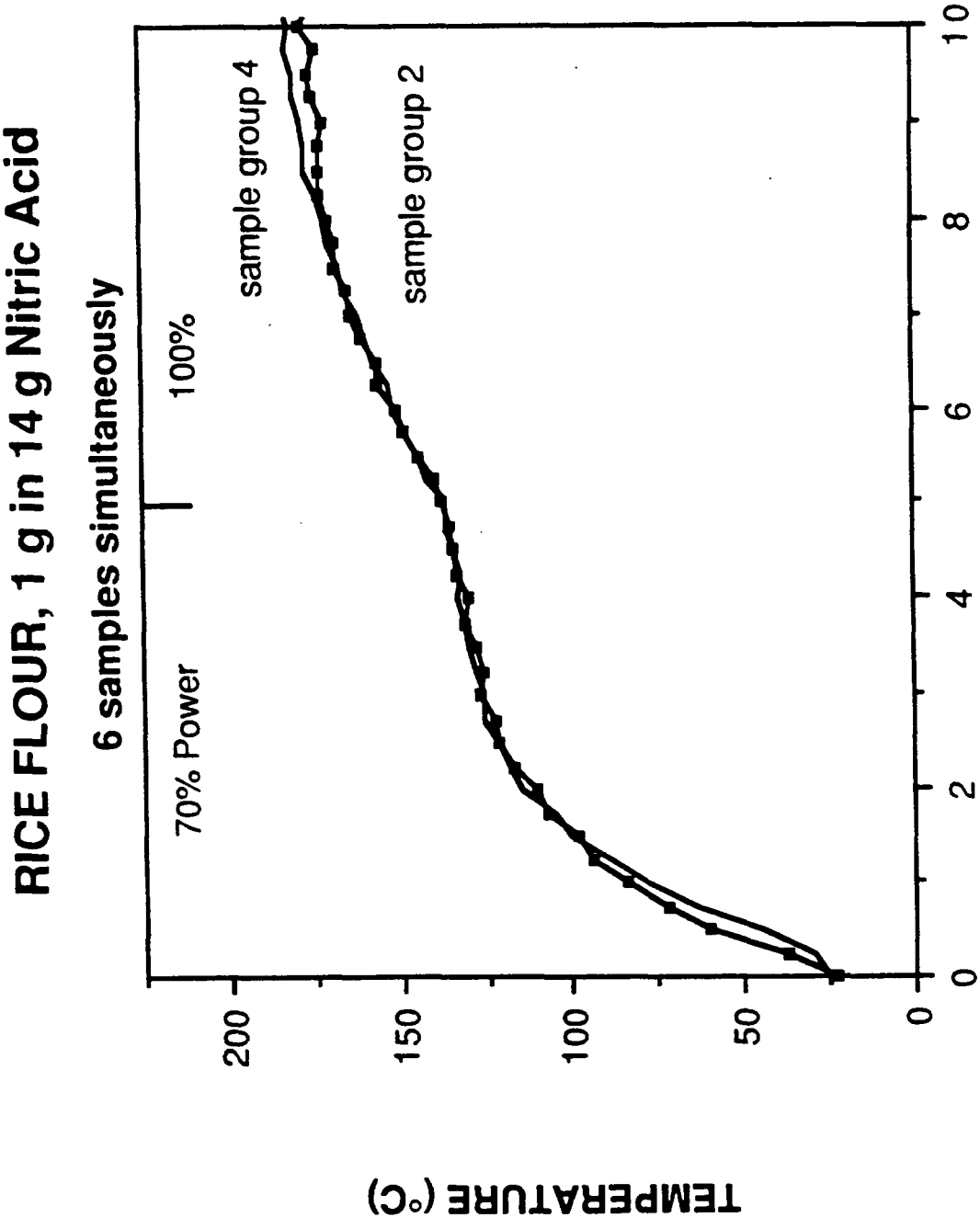
RICE FLOUR, 1 g in 14 g Nitric Acid
6 samples simultaneously
70% Power
100%
sample group 4
sample group 2
TEMPERATURE (°C)
TIME (min)
0
50
100
150
200
0
2
4
6
8
10

FIGURE 1

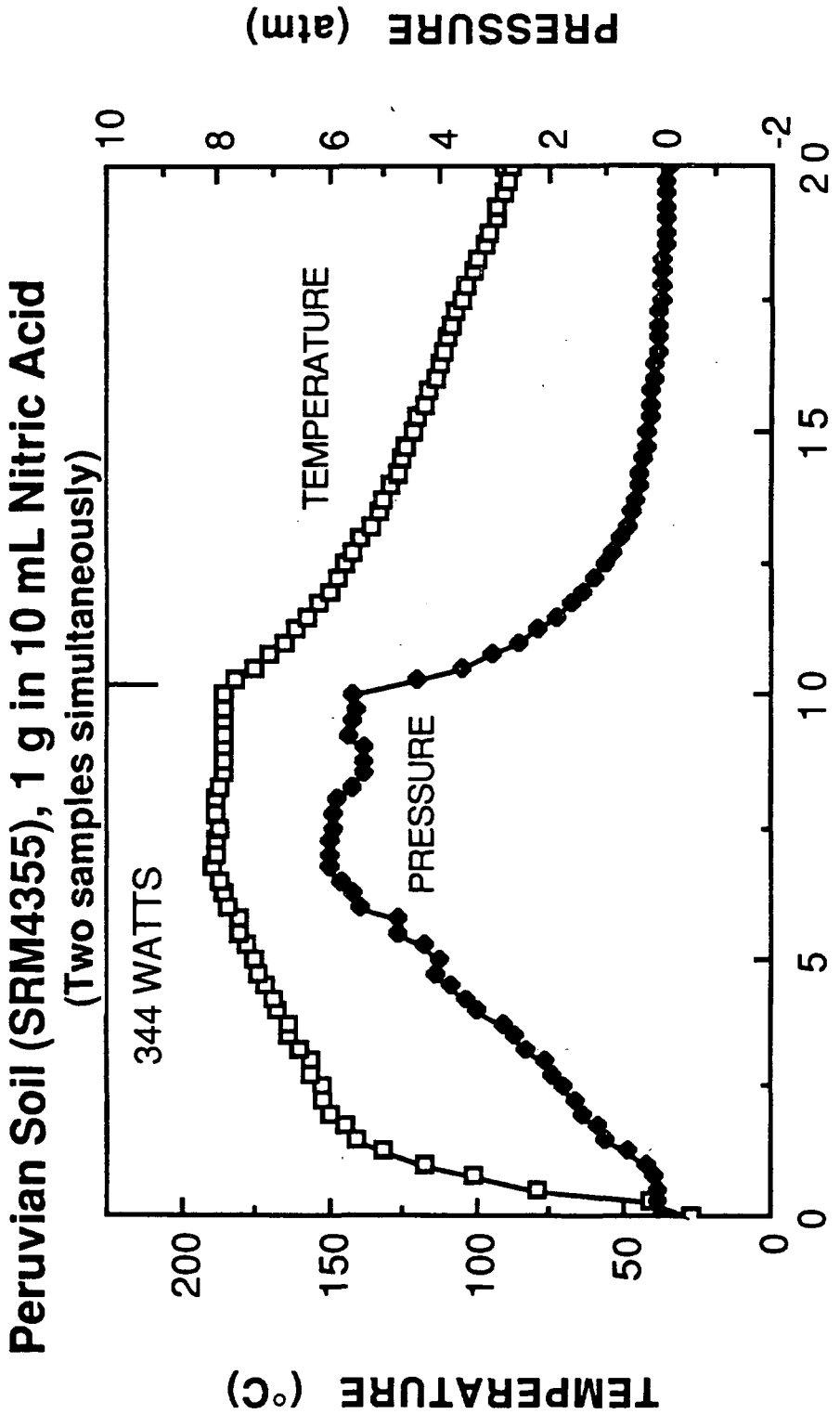
Peruvian Soil (SRM4355), 1 g in 10 mL Nitric Acid
(Two samples simultaneously)
344 WATTS
TEMPERATURE
PRESSURE
TEMPERATURE (°C)
PRESSURE (atm)
TIME (min)
0
50
100
150
200
0
2
4
6
8
10
-2
0
5
10
15
20

FIGURE 2

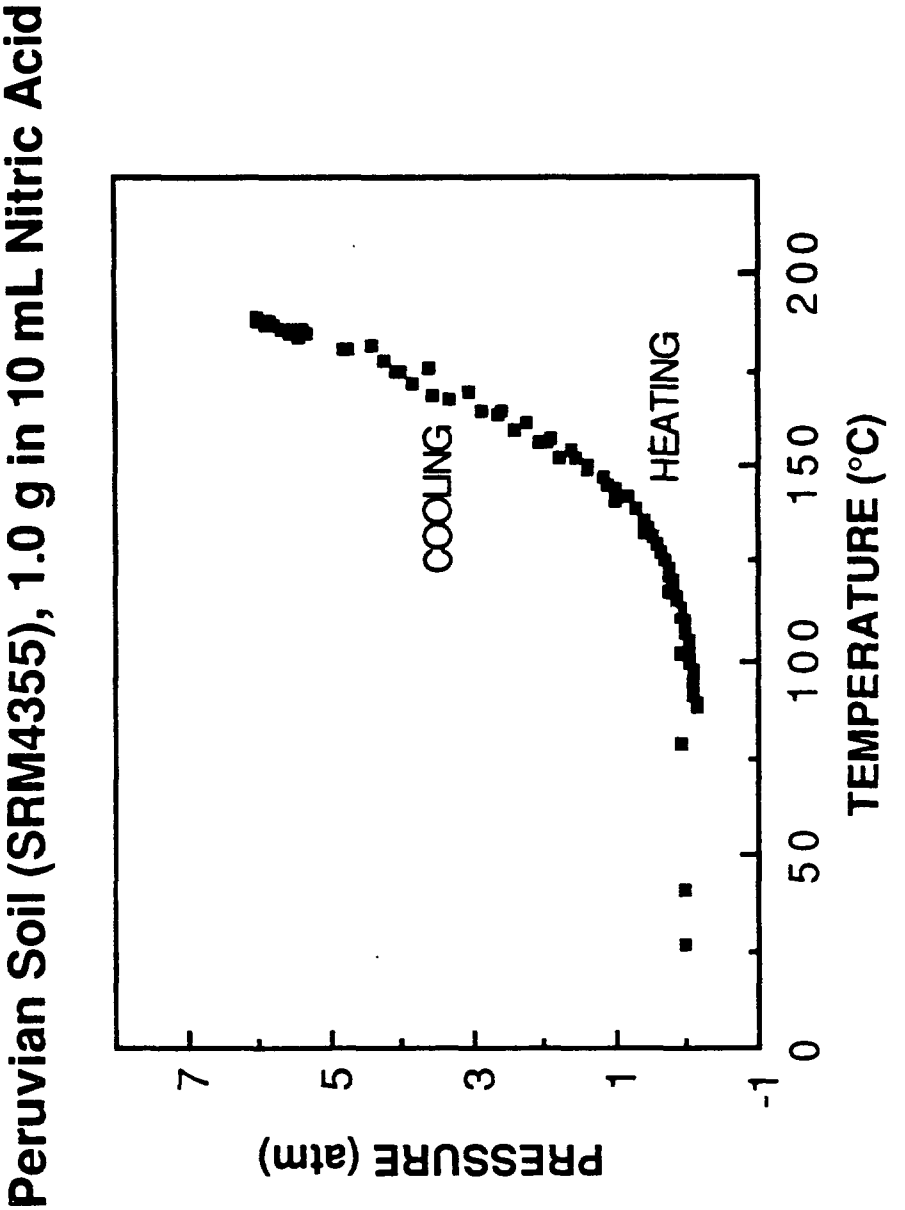
Peruvian Soil (SRM4355), 1.0 g in 10 mL Nitric Acid
PRESSURE (atm)
7
5
3
1
-1
0
50
100
150
200
TEMPERATURE (°C)
COOLING
HEATING

FIGURE 3

PORTABLE TEST METHOD FOR THE DETECTION OF HALOGENATED SOLVENTS IN USED OIL

Deborah Minucci, Quality Control Manager, Stephen Finch, Laborotory Manager, DEXSIL CORPORATION, One Hamden Park Drive, Hamden, Connecticut 06517

INTRODUCTION

In response to EPA's recent regulation of used oils containing greater than 1000 parts per million chlorine, a portable field test kit has been developed to quickly reveal whether a sample is above or below this limit. The EPA prohibits the burning of any used oil that contains more than 1000 ppm (mg/kg) of halogens measured as chlorine that cannot be shown to be from a nonhazardous source. Currently available methods of chlorine analysis are time consuming and must be performed in a laboratory by trained technicians. Foreseeing the additional testing that would be required under the new regulations, EPA Region II asked Dexsil to develop a field-portable test kit that could be used by untrained personnel. The result is a small, disposable kit that requires less than five minutes to show if an oil sample exceeds the 1000 ppm limit.

KIT DEVELOPMENT

Before the development work for this kit began, the following criteria were set forth as goals for the ideal kit. The kit should be:

1) Portable - small enough to store and carry easily
2) Easy to use - no technical training required
3) Fast - should take less than 10 minutes
4) Accurate - Accuracy should be comparable to laboratory methods
5) Safe - test kit should not be dangerous to use - if possible, flash point should be greater than 100°F.
6) Versatile - should be usable under a variety of conditions
7) Comprehensive - kit should work on fuel oils, crankcase oil, hydraulic fluid, cutting oils, and other lubricants which may eventually need testing for chlorine
8) Inexpensive - should be economical for user - not more than $10 per sample analysis

After about a year of research, a kit was developed that meets all of these criteria. The Clor-D-Tect 1000™ kit is a bit smaller than a video cassette and weighs about 50 grams (2 oz.). It can be used by anyone who can follow instructions and distinguish between yellow and purple. It takes about five minutes to run a test and is accurate to +/- 100 ppm. All reagents are contained in glass ampules so the operator never comes into contact with them. It has been shown to work at temperatures below 0°C and above 30°C. It works on fuel oil, crankcase oil, hydraulic fluid, and cutting oils. Kits cost between $5 and $7 apiece.

The kit consists of two polyethylene test tubes, each of which contains two glass ampules. These ampules contain the reagents that take part in the reaction with the halogenated compounds. Each kit also contains a small glass capillary tube attached to a syringe which is used for taking a measured oil sample.

An accurate sample of the oil is taken by completely filling the capillary tube, using vacuum supplied by the syringe. The capillary is then removed from the syringe and dropped into a polyethylene test tube. The polyethylene tube contains two ampules, the first of which holds 1.5 ml of a solution containing aliphatic solvent, napthalene and diglyme. The second ampule contains a dispersion of metallic sodium in an organic matrix. The capillary tube, full of the sample being tested, is completely crushed by squeezing the sides of the polyethylene test tube. This assures that all of the sample will be introduced into the reaction. Next, the ampule containing the solvent, napthalene and diglyme is broken. The entire tube is then well shaken to assure complete mixing of the sample. The sodium ampule is then broken and once again the tube is shaken vigorously. Covalently bonded halogens are stripped from their backbones by the active sodium.

The second polyethylene tube contains 7 ml of aqueous buffer solution, an ampule containing a fixed amount of mercuric nitrate solution and a second ampule containing s-diphenyl carbazone indicator dissolved in ethanol. After the sodium reaction has been allowed to run for one minute, the buffer solution is poured from the second tube into the first one which contains the sodium, aliphatic solvent, and oil sample. After a few seconds of shaking, the sodium reaction is quenched and any halogens that are present (now in ionic form) are extracted into the aqueous solution. The tube is allowed to settle for two minutes while the organic and aqueous phases separate. At this point, the dispenser cap on the tube is opened and the buffer solution is drained into the second tube until it is filled up to the 5 ml line. After the mercuric nitrate ampule is broken, the solution is well shaken. If any chloride, bromide, or iodide is present it will form an insoluble complex with the mercuric ions that are in solution. If these halogens are not present, then the mercuric ions will stay in solution. The last ampule to be broken contains the indicator s-diphenyl carbazone which forms a bright violet color in the presence of mercuric ions. If all the mercury has been tied up by halogen ions, then no color change occurs. Therefore, if the original halogen concentration in the sample being tested contains less than the threshold level, a violet color will result - if greater than the threshold level there will be no color change.

The kit worked well under laboratory conditions and a field study was undertaken to determine how well the kit would work with various users under varying conditions. Twenty test kits were sent to each of twenty-five companies which are involved in used oil testing. The representatives from each company were asked to use the kits to analyze various oil samples for chlorine content. After results were obtained (either greater than 1000 ppm or less than 1000 ppm), they were recorded and samples of each oil were returned to Dexsil for analysis by microcoulometry. After analysis in the laboratory, the results were compared to those that were obtained in the field using the test kit. The results for the two hundred seven samples that were returned to the laboratory were as follows:

RESULTS FROM 207 FIELD TESTED OIL SAMPLES

	<1000 ppm Cl by test kit	>1000 ppm Cl by test kit
50 samples >1000 ppm Cl by laboratory analysis	4	46
157 samples <1000 ppm Cl by laboratory analysis	152	5

TABLE I

Upon retesting with the kits those samples that gave incorrect results the first time, the following results were obtained:

RESULTS OF 207 SAMPLES AFTER RETESTING WITH TEST KIT

	<1000 ppm Cl by test kit	>1000 ppm Cl by test kit
50 samples >1000 ppm by laboratory analysis	0	50
157 samples <1000 ppm by laboratory analysis	154	3

TABLE II

The kit has been tested on four different types of oil and lubricating fluids.

FUEL OILS

The kit worked very well on all samples of #2 and #4 fuel oil. Several samples of #6 fuel oil were found to give low recovery values (70%) for chlorine. Different solvent combinations were tried and one has been found that gives greater than 90% chlorine recovery on all fuel oil samples that have been tested.

CRANKCASE FLUIDS

The kit has been found to work on all crankcase fluid samples that have been tried. It has been used on both natural and synthetic motor oils and has been found to work equally well on both. It has also been found to be accurate on oil obtained from diesel engines. We anticipate that this group of crankcase oils will constitute a majority of the oil samples that the kit will be used on.

HYDRAULIC FLUIDS

The kit has been shown to be accurate on all hydraulic fluid samples that have been tested to date.

CUTTING OILS AND METALWORKING FLUIDS

The test kit does not work on cutting oils or metalworking fluids that contain greater than 30% water. Although the kit will detect inorganic chlorine in these samples, it will not detect chlorinated organic compounds due to the interference of water with the reactions involved.

TEST KIT EFFECTIVENESS ON DIFFERENT OIL TYPES

Oil Type	Applicability of Test Kit
Fuel Oils (2,4, and 6)	Works well on all samples
Crankcase Oils (diesel and gas powered engines, natural and synthetic oils)	Works on all samples tested
Hydraulic Fluid	Works on all samples tested
Cutting Oils and Metalworking Fluid	Works on samples that contain less than 30% water.

TABLE III

Chlorinated Organic Compounds Which have been Shown to be Detectable by the Field Test Kit

Trichloroethane
Dichloroethane
Tichlorobenzene
Monochlorobenzene
Chlorooctadecane
Methylene chloride
Perchloroethylene
Freon (113)
Polychlorinated Biphenyls

TABLE IV

By verifying that the kit works on these nine compounds we have covered the major classes of chlorinated compounds that we expect to find in used oils. Volatile degreasing solvents (1,1,1 trichloroethane) are expected to be the most prevalent compounds. We have also covered chlorinated aromatics, more densely chlorinated aliphatics, cleaning solvents, refrigerants, and PCBs.

EPA AND INDUSTRY ACCEPTANCE

Those who participated in the field test reported that the kit was easy to use and that the results were easy to interpret. Some ambiguities in the instructions that resulted in some early false negative results have been cleared up. Since these changes were made, we have not recorded any false negative tests.

The field test kit, along with several other methods has been evaluated for the EPA by Research Triangle Institute of North Carolina. According to Alvia Gaskill of RTI, the kit will be included as an accepted method when the methods are published in the Federal Register and the SW-846 manual.

Some of the field test participants suggested that a quantitative kit that gives an actual number rather than a positive or negative result would be more helpful when the kit is used in a laboratory. In response to this request, Dexsil has adapted the Clor-D-Tect kit to include a small, disposable titration burette filled with standardized mercuric nitrate instead of the usual mercuric nitrate ampule. Using this burette, the sample can be titrated to a purple end point and the chlorine concentration is read directly off the titration burette. The new kit, called Clor-D-Tect Q4000, gives a quantitative reading in the range of 0-4000 ppm chlorine. The Clor-D-Tect 1000 can be used when a quick yes or no answer is desired while the Clor-D-Tect Q4000 gives an actual concentration when more exact data is requested about a sample.

CHEMOMETRICS AS APPLIED TO THE SCREENING OF HAZARDOUS WASTE

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ABSTRACT

This paper presents a unique and systematic approach for characterizing the basic physical properties of hazardous waste samples. The characterization is based on the accumulated results of specific qualitative "screening" techniques or procedures.

In screening hazardous waste, an experienced analyst evaluates the sample for pH, flammability potential, compatibility, specific gravity, cyanide, sulfide, oxidizer content, water solubility, and physical description. Looking at each of these tests individually may seem very basic but by using them together to make a decision is a sophisticated process. From these accumulated results, a pattern is generated that allows the skilled analyst to derive a conclusion. This pattern recognition is, in essence, Chemometrics.

INTRODUCTION

Chemometrics can be defined as a statistical approach of identifying environmental samples by using pattern recognition from multi-variate analyses.

The basic principles of chemometrics and pattern recognition can be applied to the screening of hazardous waste in much the same manner as a medical doctor uses chemometrics in his evaluation of a patient. This analogy is borrowed from Dr. Herbert Latinen of the University of Florida.

In medical diagnostics, a skilled doctor will run a series of basic tests such as temperature, pulse, observation of eyes, ears, throat, and testing of reflexes. The doctor uses these results to determine "wellness." By analogy, we will analyze a sample by checking the physical description, pH, flammability potential, compatibility, specific gravity, cyanide, sulfide, oxidizer content, and water solubility. In both cases, the results from the combined observations generate a pattern that allows each to derive a reasonable conclusion or diagnosis.

In screening hazardous waste, looking at each of these tests individually may seem very basic and unsophisticated. However, using them together to make a decision is, in reality, a sophisticated process. The use of screening tests is dependent on the analyst's training and experience. Just as anyone can do the things a doctor does, without his years of accumulated knowledge, they cannot make the correct evaluation or judgment.

The screening techniques give us an efficient description of the waste samples which aid in waste identification, profile verification, process compatibility, and safety in handling. They are, for the most part, preliminary qualitative tests developed from classical qualitative and quantitative chemistry techniques.

With the principle "waste is waste" taken into account, we recognize that a degree of variability and flexibility occurs in practically all waste streams. Depending on the generator's process, waste samples vary from solids to liquids to multi-phased sludges. Waste matrices vary within a process as well. We know, for example, that an API separator sludge does not look exactly the same with each shipment. The same holds true with flushings from a paintline and bottoms from a still. In each case, however, certain characteristics remain the same.

Much like the doctor that studies the patient's file, in evaluating a waste stream, the analyst studies the information supplied by the generator, the process generating the waste, and the past analysis that may have been done. By using pattern recognition, the analyst knows that a given waste stream must fit a certain mold, template, or grouping which is, in essence, chemometrics. Given the sample variability, it is the experience of the analyst, like the doctor, that allows him to make the pattern recognition decision as to whether the waste fits this "mold" or not.

Chemical Waste Management uses these screening techniques in all phases of sample analysis. The usefulness and how they apply can be seen in the following scenarios:

Preshipment
Shipment Receipt
Waste Processing
Remedial Cleanups

PRESHIPMENT

In the preshipment or sales sample laboratory, the waste screening evaluation is multi-functional:

First, through pattern recognition, the combined screening analysis gives the experienced analyst a good indication of whether the sample fits the pattern of the process that is being profiled by the generator. This is a necessary first step in the evaluation of a new waste stream. If, for example, a generating process is organic in nature and we receive an aqueous sample, it is evident that the sample does not fit the correct pattern. These types of discrepancies can be detected during this initial pattern recognition evaluation.

Secondly, the results give an important data base which is used in the chemometrics of future shipments.

Thirdly, the screening results are used in conjunction with the waste profile in prescribing additional analysis that may be required in making the proper waste management decisions. This additional analysis may include testing for PCBs, solvents, anions, and metals, compatibilities, specific gravity, and heat value.

INCOMING WASTE RECEIPT

In the production-oriented incoming waste receipt phase, a laboratory must have quick and reliable screening techniques for identifying incoming waste shipments. Not only do we identify that the incoming waste matches the waste described on the manifest and waste profile, we also ensure that the originally designated management decision can still be utilized for that waste shipment. Ensuring process compatibility and employee safety are also determined at that time.

By comparing the screening results with the preshipment results, the Waste Profile Sheet and manifest, the analyst can readily identify that the incoming waste either fits or does not fit the pattern appropriate for that waste stream.

PROCESSING

In the processing or treatment phase of a waste, it is necessary to have good control over the quality of the material being processed. When a fuel is being blended, for example, the screens help us determine if a particular blend of fuels fits the pattern for a good fuel's candidate (i.e., single phased, insoluble, lighter than water, and a positive flammability potential.

REMEDIAL CLEANUPS

In the remedial cleanup phase, these screens have proven to be invaluable in the field. Where little or no data is available on the waste, the operator is faced with an almost impossible task of identifying basic waste properties, segregating incompatible wastes, and manifesting waste shipments. With these basic screening techniques, experience, and knowledge of pattern recognition, the unknown waste can be successfully, quickly, and safely identified into basic waste types. During this identification process, the skilled analyst can segregate the unknown samples into one of several categories such as:

Inert
Flammable
Base
Acid
Oxidizer
Water Reactive
Chlorinated
Cyanide
Sulfide
Oil

Chemical Waste Management supports the concept that all methods for waste analysis must show that the analyst and the method have a prescribed degree of precision and accuracy.

The CWM Quality Control Policy requires that every tenth sample must be duplicated and then fortified with a known standard. The duplicate results show the precision of the analyst and the method for the specific matrix being tested. The fortification shows not only that the procedure works, but that it is accurate. Because the sample sequence is random, the procedure and the analyst are tested against all types of matrices.

Right now, a series of these currently used screens are in the balloting process at ASTM, Committee D-34 on Waste Disposal. ASTM is a highly recognized and accredited society, made up of people in the industry who share common needs. The screening techniques will be approved only by consensus ballot through ASTM.

In conclusion, these screens, in conjunction with a very sophisticated pattern recognition technique or "chemometrics," have proven their usefulness. Continual efforts need to be made in the development of new and more powerful screening techniques. A quick, precise screen for PCBs, Dioxin, or Lead would be a useful tool. Where we are now burdened with exorbitant costs and time consuming analytical tests that in the end tell us that PCBs are not detected, a well-developed screen could parallel these results. Any loss of sensitivity would be compensated much more efficiently by an expanded sample base, and the proverbial "hot spots" could be more clearly defined.

In addition, the development of computer software and even the use of artificial intelligence can possibly aid this pattern recognition process of hazardous waste.

A NOVEL METHOD FOR DIGESTION OF ORGANICS FOR DETERMINATION OF ARSENIC, MERCURY AND SELENIUM; A NEW METHOD FOR DETERMINATION OF MERCURY IN ORGANICS AT THE LOW PPB LEVEL

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ABSTRACT

This presentation consists of two parts.
Part 1: To those familiar with the subject, it is well known that the elements arsenic, mercury and selenium are easily lost when digested in organic systems. The reducing conditions created in the organics decomposition are probably the cause of the losses. A novel, yet simple system is described which eliminates the problem. Data are given to illustrate the effectiveness of the system.

Part 2: A new method for determination of mercury in organics at the low ppb level is described.

The method is based on heating the sample with phosphoric acid, collecting the atomic mercury produced on a gold-plated Nichrome wire, followed by thermally releasing the mercury through a UV spectrophotometer. The method has been successfully applied to samples of varying volatility such as light gasoline to heavy fuel oil. Experiments indicate that the method can be used on samples containing dimethyl mercury.

INTRODUCTION Part 1

Usually, wet oxidation of an organic sample is carried out by digesting the sample in a beaker or flask with H_2SO_4 plus an oxidizer such as HNO_3 or H_2O_2. Most metals are retained in the digestion container. However, As, Se and Hg are lost, even with an excess of oxidizer. What happens is that reducing conditions occur in the digesting medium with the formation of the volatile hydrides of As and Se along with atomic Hg.

To get around this problem, we devised a rather simple but unique digestion system which is illustrated in figure 1.

We have used the system to digest up to 1 gram of organic samples and usually requires overnight digestion. However, for 0.5 grams or less, 2-3 hours is sufficient. Table 1 contains data collected on CONOSTAN organo methallic standards using the system. After digestion, the solutions are transferred to volumetric flasks and analyzed by the hydride technique for As and Se and by the cold vapor technique for Hg. Assuming a final volume of 100 mL, one could expect a determination limit of around 0.5 ppm on a 1 gram sample. To achieve this however, ultra pure acids would be necessary because of blank problems with ordinary reagent grade acids.

CONOSTAN is a registered trademark of CONOCO, Inc.

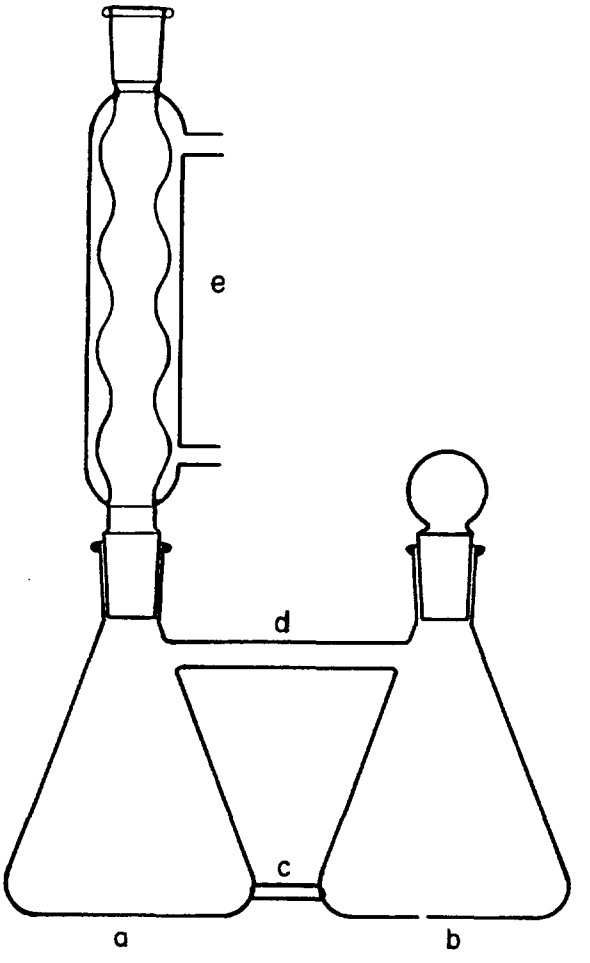

FIGURE 1. SPECIAL DIGESTOR

Explanation: (a) Sample + H_2SO_4 + HNO_3, (b) HNO_3, (c) supporting glass rod, (d) connecting glass tubing, and (e) water-cooled condenser. Sample is digested in (a). The HNO_3 in (b) provides a source of oxidizer in the condenser so that species which are reduced in (a) will be re-oxidized in the condenser.

TABLE 1

DATA FOR 100 PPM CONOSTAN METALLO ORGANIC STANDARDS

ELEMENT	MEAN	STANDARD DEVIATION	n
Mercury	100.0	2.0	16
Arsenic	98.5	1.3	4
Selenium	101.7	1.5	4

INTRODUCTION Part 2

Because of environmental concerns, the determination of mercury at very low levels has become increasingly important. Last year, when analyzing a sample of residue from a quench column from one of our chemical plants, we discovered (unexpectedly) that the sample contained percentage quantities of mercury. Where did the mercury come from? Before this question could be answered, a method was needed for the determination of mercury in hydrocarbon streams of varying volatility (light gasoline to heavy fuel oil).

We believed that a method developed in 1975, based on heating the samples in an induction furnace, would not work on the highly volatile samples[1]. Wet oxidation was another possibility but, based on our past experience, we knew that species such as mercury, arsenic, and selenium are easily lost in the digestion procedure. When digesting organics, even when one has an excess of oxidizing acid (HNO_3), the presence of carbonaceous material in the digesting container will result in chemically reducing conditions. These reducing conditions cause the formation of volatile species such as Hg°, AsH_3, and SeH_2, which are lost. Normally, one would want to avoid this type of phenomenon, but in this project, we decided to take advantage of it.

RESULTS AND DISCUSSION

It is well known that Hg° readily amalgamates with gold[1,2]. When a gas stream containing Hg° is passed through a tube containing a coil of gold-plated Nichrome wire at a flow rate of 200 mL/min or less, practically all of the mercury will be trapped on the coil. The coil used is a 6-foot, 22-gauge, hollow, helical-shaped Nichrome wire of 6 ohm resistance, having a pure gold plating thereon of approximately 5 grams. The mercury can be released from the coil by passing current (6 amps) through the coil for sufficient time (8-20 sec) to heat the coil to 500°-600°C. The gas stream can then be directed into a UV spectrophotometer where the Hg° vapor absorbs ultraviolet radiation at 2537 Angstroms. This scheme of analysis results in an extremely sensitive method for detecting Hg° (ng quantities).

Figure 1 illustrates one of the Hg° collecting coils used in this work.

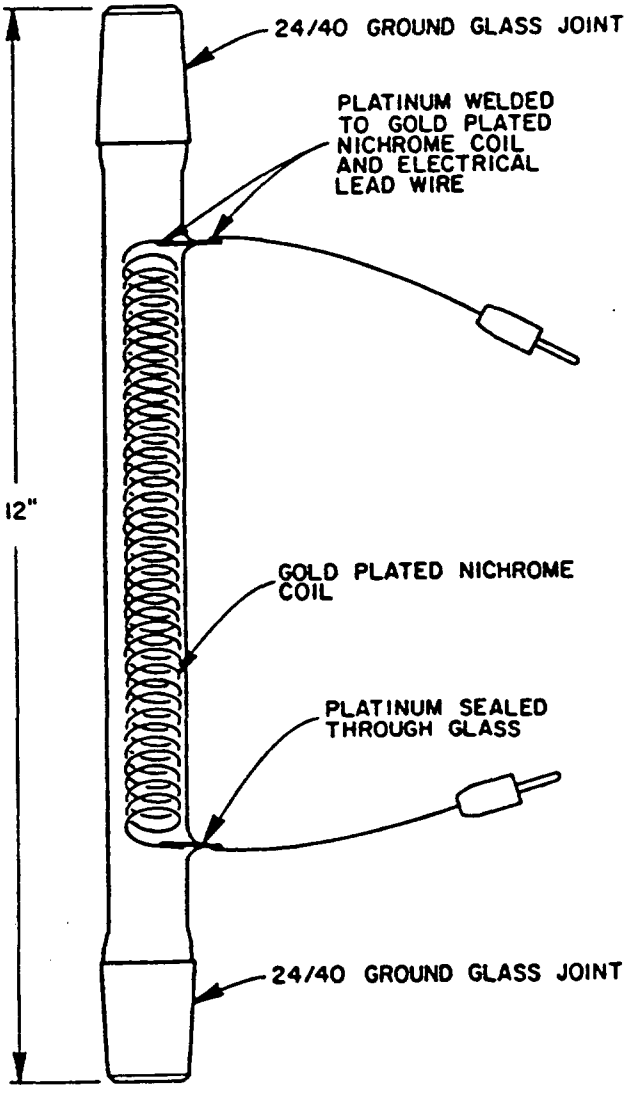

FIGURE 1: MERCURY COLLECTING COIL

In practice, one weighs a sample into a 125-mL Erlenmeyer flask which contains 2 mL of concentrated H_3PO_4. A collector coil is fitted onto the flask and heat applied. The heat, plus reducing conditions in the flask, causes Hg° to be formed. The Hg° then diffuses upward and is amalgamated with the gold. After washing and drying the coil, it is then ready for flashing off the Hg° onto a second coil followed by flashing the second coil into a Coleman MAS 50 mercury analyzer. Figure 2 illustrates the analytical set up. The analyzer is calibrated by injecting saturated Hg° in air onto a coil and measurement of peak height or area when the Hg° is flashed through the analyzer. The sensitivity is typically 1 ng Hg°/recorder division (100 divisions full scale).

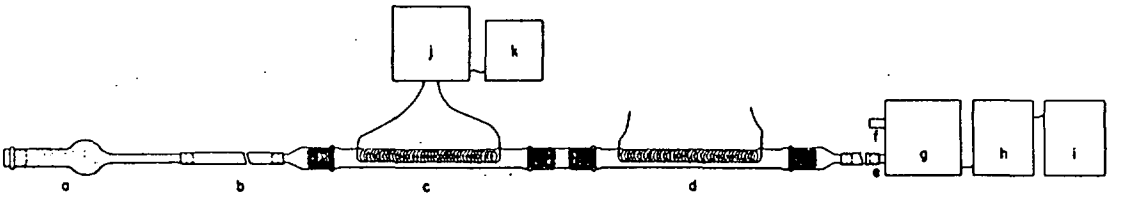

FIGURE 2: ANALYTICAL SET UP

a. Drying tube loosely packed with silver wool or wire.
b. Tygon tubing.
c. Sample collector coil.
d. Second collector coil.
e. "In" to analyzer.
f. "Out" of analyzer.
g. Coleman MAS 50 mercury analyzer.
h. Recorder.
i. Integrator.
j. Variac.
k. Timer.

Table 1 contains data on typical samples, standards prepared from CONOSTAN metallo organic standard, and National Bureau of Standards SRM 1630 (mercury in coal).

TABLE 1

Sample Description	ppb Mercury
Light fuel oil	30,36,32
Heavy bottoms	15
Drip oil	35
Heavy feed	28
Natural gasoline feed	59
Condensate No. 1	22
Condensate No. 2	50
Condensate No. 3	56
57 PPb std (from CONOSTAN)	55,57
126 PPb std (from CONOSTAN)	138,102
520 PPb std (from CONOSTAN)	512,481,533
130 PPb std NBS 1630 std	136,130,138,133,116,137

EXPERIMENTS WITH DIMETHYLMERCURY

We decided to determine what happens to dimethylmercury (dmm) when passed over a gold coil. First, dmm vapor was passed directly through the detector, and there was practically no response. Then some vapor was passed through a coil, and when flashed, a large peak was observed. In fact, it required several heating cycles to clean the coil. Then the question arose as to the efficiency of a coil for trapping dmm. A diluted solution of dmm in isooctane was prepared to test the efficiency. Volumes of 10 µL were used. The efficiency varied from 48 percent trapped at 120 mL/min to 75 percent at 50 mL/min. So one can say that the coils are definitely less efficient for trapping dmm compared to Hg°.

Another experiment was conducted to determine the efficiency of trapping dmm under the conditions of the method. An aliquot of the diluted standard mentioned above was further diluted in pure mineral oil. A sample of this (200 µL) was run through the whole procedure. Ninety-one (91) percent of the expected mercury was found (34 ng expected, 31 ng found).

PROBLEMS ENCOUNTERED

Several experiments which were done and did not work are listed below:

1. Wet digestion in a special digestor. This failed because of a combination of high blanks plus lack of sensitivity by the atomic absorption finish.

2. Combustion of samples in Parr bombs containing dilute HNO_3. Low results were obtained.

3. Using H_2SO_4 instead of H_3PO_4 in the digestion flasks. Two problems were encountered using H_2SO_4: one, some batches of H_2SO_4 were contaminated with mercury, and second, when fumes of H_2SO_4 condense on the coils, it seems to inhibit the amalgamation process. This could be due to formation of Hg ions which are not amalgamated.

4. Failure to use a second coil to collect Hg° from the digestion collector coil. It was observed that with some samples, one or more broad peaks were produced when flashing the digestion collector coil directly into the analyzer. Furthermore, the sizes of the peaks were not reproducible. It was determined that flashing the digestion collector coil through a second coil still gave a broad peak, but since the second coil is essentially 100 percent effective in trapping Hg° released from the first coil, the material causing the broad peak is not Hg°. A Hg° peak is obtained when the second coil is flashed. And as can be seen from the data, the reproducibility is generally good. The idea of using the second coil was fundamental in the success of the work. The material causing the broad peak is probably UV absorbing organics which are not easily washed off the coil in the procedure.

EXPERIMENTAL

Reagents and Equipment

1. 125-mL Erlenmeyer flask with 24/40 joints.

2. Concentrated H_3PO_4.

3. Gold-plated Nichrome coils enclosed in glass with 24/40 male joints (see Figure 1).

4. Coleman MAS 50 mercury analyzer. Install a short plastic tube containing a 1-inch, 20-gauge needle on the exit line of the analyzer.

5. Mercury in air standard. This is done by transferring a drop of mercury to a small plastic bottle fitted with a septum. The small bottle is glued into a larger bottle filled with water. A small hole in the water-filled bottle allows for a thermometer to be inserted for temperature measurement. To equalize the pressure, a syringe needle should be inserted through the septum. See Table 2 for Hg-temperature relationships.

6. Coupling glassware.

   a. Fitting with 24/40 female joints on each end, 1/4 inches long.

   b. Two female 24/40 joints pulled down to 1/4 inch. One is used to connect to the inlet of the mercury analyzer. The other is connected to a drying tube filled loosely with silver wool or wire. This is connected (via Tygon tubing) to a collector coil and used to prevent contamination of the coil when calibrating.

7. Variac - LUTRON Model PD-100 POWER DIAL, 120V, 60 amps. Set at 2.9.

8. Timer - allows current through coils for a set time either automatically or manually.

9. Recorder - Omni Scribe set at 0.1V full scale, chart speed 0.2 in/min.

10. Integrator - Hewlett-Packard HP 3390 A. Set the parameters as follows:

    ZERO = 0, -1.8
    ATT = 7
    CHT SP = 0.5
    PK WD = 0.32
    THRSH = 2
    AR REJ = 1000

PROCEDURE

Before beginning an analysis, flash the coils to be used until a peak height of one recorder division or less is obtained. Cap the coils until ready to use. Also, clean the Erlenmeyer flasks to be used by adding 20 mL OF 1:4 HNO_3 and boil for a few minutes. Rinse with deionized water, ethyl alcohol, and dry. Cap until ready to begin an analysis.

1. Into a 125-mL Erlenmeyer (with 24/40 joint) containing 2 mL of concentrated H_3PO_4, accurately weigh 0.25 g of sample. Stopper and mix by swirling.

2. Remove stopper and install a collector coil; set on a Corning PC-100 hot plate (in hood) which has been turned on for at least 10-15 minutes at a setting of 3.

3. After 20 minutes, turn the hot plate to "high" and allow to digest another 60 minutes.

4. Remove apparatus from the hot plate. Remove collector coil and wash with ethyl alcohol, followed by deionized water and again with ethyl alcohol. Set in a 100°C vacuum oven. After 5 minutes, remove and cap.

5. Flash the second collector coil to remove any contamination. Then after 5 minutes, connect the coil from the digestion onto the second coil. (See Figure 2 for apparatus setup)

6. Connect the electrical leads to the digestion coil and flash for 20 seconds. With some samples, a broad peak will result on the recorder, but it is not mercury.

7. When the recorder has returned to baseline, reconnect the wiring to the second coil and flash for 20 seconds.

8. Measure the peak height or note the peak area from the integrator and record it.

STANDARDIZATION

Before starting, make sure collector coils are not contaminated with mercury.

1. Using the mercury in air standard (note standard temperature), remove 1 cc (1 mL) with a gas syringe and slowly inject into the Tygon tubing in front of (upstream) the digestor collector coil.

2. After 15-20 seconds, flash (for 20 seconds) the digestor collector coil onto the second collector coil.

3. After 15-20 seconds, flash (for 20 seconds) the second collector coil through the analyzer.

4. Measure the peak height or note the peak area from the integrator.

5. Repeat Steps 1 through 4, except inject 3 cc of standard.

CALCULATIONS

$$\text{FACTOR (F)} = \frac{\text{ng Hg from Table 2}}{\text{Recorder divisions or peak area}}$$

The factor for the 1 and 3 cc injections should be nearly identical. Average the two values.

$$\text{ppb Hg in sample} = \frac{\text{(F) (Recorder divisions or peak area)}}{\text{Sample weight (grams)}}$$

Obviously, if one uses peak height or area for calculating F, one should use the same units for calculating the sample concentration.

REFERENCES

[1]J. W. Wimberley, Laboratory Practice, 24, 590 (1975).

[2]J. W. Wimberley, Anal. Chim. Acta., 76, 337-43 (1975).

TABLE 2

MERCURY CONCENTRATION/TEMPERATURE °C

Temperature °C	Nanograms/cc
15	8.6
15.5	9.0
16	9.4
16.5	9.8
17	10.2
17.5	10.6
18	11.1
18.5	11.6
19.0	12.1
19.5	12.6
20.0	13.2
20.5	13.7
21.0	14.3
21.5	14.9
22.0	15.5
22.5	16.2
23.0	16.9
23.5	17.6
24.0	18.3
24.5	19.1
25.0	19.8
25.5	20.7
26.0	21.5
26.5	22.4
27.0	23.3
27.5	24.3
28.0	25.2
28.5	26.3
29.0	27.3
29.5	28.4
30.0	29.5

ENVIRONMENTAL SEDIMENT EXTRACT ANALYSIS USING HYPHENATED GAS CHROMATOGRAPHY-ATOMIC EMISSION SPECTROSCOPY (GC-AES)

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ABSTRACT

Element specific analysis of complex environmental wastes improves the compound or class identification of unknown constituents. Fused silica capillary column gas chromatography is interfaced with an experimental atomic emission detector. Detection limits in the 1 to 50 picogram range of chlorine, bromine, sulfur, phosphorus, nitrogen, oxygen and other hetroatoms greatly enhances the information as demonstrated on sediment samples.

The sediments, gathered from a river bank located within a chemical dumpsite and from an agricultural area recently subjected to pesticide application, had previously been analyzed by GC-FTIR and GC-MS with 25% to 33% inconclusive confirmation of class. Information from GC-AES reduces the percent of inconclusive confirmations by compound class and gives greater confidence for postive identification of compounds.

DEVELOPMENT OF AN ENVIRONMENTAL MONITORING TECHNIQUE USING SYNCHRONOUS EXCITATION (SE) FLUORESCENCE SPECTROSCOPY

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ABSTRACT

Environmental monitoring of discharge plumes (surface and groundwater) and effluents generally involves extensive analyses for specific analytes. This monitoring frequently employs GC, GC/MS, ICAP or AA techniques. These techniques usually require extraction procedures followed by analyses for specific analytes. When monitoring complex mixtures, these procedures can become more difficult, time consuming, prone to analytical and interpretational error and limited by analytical thresholds to the ppb and ppm range. When environmental discharges occur, the dilution process exacerbates analytical problems by attenuating compounds through the environmental media and lowering concentrations. An alternative/supportive technique in these situations is the use of SE fluorescence spectroscopy. This technique can be used for fingerprinting effluents and contaminant mixtures in the ppt range and can be used to evaluate the presence of mixtures (e.g. effluents) and dilution plumes.

An SE fluorescence technique was developed specifically for monitoring effluent from a facility manufacturing a variety of dyes and ancillary products. Prior to discharge, the effluent was treated in a biological and charcoal (PAC) waste treatment system. The effects of sampling (composite, grab), sample storage, fluorescence properties of the effluent (spectral fingerprint) and fluorescence characteristics of the effluent in ambient waters was determined. Consequently, a method was established which "fingerprints" effluent at dilutions of 1:1000, can identify effluent at dilutions of 1:4000, and has fingerprinted similar source material in a rivershed adjacent to the site. This rapid sample approach can be used to fingerprint and monitor a variety of environmental discharges or plumes when appropriate contaminants are present.

INTRODUCTION

The State of New Jersey, Department of Environmental Protection (N.J.DEP) administers industrial discharge permits through the State NJPDES program. One of the permitted facilities manufactures a variety of dyes and ancillary products and employs

an ocean outfall. Concerns were expressed by the public about the environmental release of effluent from the facility. Consequently, it was necessary to develop a sensitive, specific method for the effluents identification and quantitation. Earlier studies indicated that the effluent contained various fluorescing components including azo dye and aniline derivatives. Therefore, the use of fluorescence spectroscopy was evaluated for monitoring the industrial effluent.

The study was established in several phases. The first phase of the study determined the fluorescence properties of the effluent to ascertain whether a characteristic spectal fingerprint was obtainable and whether a simple, rapid method based on Synchronous Excitation (SE) Fluorescence could be used to monitor the discharge. During this phase, the effects of sampling (composite vs. grab), sample storage, and the effects of dilution in lab and sea water were determined.

During the course of the study, Rhodamine WT was added to the industrial discharge as part of a hydrological study to determine the dilution characteristics of the waste and its fate. Consequently, the second phase of the study evaluated the use of Synchronous Excitation Fluorescence Spectroscopy to monitor the industrial discharge for rhodamine dye tissues.

The final phase of the study has continued to evaluate the SE fluorescence method established with samples taken a year later and in sea water dilutions.

WHY FLUORESCENCE SPECTROSCOPY WAS CHOSEN

Ultraviolet fluorescence spectroscopy is a useful analytical technique for the measurement of many hazardous materials. It is exceptionally sensitive and often more specific than other spectroscopic methods such as ultraviolet, visible, and infrared absorption techniques. Kullbom, et. al.[1], used fluorescence spectroscopy to analyze for phenols and lignins in water. Other investigators[2-6] used it to measure petroleum and non-petroleum hydrocarbons in water. Some of the later investigators[2,3,5,6], extracted the toxicants from water with solvents prior to measurement. Kullbom, et. al[1] and Frank[4], measured toxicants directly in water. All of these investigators used conventional single wavelength excitation (SWE) techniques.

SWE fluorescence spectroscopy is not satisfactory for in-situ measurements of hazardous materials in water. Spectra derived from use of the SWE technique are distorted by Rayleigh-Tyndall and Raman scatter radiations, especially when these spectra are

obtained from samples containing only mg/l of toxicants. Consequently, the use of "Synchronous Excitation" (SE) fluorescence spectroscopy for rapid in-situ quantification of hazardous materials in water has been demonstrated by several authors[7,8] to yield substantially improved spectral resolution.

The authors demonstrate that this technique also yields spectra free of Rayleigh-Tyndall and Raman scatter when used for in-situ quantification of hazardous materials in water.

Differences between SWE procedures and the SE technique and the advantages of the latter for in-situ quantification of hazardous materials in water are discussed within the context of a three dimensional coordinate system. This system was previously described by Frank[9], and Frank and Gruenfeld[10]. Three interdependent variables that are inherent to fluorescence spectroscopy are used as axes: (1) excitation wavelength (x), (2) emission wavelength (y), and (3) fluorescence intensity (z). Within this context, the fluorescence properties of hazardous materials are viewed as "total fluorescence spectra" and the SE and SWE fluorescence approaches are readily compared.

A three dimensional total spectrum of a compound that resembles a mountainous region is shown in Figure 1. The two mountain peaks (solid lines) are in fact the fluorescence maxima of this compound. SWE spectra are two dimensional slices through the three dimensional spectrum, parallel to the (y) axis. According to Lloyd[7], such spectra provide only limited useful information. SE fluorescence spectra, however, are slices oriented at a 45° angle to the (x) and (y) axes and often yield more useful information.

The ability of SE fluorescence spectroscopy to avoid Rayleigh-Tyndall and Raman scatter distortions of spectral profiles is demonstrated within the context of the three dimensional coordinate system. The combined effect of these scattering phenomena can be viewed as resembling a three dimensional "interference wedge" that bisects total-fluorescence spectra (Figure 2). Although this interference wedge causes detrimental spectral distoritions when using SWE techniques, it can be avoided by using the SE procedures (Figure 3). Rayleigh-Tyndall (reflected) scatter radiation is especially troublesome when measuring natural waters that contain suspended particulates. Spectra obtained with the SE and SWE procedures are contrasted in Figure 4, a synthetically prepared sample of phenol in blackish marsh water containing suspended particulates was measured. This example illustrates the obvious advantage of the SE technique, i.e., its elimination of scatter interferences.

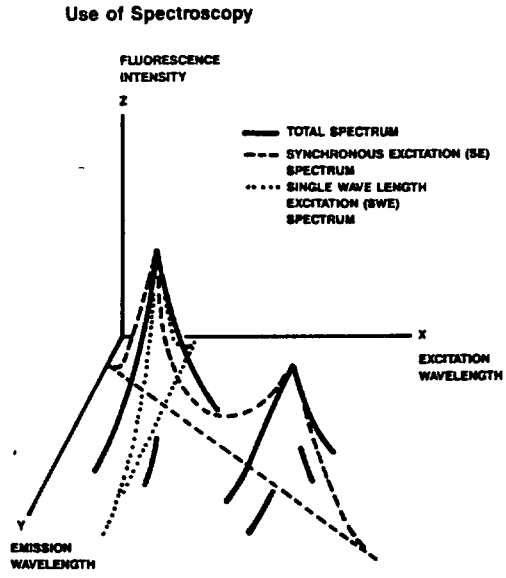


FIGURE 1: Comparison of SE, SWE and Total Fluorescence Spectra Within the Context of the Three Dimensional Co-ordinate System

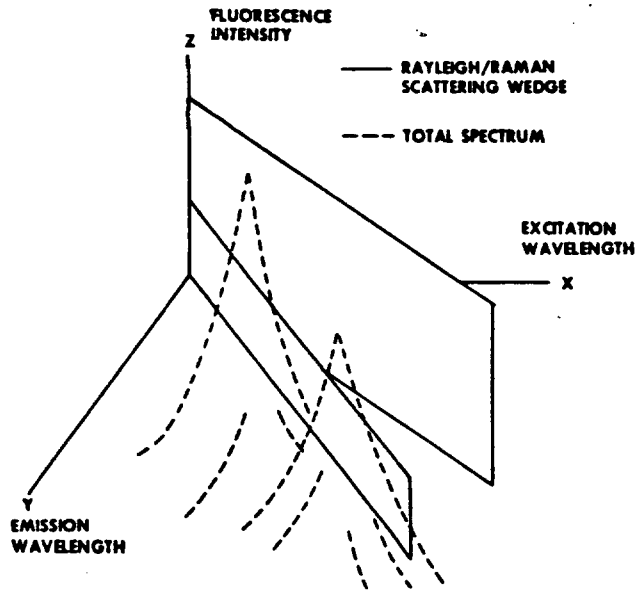


Figure 2: A Three Dimensional Presentation of a Rayleigh-Tyndall/Raman Radiation "Scattering Wedge", Superimposed on a Total Fluorescence Spectrum of a Hazardous Material

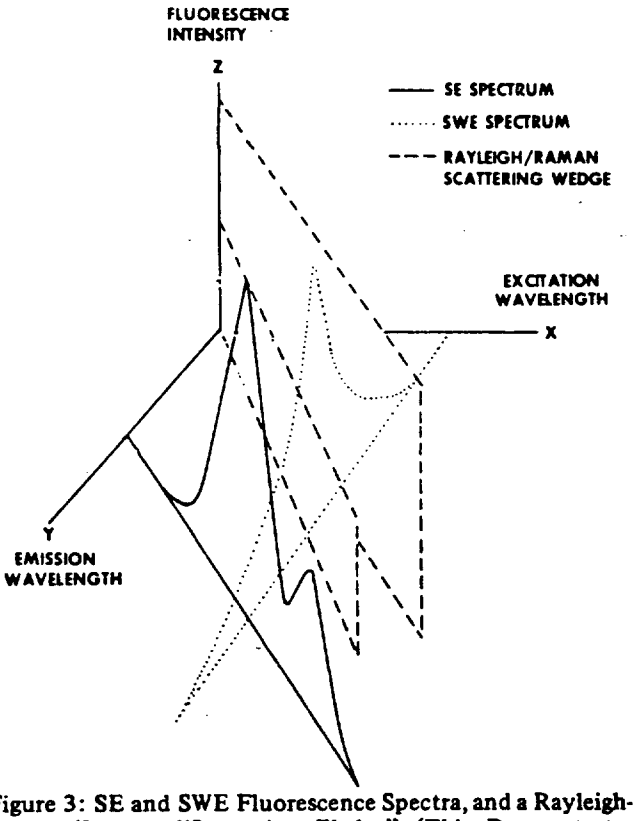


Figure 3: SE and SWE Fluorescence Spectra, and a Rayleigh-Tyndall/Raman "Scattering Wedge" (This Demonstrates that the Scattering Wedge is Readily Avoided by Use of the SE Technique)

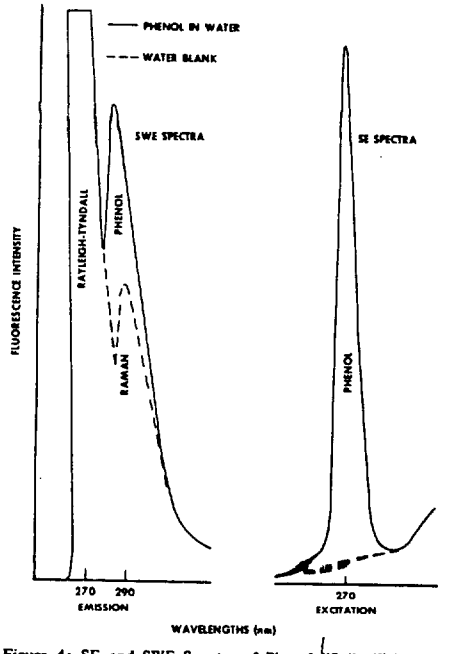


Figure 4: SE and SWE Spectra of Phenol "Spiked" into Water Obtained from a Marsh in Sayreville, New Jersey (The Water Contains Suspended Colloidal Particulates. Phenol Concentration is 1mcl/l)

Raman scatter interferences emanating from water are less pronounced than Rayleigh-Tyndall scatter. They become apparent, however, when using high instrument sensitivities for measuring low toxicant concentrations. The SWE technique fails to avoid this scatter problem, while the SE procedure avoids it readily. Elimination of scatter interferences by the SE procedure also minimizes the need for blank or backgrounds sample analyses. SWE techniques require blank sample measurements whenever instrument parameters are changed resulting in a large number of additional and time consuming analyses.

Elimination of scatter interferences by using the SE procedure also permits increased sensitivity. While actual detection limits obtained with the SE procedure are determined by a fluorescence instrument's design, SWE techniques detection limits are severly influenced by scatter interferences. Detection limits of several hazardous materials in distilled water, determined by the SE technique, are listed in Table 1.

The effect of indigenous fluorescent species in natural waters, on analysis results is illustrated in Figure 5. The SE fluorescence spectrum of aniline in Raritan River water is shown in Figure 5a; while the SE fluorescence spectrum of aniline in Arthur Kill River water is shown in Figure 5b. These five represent heavily trafficked and bodies of water in New Jersey. The SE fluorescence spectrum of aniline in distilled water is shown in Figure 5c. The spectrum of the latter figure was used as standard for quantifying aniline in Figures 5a and 5b; recoveries were 96% and 94% respectively. In effect, the aniline in distilled water solution was used as the standard for quantifying the aniline content of synthetically prepared samples containing this hazardous material in two polluted waters.

In addition, the use of synchronous excitation fluorescence spectroscopy has also been preferred for monitoring Dye Tracers in hydrology studies. Andre et. al.[11] points out that this techniques gives a preceptible increase in sensitivity and reduces experimental errors in the qualitative and quantitative determination of Rhodamine (G,B, and WT) in water samples.

METHODS

1. OVERVIEW

Synchronous Excitation Fluorescence Spectroscopy (SEFS) was used as the analytical procedure for the direct in-situ measurement of fluorescing industrial discharge (effluents) and Rhodamine Dye

Tracer in Ocean Surf Water Samples. The SEFS method was also used as an analytical procedure for direct in-situ screening of hazardous materials in water and includes the following:

2. CAUTIONS AND LIMITATIONS:

2.1 All glassware must be rinsed (three times) with carbon filtered, distilled water and air dried prior to use.

2.2 Preparation of standards and samples should be performed inside a fume hood to minimize exposure of laboratory personnel to hazardous substances.

2.3 All measurements of standard and sample solutions and solvent blank must be conducted under identical instrumental conditions.

2.4 The instrument recorder baseline should be deflected at least ten percent of full scale to avoid erroneous zero measurements.

3. APPARATUS AND REAGENTS:

3.1 APPARATUS

1. Beaker, 50 ml
2. Fluorescence spectrophotometer equipped with dual monochromators for synchronous measurement (Perkin-Elmer Model MPF-44B)
3. Micropipets, digital adjust (Scientific manufacturing Industries, or equivalent)
4. Quartz cuvette
5. Volumetric flasks, assorted volumes

3.2 REAGENTS

1. Distilled water, carbon filtered
2. Pure material for standard preparation

4. PROCEDURES:

4.1 STANDARD PREPARATION

1. Place 90 ml of distilled water into a 100 ml volumetric flask.
2. Weigh 20 mg of the standard material and quantitatively transfer the material to the volumetric flask.
3. Stopper the first and invert repeatedly to ensure uniform mixing.

TABLE I. DETECTION LIMITS FOR SEVERAL HAZARDOUS MATERIALS IN WATER

Hazardous Material	Detection Limit (ppm)
Aniline	0.005
O-Cresol	0.1
Dodecyl Benzene Sulfonic Acid	0.1
Naphthalene	0.02
Naphthenic Acids	0.1
Phenol	0.01
Quinoline	0.005
Resorcinol	0.02
Styrene	0.005
Toluene	0.1
Xylene	0.1
Xylenol	0.1

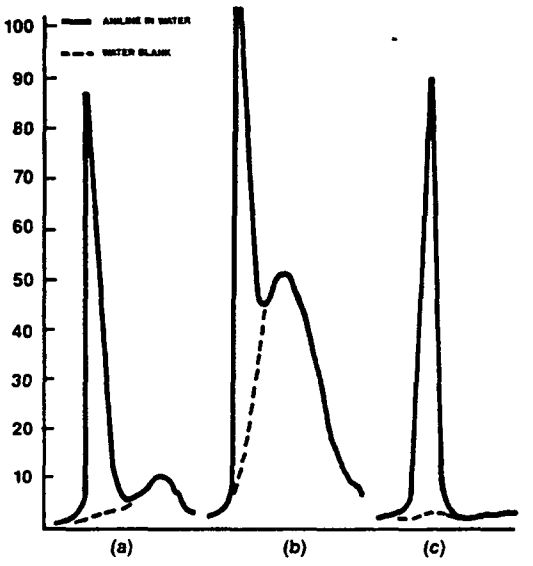


FIGURE 5: SE Spectra of Aniline "Spiked" into River Waters (A) and (B) and Distilled Water (C). Aniline in Water Concentration is 1 mcl/l. The Waters used to Obtain (A) and (B) were Collected from the Raritan and Arthur Kill Rivers in New Jersey, Respectively. Both Rivers are Subject to Heavy Marine Traffic and Chronic Pollution from Petroleum and Industrial Chemicals

4. Fill the volumetric flask to the fiducial mark with water; the concentration of the stock solution is 200 mg/L.
5. By dilution, prepare a calibration solution and establish a calibration range incorporating the expected concentration of the samples.

4.2. SAMPLE PREPARATION

1. Samples must be stored in glass bottles and refrigerated prior to analysis.
2. Direct in-situ measurement of hazardous materials precludes the need for extensive sample preparation preceeding fluorescence analysis.

4.3 FLUORESCENCE ANALYSIS

1. Use the following instrumental conditions:

Excitation wavelength	200 nm
*Initial wavelength interval (Δ)	20 nm
Initial emission wavelength	200 nm
Excitation slit width	10 nm
Emission slit width	10 nm
Wavelength drive	Excitation
Mode	Dual
Amplifier dynode voltage	Ratio
Recorder power	Servo

*NOTE: The initial wavelength interval of 20 nm requires an initial emission wavelength of 220 nm. The emission wavelength is adjusted to 230 nm, 240 nm, etc., to record synchronous excitation spectra for each set of wavelength intervals (30, 40, 50, etc.). The excitation monochromator is scanned in synchronization with the emission monochromator over the wavelength range of 200 nm - 500 nm to obtain a complete fluorescence spectrum of the test material.

2. Select the best wavelength interval to yield optimum spectral results.

1. Observe the synchronous excitation spectra for each set of wavelength intervals (20, 30...., 100). The best wavelength interval is a compromise between maximum fluorescence intensity and optimum peak resolution.
2. Establish the best wavelength interval and record the synchronous excitation spectra of the calibration solution and calibration standards under these conditions.

3. Record the synchronous excitation spectra of the solvent blank (distilled water), the sample solution(s), and the sample blank (if available) under identical instrumental conditions.
4. Assure instrument stability by scanning the calibration solution and solvent blank following each fifth sample measurement.

DISCUSSION/RESULTS

PHASE I

The primary objective in Phase I was to determine whether a simple and rapid method based on SEFS could be used for monitoring industrial discharge (effluent) in ocean water without the addition of dye tracers. The approach was based on the assumption that the fluorescing components in the industrial discharge would provide a spectral fingerprint traceable to the source in ocean water. Furthermore, it was anticipated that the fluorescence fingerprint could be used to follow the migration of chemical discharge plumes from the source and determine its ultimate fate along the New Jersey shore.

COMPARISON OF SINGLE WAVELENGTH EXCITATION AND SEFS

Figure 6 illustrates the type of fingerprint obtained by using single wavelength excitation to obtain a fluorescence fingerprint. The first peak in Figure 6 is caused by Rayleigh-Scatter interference in the Single Wavelength excitation spectrum. Also, note the increased spectral resolution (and improved fingerprint pattern) in the SEFS spectrum. Figure 7 showns the same effluent grab sample but the spectrum was obtained using the SEFS method. Note the absence of spectral distortions and interferences in the SEFS spectrum.

Figure 6 illustrates the type of fingerprint obtained by using single wavelength excitation to obtain a fluorescence fingerprint. The first peak in Figure 6 is caused by Rayleigh-Scatter interference in the spectrum. Figure 7 showns the same effluent grab sample but the spectrum was obtained suing the SEFS method. This spectrum exhibits increased spectral resolution and the fingerprint represents a summation of all fluorescing materials under these instrument and effluent conditions. This synchronous fluorescence scan is a reflection of the batch production processes at the plant. Although specific facility production varied over time, a combination of factors - waste treatment system, constrancy of chemical groups (i.e. from dyes, phthalates), low process flow, tended to yield

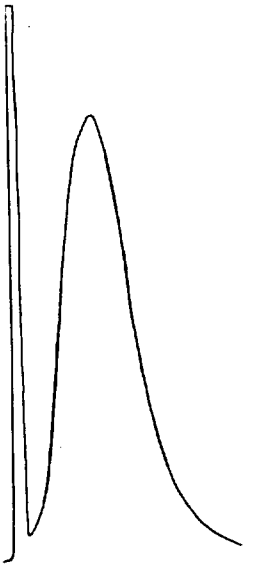


FIGURE 6. SINGLE WAVELENGTH EXCITATION FLUORESCENCE SPECTRUM OF GRAB 1.

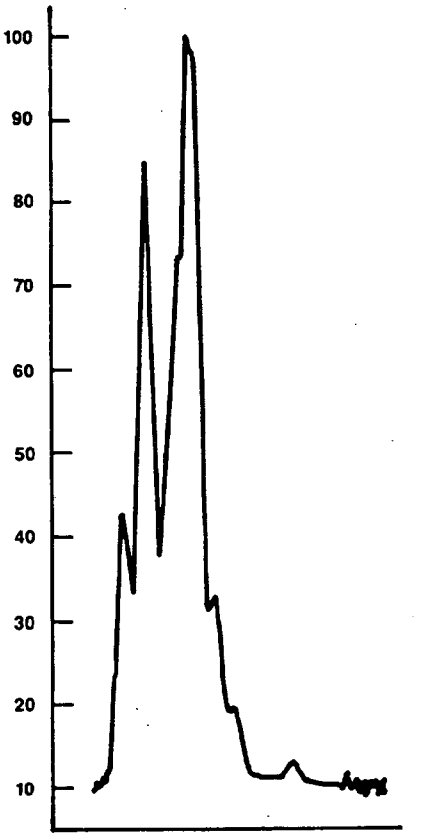


FIGURE 7: SEF spectrum of Grab 1.

D-89

similar effluent fluorescence spectra over months of sampling. Results indicated that similar spectra were obtained from composite and grab samples. In addition, samples were unusually stable. With refrigeration and no preservative, similar spectra were obtained almost one year later.

SELECTION OF OPTIMUM WAVELENGTH INTERVAL (DELTA)

Wavelength intervals ranging form 10 to 120 nm were evaluated to determine which interval (delta) yields the optimum spectral resolution for fingerprinting (qualitative analysis) and which delta yields the maximum spectral intensity (quantitative analysis). Figure 8 illustrates that the delta of 20 nm provides the greatest spectral resolution for fingerprinting the industrial discharge of Grab 1. A delta of 80 nm provides the greatest fluorescence intensity for quantitative analysis.

SPECTRA COMPARISON

For samples containing elevated levels of industrial effluent a delta of 20 nm was used to obtain the maximum amount of spectral detail for fingerprinting purposes. To assure that concentration quenching effects did not interfere with the sample spectra most effluent samples were diluted 1 to 10.

The SEFS fingerprint patterns of the effluent appear to be relatively constant, with the same peaks present and minor variations in peak intensity. This is shown in Figure 9 which compares the spectra obtained of samples collected a month apart (Composite/vs. Grab 2). Figure 10 illustrates SEFS fingerprints of effluent sampled over a month later (6/12/86). At this time Rhodamine WT was also added to the effluent as a dye tracer at the source of discharge. The fingerprint of these samples all appear similar, even though sampled at different dates. In addition, the Rhodamine peak does not appear to interfere with the effluent SEFS fingerprint.

The SEFS fingerprinting technique was also applied to samples of swamp water taken from Winding River Park adjacent to the facility. The spectra (Figure 11) indicate the same presence of fluorescence peaks as in the effluent, although the peak height maxima have changed. These Winding River samples represent the fluorescence spectra of material which are presumed to derive from the facility and do not occur naturally in swamp or ground water. (The Winding River sample fluorescence is presumed to derive from the same generic composition of varying concentrations of dyes, phthalates, plasticizers and nitrophenols. These materials are believed to be entering the river through contaminated groundwater).

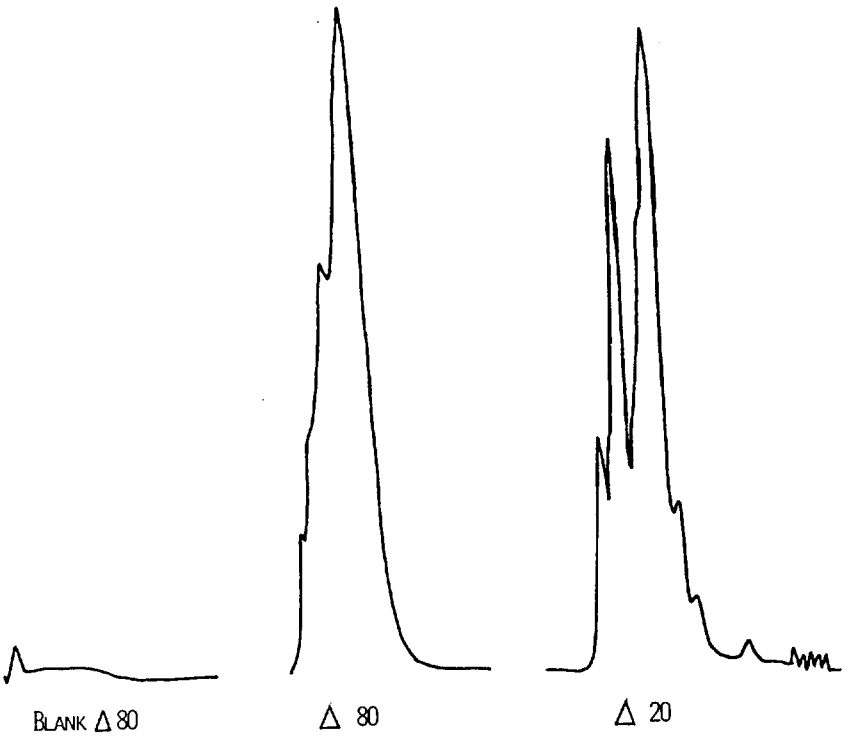


FIGURE 8. COMPARISON OF WAVELENGTH INTERVAL USING SEFS FOR GRAB 1. DELTA 20 NM WAS THE OPTIMUM INTERVAL FOR FINGERPRINTING.

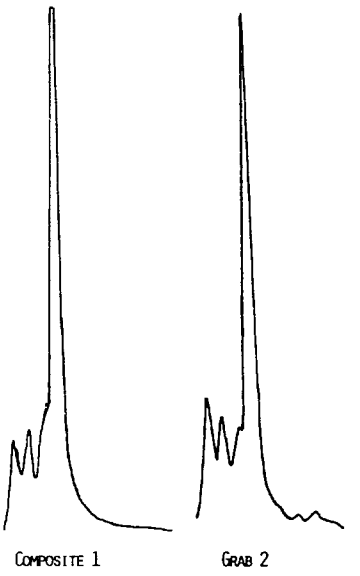
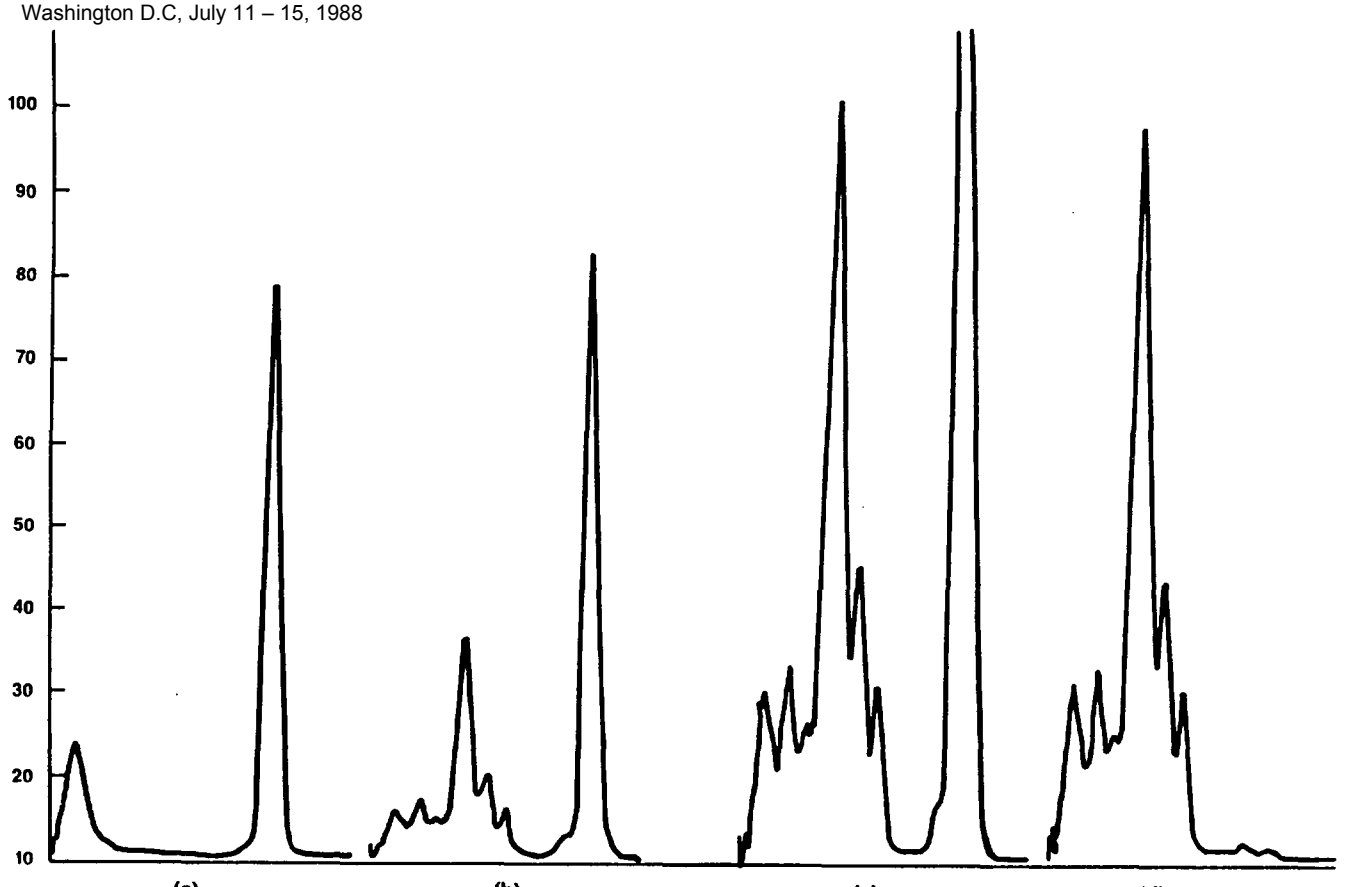


FIGURE 9. SEFS FINGERPRINTS OF EFFLUENT DERIVED FROM A DELTA OF 20 NM. THE COMPOSITE WAS SAMPLED 4/9/86; GRAB 2 WAS SAMPLED 4/28/86



(a) Rhodamine Standard
(b) Grab DS-4-A Effluent Illustrating SEFS Fingerprint and Rhodamine Dye Tracer
(c) Grab DS-4-A after addition of Rhodamine Dye Tracer
(d) Grab DS-4-A Effluent

Figure 10. SEFS fingerprint of effluent.

TABLE II. RESULTS OF GRAB SAMPLES TAKEN PRE AND POST DOSING OF EFFLUENT WITH RHODAMINE WT.

Sample	Description	Measured Rhodamine WT Concentration
11685	Effluent Grab Pre-Rhodamine Dosing	0.05 ppb
11686	Effluent Grab Post-Dosing	5.03 ppb
11687	Field Blank	0.02 ppb (None Detected)
11688	Effluent Grab Pre-Dosing	1.11
11689	Effluent Grab Post-Dosing	210.00 ppb
11690	Trip Blank	0.02 ppb (None Detected)

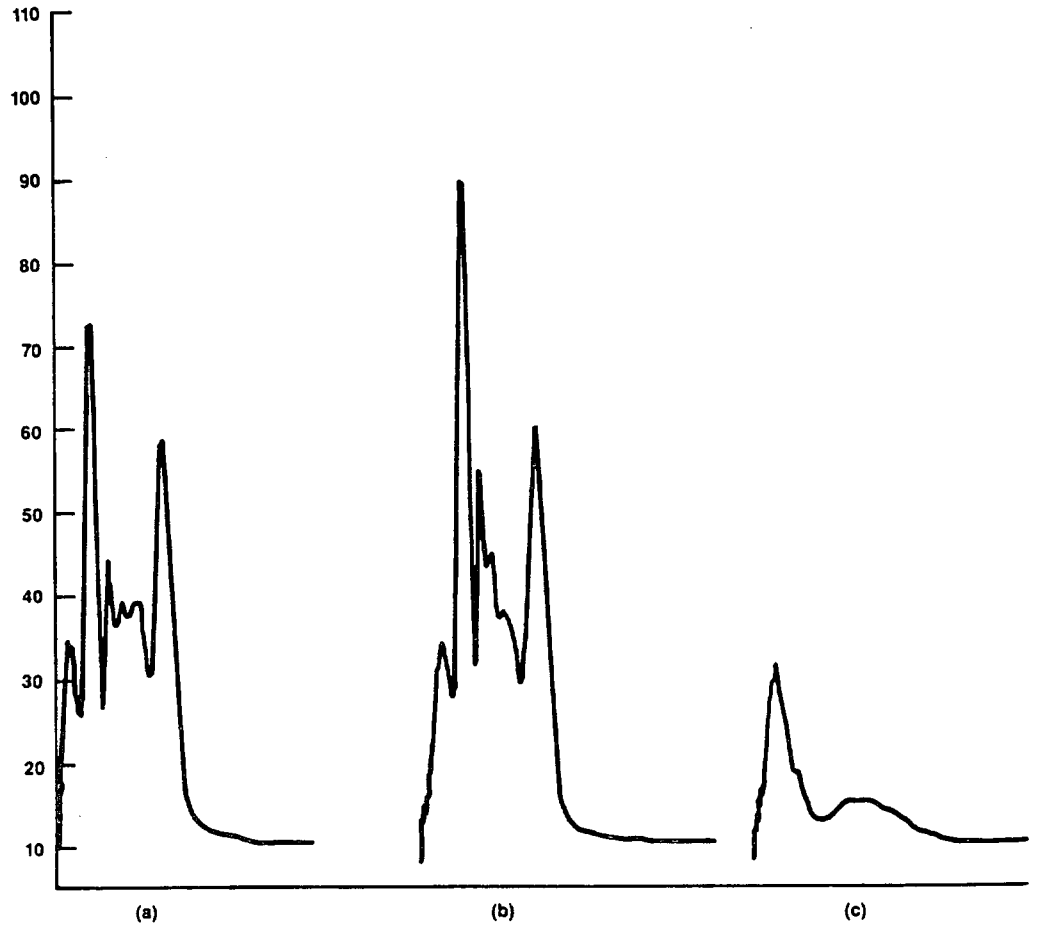


Figure 11.

SEFS Fingerprints of "Winding River Park" Water Samples.
(a) Grab 44202
(b) Grab 44203 taken downstream of effluent source
(c) Grab 44205 taken upstream of effluent source (Control Sample)

PHASE II

The primary objective in Phase II was to evaluate the use of SEFS to monitor for Rhodamine Dye Tracer in effluent and ocean water samples. The standards were prepared from a 20% Rhodamine WT solution supplied from the actual stock drum used on-site at the facility.

Prior to use all glassware was cleaned with chromerge solution, rinsed thoroughly with deionized water and air dired. Standards were prepared using class A volumetric flasks and Scientific Manufacturing Industries digital adjust micropetters. A 200 ug/ml stock solution was prepared by dilution 100 ul of the 20% Rhodamine WT solution in a 100 ml volumetric flask with deionized water. All standards were prepared from this stock solution. A five point calibration curve was run in triplicate as well as a laboratory water blank and an ocean water blank.

The samples and standards were scanned synchronously on the excitation wavelength drive from 200 nm to 640 nm with a delta (Δ) of 20 nm. The instrument conditions were:

Excitation wavelength	200 nm
Wavelength interval (Δ)	20 nm
Emission wavelength	220 nm
Excitation slit width	10 nm
Emission slit width	10 nm
Wavelength drive	Excitation
Mode	Dual
Amplifier dynode voltage	Ratio
Recorder power	Servo

The instrument sensitivity was adjusted near the maximum settings to achieve the highest possible detection limits of Phodamine WT where necessary.

Figure 12 illustrates the type of SEF spectra obtained for Rhodamine WT of Surf Samples collected by the N.J.DEP along the New Jersey Coast near the source of effluent discharge. These specific analyses were performed to determine the occasional impingement of the oceanic discharge plume on the beach. Calculated concentrations of Rhodamine WT in Figure 12 were 0.05-0.08 ppb.

Figure 13 illustrates an SEF spectrum of an effluent sample dosed with Rhodamine WT. Table 2 summarized the results of grab samples taken pre and post dosing of effluent with Rhodamine at the source of discharge. Note that Rhodamine was apparently

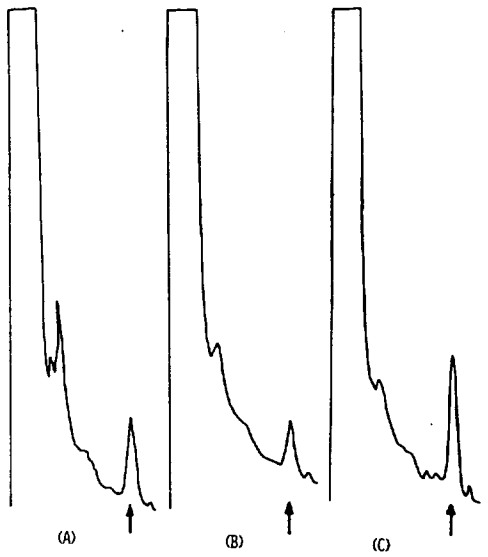


FIGURE 12. SEF SPECTRA FOR QUANTITATION OF RHODAMINE WT IN SURF SAMPLES. ARROW INDICATES RHODAMINE. SAMPLE C SPIKED WITH RHODAMINE WT.

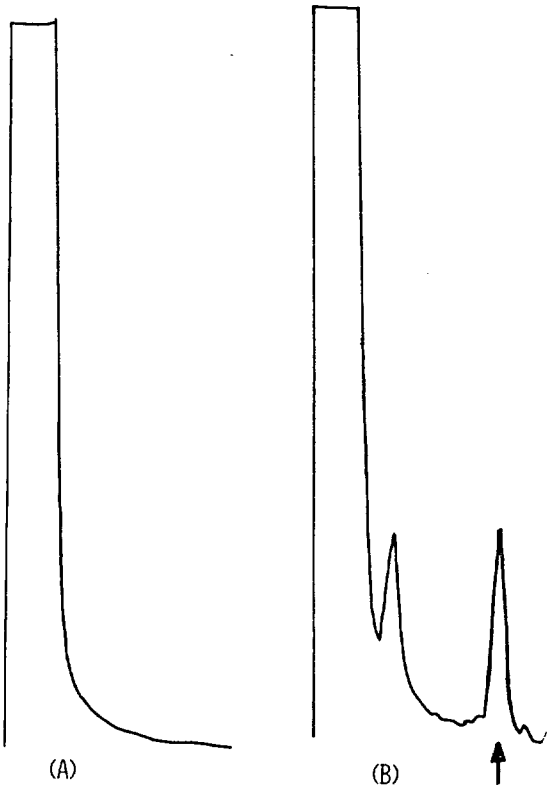


FIGURE 13. SEF SPECTRA OF
(A) FIELD BLANK AND
(B) EFFLUENT GRAB POST DOSING WITH RHODAMINE. ARROW INDICATES RHODAMINE. NOTE THAT SAMPLE WAS DILUTED 1 TO 2,500 TO AVOID CONCENTRATION QUENCHING EFFECTS.

detected at low levels in "Pre-Rhodamine Dosed" samples. Dye constituents and by-products of similar composition to Rhodamine (that are usually present in this particular type of effluent) could account for these values. This effluent is reported to emanate from dye manufacturing processes.

Results of samples taken at sea at various depths and dilution ratios of Rhodamine WT are listed in Table 3. These samples were a vertical profile within the oceanic discharge plume. These ratios were estimated in the field during sampling using a Turner Flow Through Fluorimeter. Typical SEF spectra of these samples are demonstrated in Figure 14.

Detection limits of Rhodamine WT that are practically achieveable in actual sample matrices were estimated to be about .001 ppb (1 ppt). Lower limits are probably achieveable by direct in-situ measurement using the SEFS technique depending on interferences from fluorescing compounds indigenous to ocean water matrices.

PHASE III

The objective in this phase was to continue to evaluate the use of SEFS for monitoring industrial effluent by obtaining spectra from the same source over an extended time interval and to determine practical detection limits.

SPECTRA COMPARISON OVER TIME

In samples taken one year later (Figure 15) the SEF fingerprint remains similar to the previous year (Figure 7). There has been a shift in the relative peak heights which may indicate a slight change in effluent content of the various compounds produced by the facility.

DETECTION LIMIT

The practical detection limit where industrial effluent can still be detected and possibly fingerprinted was determined by serially diluting sample effluent in sea water, i.e., 1 to 5, 1 to 10, 1 to 50, 1 to 100, etc. (Figure 16). Although a delta of 20 nm provides a more detailed fingerprint for samples containing higher levels of effluent, a delta of 80 nm yields significant lower detection limits. Some spectral resolution is lost but sufficient detail is retained for a distinct fingerprint pattern (see Figure 9). This fingerprint pattern is retained to a dilution of 1 to 4000 for Grab 1 (see figure 10). The subsequent utility of the fingerprint pattern obtained at delta 80 nm was confirmed in surf samples (Figure 12).

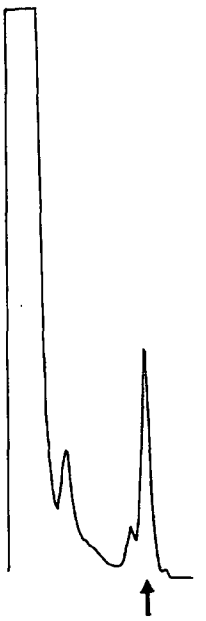


FIGURE 14. TYPICAL SEFS OF OCEANIC SAMPLE No. FPF-1 (ARROW INDICATES RHODAMINE WT)

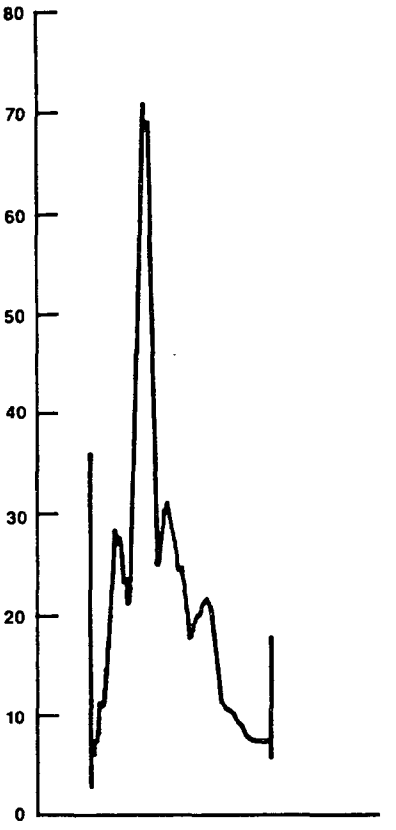


Figure 15. 24 hour effluent composite sampled April 1987

CONCLUSION

A sensitive monitoring procedure was developed based on fluorescence measurements which can reliably quantitative and fingerprint effluent at concentrations ppb levels. The procedure makes use of synchronous excitation fluorescence spectroscopy to monitor the fluorescent properties of the effluent. This procedure can be used to monitor the discharge of actual effluents in groundwater, river water and sea water.

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MICROWAVE AIDED DIGESTION OF ORGANIC/WASTE BLENDS PRIOR TO METALS ANALYSIS

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ABSTRACT

Acid digestion of samples containing mixtures of organics, and inorganic materials for metals analysis by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) and/or Atomic Absorption Spectroscopy (AAS) is often difficult and very time consuming. Complete digestion of the organic matrix, using concentrated nitric acid and 30% hydrogen peroxide, can require 24 to 48 hours of continuous reflux. The long digestion time increases sample turnaround time, the chance of contamination, and loss of volatile elements. Microwave aided mixed acids digestion, using the CEM system of sealed low pressure Teflon digestion bombs, has been investigated by Chemical Waste Management, Inc. (CWM). The microwave aided digestion results indicate that recoveries of approximately 100% and an average relative standard deviation of better than 8% can be obtained in only two hours, even without the complete destruction of the organic matrix.

The sample matrix used for the study was a blend of fuel oil and still bottoms from solvent recovery distillation units. This matrix is a complex one containing fuel oil, a variety of different residual solvents and heavy organics, and frequently a substantial quantity of inorganic materials.

INTRODUCTION

Organic solvents are used for many cleaning purposes in the electronics and automobile repair industries and in the production products such of paints or inks. Frequently, the solvents contained in these used cleaners or waste paints and inks can be reclaimed by simple distillation, and Chemical Waste Management has an active and growing involement in this area. Solvent recovery stills are located at our Solvent Resource Recovery Facility in West Carrollton, Ohio as well as at our OSCO facilities in Azusa, California and Henderson, Colorado.

After solvent recovery, the still bottoms contain dirt, pigments, metal wear debris, residual solvents, and high molecular weight organics. These still bottoms are a hazardous waste which can not be recycled and must be disposed of in a safe manner. Blending these still bottoms with fuel oil prior to burning the mixture in a properly designed incinerator is an environmentally safe disposal procedure.

Two stage incinerators with stack scrubbing facilities can be used for burning these blends, as well as for incineration of other hazardous materials such as PCB contaminated wastes. CWM operates this type of incinerator at several sites.

Burning the mixture in a cement kiln is another way of safely disposing these fuel oil/still bottom blends. A major advantage of this type of incinirator is that the heating value of both the fuel oil and the residual organic wastes are recovered and used in the calcination process. While the organics are completely destroyed, as in the two stage incinerator, an additional benifit is that the metals and halogens react with, are diluted in, and are immobilized by the cement, therefore, there is no ash disposal problem.

No mater which type of incinerator is used, it is important that the metals content of this fuel oil/waste mixture be limited in order that the capacity of the incinerator scrubbers or the cements ability to bind and dilute these metals not be exceeded. Metals analysis by dilution in an organic solvent such a xylene and direct aspiration into an AA or ICP is generally not possible because of the presence of large particles in these materials, therefore, the samples are commenly acid digested prior to analysis.

Since these fuel oil/still bottom blends contain both organic and inorganic materials they do not fit neatly into any of the EPA recommended digestion methods for hazardous waste. At CWM we have found that a slightly modified version of EPA SW-846 Method 3050 for Sludge Digestion, which is a nitric acid/hydrogen peroxide digestion, can be successfully applied to these materials. Complete matrix destruction, however, can require refluxing in concentrated nitric acid for 24 to 48 hours on a hot plate, followed by several treatments with 30% hydrogen peroxide.

In order to streamline the digestion process, we have investigated the use of microwave aided digestion, using the CEM low pressure Teflon bomb system. The method we have developed (Appendix 1) is simple, general, and rapid. The digestion time is reduced from 24 to 48 hours for the hot plate digestion method to only 2 hours with microwave heating. Although this short digestion time does not completely destroy the oil matrix, we have found the the results are equivalent to, or superior to, those obtained with hot plate digestion. A complete breakdown of sample digestion times are shown in Table I.

EXPERIMENTAL SECTION

Apparatus: A Model 3200 Bransonic Ultrasonic Cleaner was used to disperse the solid particles in the fuel oil matrix prior to aliquoting the samples. (Branson Ultrasonics Corporation, Eagle Road, Danbury CT 06810-1961, (203)796-0400)

A Model MDS-81D Microwave Digestion System consisting of 700 Watt Teflon-coated microwave oven, twelve 120 mL low pressure Teflon sample vessels, a turntable/sample vessel carrier, and a capping station was used for the digestions. The Teflon sample vessels are equipped with relief valves which vent at approximately 100 psi. The vented vapors are transferred through Teflon tubes to a collection vessel where they condense. The temperature of the digestion mixture increases to about 160-180^{o}C as the pressure inside the vessels increases to 100 psi. Since the acid is much

more reactive at this higher temperature, the digestion time is greatly reduced . Figure 2 (Ref.1) shows a temperature vs. pressure curve for a CEM microwave vessel containing a mixture of HNO_3 and H_2O_2. The digestion mixture used in this study is different, however similar temperatures should be obtained during the digestion. (CEM Corporation, P.O. Box 9, Indian Trail, NC 28079, (800)334-6317)

A Jarrell-Ash Model 1100 Inductively Coupled Argon Plasma direct reading spectrophotometer was used for all sample analysis work. (Thermo Jarrell-Ash Corporation, 8 E Forge Parkway, P.O. Box 9101, Franklin, Ma 02038-9101, (617)520-1880)

Covers for Erlenmeyer flasks (Reflux Caps) designed by Professor F. E. Tuttle (Catalog No. 10-042A, Allied Fisher Scientific, 1600 W. Glenlake Avenue, Itasca, IL 60143, (312)773-3050)

REAGENTS

Baker Analyzed Reagent, Nitric Acid, 70.0-71.0%, and if appropriate,Baker Analyzed Reagent, Hydrochloric Acid, 37.6%, was used to prepare all ICAP calibration standards. Baker Analyzed Reagent, Nitric Acid and Baker Analyzed Reagent, Hydrogen Peroxide, 30%, or Baker Analyzed Reagent, Hydrochloric Acid were used for all sample digestions. (J. T. Baker Chemical Co., 222 Red School Lane, Phillipsburg, NJ 08865, (201)859-2151)

Conostan Metallo-Organic Standard, S-21, 900 ppm and Conostan Base Oil were mixed and used in the initial recovery study. The 900 ppm Conostan S-21 was then used to spike all samples analyzed in the equivalency study. (Conostan Division, Conoco Specialty Products, Inc., P.O. Box 1267, Ponca City, OK 70603, (405)767-3078)

Liquid argon of typical 99.997% purity was used for all ICAP analysis (Liquid Carbonic, 12054 S. W. Doty Ave., Chicago, IL 60627, (312)568-8840)

The ICAP calibration standards for the Jarrell-Ash Model 1100 ICAP were made by diluting concentrated mixed standards from Leeman Labs. All calibration standards were made up in 10% nitric acid or in 10% nitric acid/10% hydrochloric acid as appropriate. The calibration blank was 10% nitric acid or 10% nitric acid/10% hydrochloric acid. (Leeman Labs, 600 Suffolk St, Lowell, MA 01854, (617)454-4442)

PROCEDURE

The fuel oil/organic/inorganic waste mixtures are inhomogeneous and must be well shaken and mixed in an ultrasonic cleaner prior to analysis. A 0.1g to 0.5g sample is transferred to either a CEM Teflon digestion vessel or a 125 mL Erlenmeyer flask for analysis.

When all samples are weighed, 5mL of concentrated HNO_3 is

added. Immediately swirl the samples to mix and then allow them to set for about 5 minutes (Caution: A vigorous reaction may take place between the HNO_3 and any easily oxidizable residual solvents in the sample with an accompanying release of nitrogen oxides, therefore this step must be performed in an exhaust hood.).

For samples to be digested on a hot plate, a Reflux Cap is placed on each Erlenmeyer as soon as the acid has been added. When any reaction has subsided, the Erlenmeyer flasks are placed on a hot plate which has been adjusted to a temperature at which the samples will reflux slowly. Digestion of the samples in the Erlenmeyer flasks then proceeds with HNO_3 and H_2O_2 according to EPA SW-846 Method 3050.

For the samples in the CEM microwave oven digestion vessels, after any reaction has subsided, 5mL of concentrated HCl is added to each. Immediately the samples are swirled to mix and then allowed to set for at least 5 minutes (Caution: A vigorous reaction may take place between the HNO_3/HCl mixture and any easily oxidizable residual solvents in the sample with an accompanying release of nitrogen oxides, therefore this step must be performed in an exhaust hood.).

After any reaction has subsided in the CEM vessels, they are capped with the capping station. The use of the capping station is critical since it torques the caps to the same tightness so that all vessels will vent at approximately the same pressure. After capping, the vessels are placed in the turntable, connected to the vent collection vessel, and the entire assembly placed in the microwave oven.

Twelve vessels should be digested for 10 minutes at 10% power, 10 minutes at 30% power, and 10 minutes at 50% power. The conditions have been choosen so that the vessels will vent only a small amount of vapors. Caution should be used in selecting power levels since excessive venting can lead to a loss of sample. If fewer than 12 vessels are used the power levels should be adjusted proportionately downward to prevent excessive venting. Exact power levels must be determined experimentally.

At the completion of the digestion, the Erlenmeyer flasks or the turntable and vessels are removed from the hot plate or the microwave oven and cooled. A water bath may be used to reduce the cooling time if desired. After cooling, the CEM vessels must be vented inside an exhaust hood by pressing the tubing connection at the top of the vessel cap to the side (Caution: The CEM vessels may contain up to 100 psi of pressure). The cooled CEM vessels should be opened with the capping station only after venting.

The digested sample should be transfered to a 50 mL volumetric flask. The Erlenmeyer flask and reflux cap or the CEM Teflon digestion vessel, pressure release valve, and cap should be carefully washed into the volumetric flask to insure that sample transfer is quantitative. After dilution to volume the sample should be filtered through #1 filter paper (Whatman or equivalent) to remove any particles or oil droplets that might clog the ICP

nebulizer.

RESULTS AND DISCUSSION

Hot plate digestion, by refluxing in concentrated nitric acid followed by additions of hydrogen peroxide to totally destroy the matrix, has historically worked well for blended fuel oil/waste samples, however, this method can require as long as one week of digestion time. Microwave aided low pressure bomb digestion has been examined as an alternative digestion method.

Initially, microwave aided nitric acid/hydrogen peroxide digestions of a 900 ppm CONOSTAN S-21 metals in oil standard were performed for comparison with the hot plate digestion method. Fifteen aliquots of about 0.1 g and 0.5 g, with CONOSTAN base oil added to maintain a total weight of approximately 1 g., were digested and analyzed by ICP over a ten day period of time. The hot plate samples were allowed to reflux with 5 mL of concentrated nitric acid for 20 hours before 3 mL of 30% hydrogen peroxide was added. An additional aliquot of 3 mL of 30% hydrogen peroxide was added at 22 hours and the digestion terminated at 24 hours. The microwave aided digestion was with 5 mL of nitric acid and 5 mL of Hydrogen peroxide with the oven programed in three stages: 10% power for 10 minutes, 30% power for 10 minutes, and 50% power for 40 minutes.

The compiled results for 9 metals are shown in Table II. The % recovery for 0.1 g of S-21 standard is 107% $\pm$ 7% with microwave aided digestion versus 110% $\pm$ 14% with hot plate digestion. With a 0.5 g sample of S-21 the results are 102% $\pm$ 8% and 102% $\pm$ 15% respectively. The % recovery results for both digestion methods are similar and quite acceptable, but the standard deviations for microwave aided digestion are significantly lower. This may in part be due to the fact that the hot plate digestions were terminated at 24 hours even though the matrix was not totally destroyed, however, it should be noted that the matrix was also not totally destroyed in the microwave aided digestions.

The % recoveries for S-21 spikes in 1 g samples of ten different fuel oil/waste blends were also measured over the 10 day period. A few of the results for the 0.1 g spike are also given in Table II. The results for the samples show much more scatter than for the CONOSTAN S-21 standard, with the microwave aided digestion % spike recoveries ranging from -106% $\pm$ 332% for barium to 102% $\pm$ 10% for cadmium.

Two possible explanations for the erratic results were either incomplete digestion, or non-homogeneous samples. In order to test these possibilities, five aliquots of several samples were digested under several conditions. The sample size was reduced to 0.5 g to accentuate any inhomogeneity in the samples while at the same time the amount of nitric acid and hydrogen peroxide was held at 5 mL each, thus increasing the digestion mixture to sample ratio, and the digestion time extended to 2 hours. A digestion mixture of 5 mL of concentrated nitric acid and 5 mL of concentrated hydrochloric acid was also tested to determine its effect on the

digestion.

Results for nitric acid/hydrogen peroxide and nitric acid/hydrochloric acid digestions for one of the samples are shown in Table III. As can be readily seen the standard deviations are quite acceptable for these samples. The substitution of hydrochloric acid for hydrogen peroxide had a large effect on the measured iron content and a small, but mathematically significant, effect on several other the elements. The general conclusions are that the samples are homogeneous enough to allow determinations with good precision and that the use of a nitric acid/hydrochloric acid gives better recoveries than a nitric acid/hydrogen peroxide mixture.

Further work is currently underway to extend this study. The first aim is to better define the digestion conditions relating to the needed digestion time and the optimum acid mixture. The study will also be expanded to include additional sample types.

TABLE I

SAMPLE PREPARATION STEP	REQUIRED TIME HOT PLATE	REQUIRED TIME MICROWAVE OVEN
1. Preparation	10 minutes	10 minutes
2. Weigh 10 Samples plus Dup. and Spike	20 minutes	20 minutes
3. Add Acid(s)	5 minutes	10 minutes
4. Cover and Place in Turntable		10 minutes
5. Digest	24-48 hours	2 hours
6. Cool, Dilute, Filter	1 hour	1 hour
TOTAL TIME	26-50 hours	4 hours

TABLE II

MEASURED % RECOVERIES FOR CONOSTAN S-21 AND FOR SPIKED SAMPLES

CONOSTAN S-21 (900 ppm) , ~0.1g
PLUS BASE OIL TO 1 g TOTAL

	Microwave Digest		Hot Plate Digest	
	Recov	Std Dev	Recov	Std Dev
Ba	102%	11%	111%	26%
Cd	107%	8%	103%	15%
Cr	93%	10%	111%	11%
Pb	102%	6%	100%	12%
Cu	115%	4%	117%	11%
Ni	111%	10%	113%	12%
Fe	107%	4%	111%	10%
Zn	109%	5%	105%	15%
Mn	119%	4%	119%	11%
Av	107%	7%	110%	14%

SAMPLE, 1 g, PLUS SPIKE OF
CONOSTAN S-21 (900 ppm) , ~0.1g

	Microwave Digest		Hot Plate Digest	
	Recov	Std Dev	Recov	Std Dev
Ba	-106%	332%	12%	455%
Cd	102%	10%	107%	18%
Cr	98%	41%	124%	53%
Pb	107%	107%	197%	187%
Cu	110%	27%	104%	10%

CONOSTAN S-21 (900 ppm) , ~0.5g
PLUS BASE OIL TO 1 g TOTAL

	Microwave Digest		Hot Plate Digest	
	Recov	Std Dev	Recov	Std Dev
Ba	81%	23%	90%	44%
Cd	102%	9%	100%	10%
Cr	94%	7%	102%	10%
Pb	91%	8%	89%	20%
Cu	112%	5%	110%	8%
Ni	110%	6%	106%	10%
Fe	102%	4%	105%	10%
Zn	108%	6%	102%	11%
Mn	114%	6%	112%	10%
Av	102%	8%	102%	15%

TABLE III

HOMOGENEITY STUDY, 5 ALIQUOTS OF SAMPLE #5
0.5 g SAMPLE, 2 HOURS TOTAL DIGESTION TIME

	DIGESTION WITH NITRIC ACID/HYDROCHLORIC ACID			DIGESTION WITH NITRIC ACID/HYDROGEN PEROXIDE			t TEST
	Conc.	%RSD	Std Dev	Conc.	%RSD	Std Dev	Hcl SIGNIF?
BA	580	2%	10	559	2%	10	yes
CD	1.97	3%	0.06	1.78	3%	0.05	yes
CR	222	3%	6	223	2%	4	no
CU	224	1%	3	216	2%	4	yes
NI	16.8	21%	3.4	17.0	12%	2.1	no
PB	433	2%	10	412	2%	7	yes
ZN	228	2%	5	220	2%	5	yes
AL	1293	3%	41	1266	2%	24	no
B	12.2	6%	0.7	10.4	2%	0.2	yes
CA	2568	2%	41	2472	3%	69	yes
FE	1432	1%	16	731	11%	79	yes
MG	175	3%	4	172	9%	16	no
MN	15.1	2%	0.3	15.3	3%	0.4	no
MO	79.5	3%	2.3	70.4	3%	2.0	yes
TI	37.7	6%	2.3	28.8	4%	1.1	yes
V	39.5	3%	1.2	35.7	3%	0.9	yes

Appendix I

Microwave Aided Digestion of Fuel Oil/Waste Mixtures

1.0 SCOPE AND APPLICATION

1.1 This method is a microwave aided acid digestion procedure used to prepare mixtures of fuel oil and organic and/or inorganic wastes for analysis by atomic absorption spectroscopy (AAS) or inductively coupled argon plasma spectroscopy (ICP). Samples prepared by this method may be analyzed by ICP for all the listed metals.

Aluminum	Lead
Barium	Magnesium
Boron	Manganese
Cadmium	Molybdenum
Calcium	Nickel
Chromium	Titanium
Copper	Vanadium
Iron	Zinc

2.0 SUMMARY OF METHOD

2.1 A representative 0.1-g to 0.5-g sample is digested in a microwave heated low pressure Teflon bomb containing nitric and hydrochloric acids as a rapid means of digesting the sample for metals analysis by AAS or ICP.

3.0 INTERFERENCES

3.1 Fuel oil/organic waste/inorganic waste samples can contain diverse materials, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether this method is applicable to a given waste stream.

4.0 APPARATUS AND MATERIALS

4.1 CEM Model MDS-81D Microwave Digestion System: MDS-81D Oven, 120 mL Teflon sample vessels, turntable/sample vessel carrier, and capping station.

4.2 Ultrasonic Cleaner: Capable of dispersing solid waste material in the fuel oil/waste mixture.

4.3 No. 1 filter paper (Whatman or equivalent).

5.0 REAGENTS

5.1 ASTM Type II water (ASTM D1193): Water should be monitored for impurities.

5.2 Concentrated hydrochloric acid, reagent grade or better(HNO_3): Acid should be analyzed to determine level of impurities. If method blank is <10 times the MDL, the acid can be used.

5.3 Concentrated nitric acid, reagent grade or better(HNO_3): Acid should be analyzed to determine level of impurities. If method blank is <10 times the MDL, the acid can be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses all Federal, State, Local, or company requirements.

6.2 All sample containers must be prewashed with detergents, acids, and Type II water unless previously shown to be non-contaminating. Glass containers are preferable.

7.0 PROCEDURE

7.1 For each digestion procedure, weight to the nearest 0.001-g and transfer to a CEM 120 mL Teflon sample vessel a 0.1-g to 0.5-g portion of the sample (Note: The sample must be mixed thoroughly to achieve homogeneity. Vigorous shaking and the use of an ultrasonic cleaner is recommended).

7.2 Add 5 mL of concentrated HNO_3, swirl to mix, and allow the sample to set for 5 minutes (Caution: The presence of easily oxidizable residual organic solvents in the mixture may cause a vigorous reaction and care must be taken to insure that no sample is lost.). Add 5 mL of concentrated HCl, swirl to mix, and allow the sample to set for 5 minutes (Caution: The presence of easily oxidizable residual organic solvents in the mixture may cause a vigorous reaction and care must be taken to insure that no sample is lost.). When any reaction occurring has subsided assemble the digestion vessel and torque the cap to the correct tightness using the CEM capping station. Place the vessels in the turntable and connect them to the vent collection vessel using the Teflon tubes. Place the turntable and vessels in the CEM MDS-81D Microwave oven and heat 10 minutes at 10% power, 10 minutes at 30% power, and 100 minutes at 50% power (Caution: Lower power levels will be required for less than 12 vessels. The use of stepped power increases are also important since they allow the reaction of easily oxidizable residual organic solvents to be completed slowly and minimize venting.).

7.3 After Step 7.2 has been completed, remove the turntable and vessels from the microwave oven and cool the samples. Place the turntable and vessels in an exhaust hood and vent the sample vessels by pushing the tubing connector at the top of the vessel to the side to release any nitrogen oxides inside the vessels. Remove the vessels from the turntable and open then using the capping station.

7.4 Quantitatively transfer the digested sample to a 50 mL volumetric flask and dilute to volume with Type II water. Particulates or residual oil can clog the nebulizer and must be removed by filtration.

7.4.1 Filtration: Filter through No. 1 filter paper.

7.5 Calculation:

7.5.1 The concentrations determined are to be reported on the basis of the actual weight of the sample.

8.0 QUALITY CONTROL

8.1 For each group of samples processed, preparation blanks (base oil and reagents) should be carried throughout the entire sample preparation and analytical process. These blanks will be useful in determining if samples are being contaminated.

8.2 Duplicate samples should be processed with each group of samples digested. Duplicate samples will be used to determine precision. The sample load will dictate the frequency, but at least 10% is required.

8.3 Spiked samples must be employed to determine elemental recoveries. Exact spiking levels will be sample dependent, but the samples should be spiked with Conostan S-21 (900 ppm) to levels that will allow accurate determination of the recoveries. A spiked sample should be included with each group of samples processed.

8.4 A matrix matched quality control check sample or standard reference material sample must also be analyzed with each batch of samples to insure that analytical accuracy and precision are maintained within acceptable limits.

9.0 METHOD PERFORMANCE

9.1 No data provided.

10.0 REFERENCES

10.1 None required.

A RAPID METHOD FOR THE QUANTITATIVE DETERMINATION OF HALOGEN IN USED OILS

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INTRODUCTION

EPA regulation 40 CFR 261 bars the sale of used oil for fuel if it is contaminated with halogens measured as chlorine at levels exceeding 1000 ppm. Such oil is considered to be a hazardous waste unless it can be proven that the chlorine content is inorganic or that the halogenated organics are not hazardous constituents. The cost of disposing of a hazardous waste is many times higher than the cost of used oil disposal. Therefore, it is critical for users, generators, haulers, reprocessors, and collectors to test the material they handle in order to comply with regulations, maintain safe operations, and avoid high disposal costs. The analytical burden on these various types of companies becomes very significant and costly.

The EPA contracted for an evaluation of analytical technology in order to recommend acceptable methodology. Neutron activation analysis, microcoulometric titration and X-ray fluorescence were found to be acceptable instrumental approaches, while the LECO chlorine determinator and the Beilstein test device were found to need more development. All of these instrumental approaches require a significant capital investment, trained personnel, and a sample load greater than 10,000 / year to justify the investment.

While some instrumental approaches permit the direct determination of total chlorine in petroleum products, most laboratory methods need to convert organic chloride to inorganic chloride using the ASTM D-808-63 oxygen bomb method as a prerequisite for analysis. The "bomb method" requires a capital purchase of equipment, is time consuming (about 45 minutes per sample), and is highly technique oriented. The chemical approaches evaluated were the finishing ASTM methods following the bombing prerequisite. These methods included ion chromatography, turbidimetric, mercuric nitrate, silver nitrate, ferricyanide, potentiometric and amperometric methods. All of these approaches worked if the ASTM methods were modified in some way, such as filtering, drying, extracting or various other pretreatments. The major problem with all of the chemical approaches is the requirement of ASTM - D - 808 - 63, the use of the oxygen bomb.

KIT DEVELOPMENT

A method has been developed that replaces the lengthy oxygen bomb prerequisite for analysis. This method involves a chemical removal of chlorine, rather than a combustion oxidation removal. Analysis time is approximately ten minutes and the necessary reagents are all contained in a small test kit. Each kit contains sufficient materials for ten analyses.

The materials required for one analysis are two plastic tubes containing predispensed, encapsulated reagents, a filter assembly, and standardized silver nitrate titrant.

Approximately 0.35 grams of sample is weighed into the first tube using an analytical balance. After the tube is recapped, the bottom glass ampule is broken and the tube is shaken for ten seconds. This ampule contains 1.5 ml of a solution containing aliphatic solvent, napthalene and diglyme, which

conditions the waste oil for the analysis. The second ampule is then broken and the tube is shaken for ten seconds, and allowed to react for one minute, shaking intermittently. This ampule contains a dispersion of metallic sodium in an organic matrix that strips the organic chloride from the sample.

The second tube contains seven ml of aqueous buffer solution. After the sodium reaction has been allowed to run for one minute, the buffer solution is poured from the second tube into the first one which contains the sodium, aliphatic solvent, and oil sample. After a few seconds of shaking, the sodium reaction is quenched and any halogens that are present (now in the ionic form) are extracted into the aqueous solution. The tube is then inverted and allowed to settle for two minutes while the organic and aqueous phases separate. At this point, the dispenser cap on the tube is opened and the buffer solution is through the filter assembly into the second tube. Five ml of the aqueous phase is then pipetted from the second tube into a titration beaker and approximately 40 ml distilled water is added. An automatic or manual potentiometric titration can now take place using a Ag/AgCl electrode.

Figure 1 illustrates a typical titration curve plotted by the Mettler DL20 automatic titrator equipped with a DM141 electrode and 5 ml dispensing burette. The sample tested consisted of 1000 ppm trichlorobenzene in used lube oil. This method typically gives a 100 mV spread at the point of inflection.

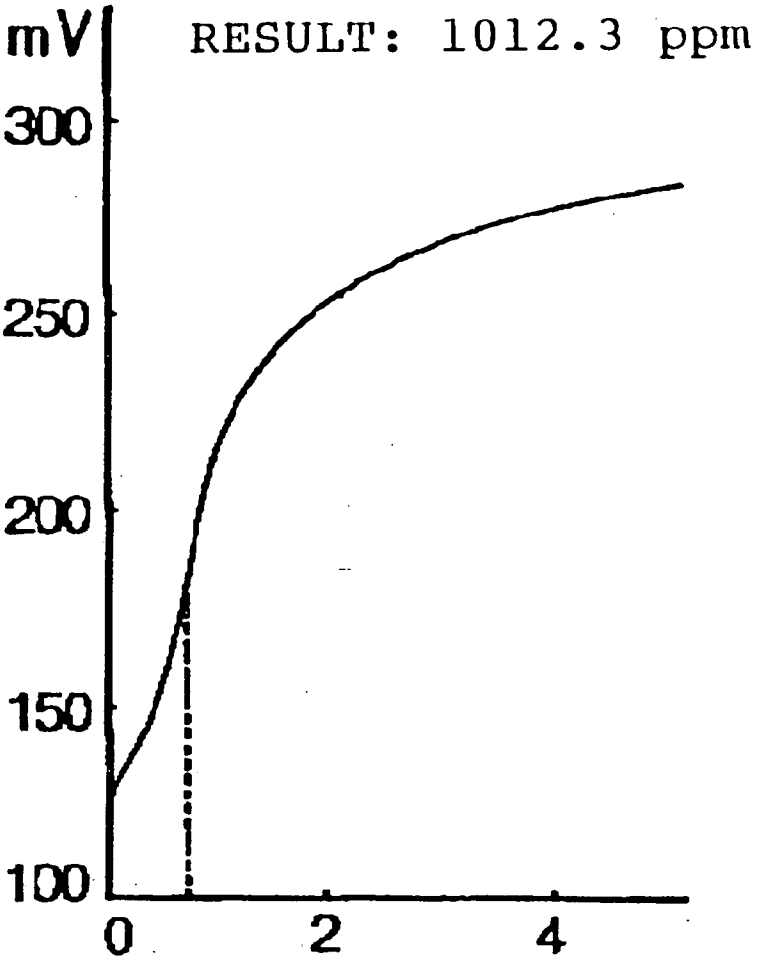

FIGURE 1

D-113

Table 1 lists some typical results obtained on various types of petroleum products. The results are very acceptable and precision is +/- 7 %.

Sample Type	Cl^- added (ppm)	Cl^- found (ppm)
Used Lube Oil	1100	1184
	1000	1001
	832	841
Virgin Lube Oil	970	923
#6 Fuel Oil	40	44
	1000	1035

TABLE 1

This method has been evaluated on virgin lube oil, virgin hydraulic oil, used lube oil, fuel oils #2 - #6, kerosene and transformer oils. The samples must, however, contain less than thirty percent water. If the samples contain greater than thirty percent water, the sodium reacts preferentially with the water rather than with the halogenated organics in the oil.

This method detects iodine, bromine and chlorine equally. It does not detect fluorine because AgF stays in solution during the titration, while AgI, AgBr, and AgCl precipitate out and can therefore be detected.

Fluorine does not interfere with the detection of the other halogens present. Table 2 lists recovery of other halogen types in a #6 fuel oil and recovery of halogen from a mixed halogen in used lube oil.

Material Added	Cl^- Added (ppm)	Cl^- Found (ppm)
Bromobenzene	1100	1151
Iodobenzene	1100	1033
Fluorobenzene	1100	15.9
Trichloro-	832 (added as Cl^-)	841
trifluoroethane	446 (added as F^-)	0

TABLE 2

Table 3 lists some chlorinated organic compounds which have been shown to be detectable by the potentiometric titration.

Trichloroethane
Dichloroethane
Trichlorobenzene
Monochlorobenzene
Chlorooctadecane
Methylene Chloride
Perchloroethylene
Freon (113)
Polychlorinated Biphenyls

TABLE 3

By verifying that the kit works on these nine compounds we have covered the major classes of chlorinated compounds that we expect to find in used oils. Volatile degreasing solvents (1,1,1-trichloroethane) are expected to be the most prevalent compounds. We have also covered chlorinated aromatics, more densely chlorinated aliphatics, cleaning solvents, refrigerants, and PCBs.

This method is now available from Dexsil in kit form. Complete, each kit contains sufficient materials for ten analyses. The cost is $60 per kit, or $6 per analysis. Total cost per analysis is significantly reduced by the four fold (400%) reduction in analysis time over conventional methods.

LABORATORY INFORMATION MANAGEMENT

AUTOMATION OF REGIONAL DATA VALIDATION STUDIES

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ABSTRACT

A computerized system has been developed to assist in U.S. Environmental Protection Agency data validation studies of organic analytical data collected for hazardous waste site assessments. The Regional Automated Data Auditing System (RADAS) is a data evaluation and management computer program that provides data entry, file maintenance, data qualifying, and report generation for results from gas chromatography and gas chromatography/mass spectrometry analyses.

The RADAS system determines the validity of individual analyte concentrations reported by the laboratory. This determination is based on instrument calibration, sample holding time, blank contamination, and contract required detection limit. Delivery-ready reports are produced, including data tables, sample and calibration listings, and a documented summary of data validation.

The system developed has been found to perform appropriately and its use represents a significant improvement in terms of turn-around time, completeness, and consistency of data validation studies.

INTRODUCTION

As a result of the enactment of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980 and the Superfund Amendments and Reauthorization Act (SARA) in 1986, the U.S. Environmental Protection Agency (U.S. EPA) has developed the Contract Laboratory Program (CLP) to analyze Superfund samples [1].

An extensive Quality Assurance/Quality Control (QA/QC) system is in place to guarantee that CLP data is of known quality [2, 3]. The CLP specifies the use of gas chromatography/mass spectrometry (GC/MS) and electron capture gas chromatography (GC/EC) analytical techniques for the analysis of organic samples. Volatile [4] and semi-volatile [5] compounds are analyzed by GC/MS and pesticides [2] by GC/EC.

The availability of environmental data that identify and quantify contamination at hazardous waste sites is a key element in the remediation process. Sampling [6], analysis, and data evaluation have been identified as the rate-limiting steps in the environmental measurement process [1].

This rate-limiting condition has been addressed by revising analytical methods [7], automating data reporting [8], and providing standard data review procedures [9]. This paper describes the Regional Automated Data Auditing System (RADAS), a program developed for automating the evaluation of organic data validation.

The system described was developed by Lockheed EMSCO for use in data validation studies performed for U.S. EPA Region 9 and for Resources Conservation and Recovery Act (RCRA) groundwater monitoring, through the Quality Assurance Branch of the U.S. EPA Environmental Monitoring Systems Laboratory at Las Vegas (EMSL-LV).

REGIONAL DATA VALIDATION STUDIES

Identification of hazardous waste and quantification of the extent of site contamination must be performed based on scientifically valid data. Tasks such as health risk assessments or determination of potentially responsible parties, with the associated liabilities, must be founded upon high quality data determined by the best available analytical technology.

Determination of the quality of data generated by the analytical laboratory is critical in defining the usefulness of the data in question. The criteria employed in these assessments are dependent not only upon the method-established QA/QC parameters but also upon the intended use of the analytical data. The environmental community has many users of analytical results, and each user has characteristic objectives and needs [10]. Different data quality levels may be required for site characterization than for risk assessment or monitoring projects, for example. The ten U.S. EPA Regional Offices conduct data validation reviews of data that will be used for many different purposes. General guidelines [9] exist to assure consistent application of the same criteria to data used for environmental decisions.

Technical factors that affect the validity of organic analytical data include the following: sample holding time, tuning and calibration of the analytical instrument, contamination detected in blank samples, percent recovery on extraction (as evaluated by matrix and surrogate compounds spike recovery), analyte identification criteria, analytical system performance, and overall data assessment. These items are examined for each batch of samples (case) analyzed and their adherence to pre-established QA/QC criteria is determined. Anomalies are documented and reported, and the affected portions of the data are flagged accordingly using data validation codes. This data qualification allows the Regional decision makers to take more appropriate actions concerning the environmental project, or require reanalysis when necessary, based on data of known quality.

AUTOMATION OF DATA VALIDATION ASSESSMENTS

The computerized automation tool described in this paper (RADAS) was developed to reduce the turn-around time of regional data validation studies performed by the Lockheed EMSCO Quality Assurance Department for U.S. EPA Region 9 and RCRA. This time reduction is important because data review is a relatively slow step in the environmental work cycle [11]. Prior to the development of the RADAS program, data validation studies were performed manually, with the help of spreadsheet and word processing software. Resource limitations and time constraints imposed a partial audit method, where a representative portion of the data package was reviewed and the results considered to be indicative of the general quality of the entire sample batch [12]. Although this is a reasonable approach to the problem given the resource limitations, exhaustive reviews obviously yield a more accurate assessment.

Some of the review process requires visual inspection of chromatograms or mass spectra by a skilled chemist, and automation is unsuitable for such an evaluation within the present framework [13]. The availability of a system that automates the more routine and repetitive tasks allows the data evaluators to concentrate the bulk of their time on the higher-level tasks.

The QA/QC checks automated in RADAS to date include those for sample holding time, blank contamination, instrument calibration, and Contract Required Quantitation Limits (CRQL). The tasks that require the most time from the most skilled (and expensive) reviewers were selected to be automated first. RADAS uses information from each sample, such as date and time of extraction and analysis, and instrument employed (see Table 1). The system also uses information from the associated instrument calibrations performed by the laboratory during the period of analysis (Table 2). Relevant QA/QC samples are included, such as trip blanks, field blanks, method blanks, reagent blanks, and field duplicate samples. The system uses data files that contain information on the analytes, their QA/QC parameters, and the particular data validation guidelines to be employed.

Compound concentrations found to be suspect, including compounds reported as not detected (non-detects), are assigned a data quality label according to the severity of any deficiencies (Figure 1), based on the quality assurance requirements of the method and U.S. EPA Laboratory Data Validation Functional Guidelines [9]. Table 3 is a sample output depicting case data with corresponding data quality flags for the reported compound concentrations. Values that have deficiencies with low impact on data quality are classified as estimated quantities (J). These values are considered to be qualitatively acceptable but quantitatively unreliable, and are

CASE ID: 8780
FILE NAME: RADAS.SAM

SAMPLE LISTING

	SAMPLE		VOA				BNA					PES				
			REMARKS	ANALYSIS			REMARKS	EXTRACTION	ANALYSIS			REMARKS	EXTRACTION	ANALYSIS		
ORD	SAMPLE NUMBER	RECEIVED		DATE	TIME	INSTR		DATE	DATE	TIME	INSTR		DATE	DATE	TIME	INSTR
1	VMB9238	3/16/87		3/23/87	11:24	F4	NOT ANALYZED					NOT ANALYZED				
2	VMB2954	3/16/87		3/23/87	11:19	F9	NOT ANALYZED					NOT ANALYZED				
3	SMB7304F8		NOT ANALYZED				NONE DETECTED	3/18/87	4/21/87	15:06	F8	NOT ANALYZED				
4	SMB7304F12		NOT ANALYZED				NONE DETECTED	3/18/87	4/21/87	22:05	F12	NOT ANALYZED				
5	SMB7304F15		NOT ANALYZED				NONE DETECTED	3/18/87	4/21/87	19:07	F15	NOT ANALYZED				
6	SMB7334F12		NOT ANALYZED					3/18/87	4/22/87	13:36	F12	NOT ANALYZED				
7	PMB7304		NOT ANALYZED				NOT ANALYZED					NONE DETECTED	3/18/87	4/11/87	9 ;42	GC6
8	KB342	3/16/87	NONE DETECTED	3/23/87	15:21	F9		3/18/87	4/21/87	8:52	F15	NONE DETECTED	3/18/87	4/11/87	10:13	GC6
9	KB343	3/16/87		3/23/87	12:47	F4	NONE DETECTED	3/18/87	4/21/87	23:04	F12	NONE DETECTED	3/18/87	4/12/87	9:30	GC6
10	KB344	3/16/87		3/23/87	12:06	F4	NOT ANALYZED					NOT ANALYZED				
11	KB345	3/16/87		3/23/87	12:14	F9	NOT ANALYZED					NOT ANALYZED				
12	KB346	3/16/87		3/23/87	12:53	F9	NONE DETECTED	3/18/87	4/21/87	14:14	F8	NONE DETECTED	3/18/87	4/11/87	10:11	GC6
13	KB347	3/16/87		3/23/87	13:32	F9	NONE DETECTED	3/18/87	4/21/87	15:23	F15	NONE DETECTED	3/18/87	4/11/87	10:59	GC6
14	KB347MS	3/16/87	NOT ANALYZED				NONE DETECTED	3/18/87	4/21/87	16:43	F15	NOT ANALYZED				
15	KB347MSD	3/16/87	NOT ANALYZED				NONE DETECTED	3/18/87	4/21/87	17:56	F15	NOT ANALYZED				
16	KB348	3/17/87		3/23/87	16:03	F9		3/18/87/	4/21/87	21:13	F15	NONE DETECTED	3/18/87	4/11/87	11:37	GC6
17	KB349	3/17/87		3/23/87	18:00	F9		3/18/87	4/21/87	22:31	F15	NONE DETECTED	3/18/87	4/11/87	12:09	GC6
18	KB350	3/16/87		3/25/87	14:26	F9	NONE DETCTED	3/30/87	4/21/87	23:18	F15	NONE DETECTED	3/18/87	4/11/87	12:50	GC6
19	KB350MS	3/16/87	NOT ANALYZED				NOT ANALYZED					NONE DETECTED	3/18/87	4/12/87	13:42	GC6
20	KB350MSD	3/16/87	NOT ANALYZED				NOT ANALYZED					NONE DETECTED	3/18/87	4/12/87	14:27	GC6
21	KB351	3/16/87		3/23/87	15:05	F9		3/18/87	4/22/87	16:44	F12	NONE DETECTED	3/18/87	4/12/87	10:56	GC6
22	KB352	3/17/87		3/23/87	18:54	F4	NONE DETECTED	3/18/87	4/21/87	23:31	F15	SEE KB352-PES				
23	KB352-PES	3/17/87	SEE KB352				SEE KB352					NONE DETECTED	3/18/87	4/18/87	9:40	GC6
24	KB353	3/17/87	NONE DETECTED	3/23/87	16:52	F4		3/18/87	4/22/87	10:49	F12	SEE KB353-PES				
25	KB353-PES	3/17/87	SEE KB353				SEE KB353					NONE DETECTED	3/18/87	4/18/87	10:15	GC6
26	KB354	3/17/87		3/23/87	17:18	F9		3/18/87	4/22/87	14:29	F12	NONE DETECTED	3/18/87	4/12/87	11:44	GC6
27	KB355	3/17/87		3/23/87	16:03	F4	NOT ANALYZED					NOT ANALYZED				
28	KB356	3/16/87	NONE DETECTED	3/23/87	14:04	F4		3/18/87	4/22/87	12:43	F12	NONE DETECTED	3/18/87	4/12/87	12:27	GC6
29	KB356MS	3/16/87	NONE DETECTED	3/23/87	14:43	F4	NOT ANALYZED					NOT ANALYZED				
30	KB356MSD	3/16/87	NONE DETECTED	3/23/87	15:20	F4	NOT ANALYZED					NOT ANALYZED				
31	KB357	3/16/87	NONE DETECTED	3/23/87	17:29	F4		3/18/87	4/22/87	11:51	F12	NONE DETECTED	3/18/87	4/12/87	13:13	GC6

Table 1. Sample information listing.

CASE ID: 8780
FILE NAME: RADAS.SAM

ORDER	FRACTION	INSTRUMENT	TYPE	DATE	TIME	COMPOUND	RRF	%RSD (%D)
1	VOA	F4	INITIAL	3/18/87	15:58			
						2-BUTANONE	0.022	
2	VOA	F9	INITIAL	3/19/87	14:06			
						2-BUTANONE	0.041	
3	VOA	F4	CONTINUING	3/23/87	10:33			
						2-BUTANONE	0.024	
4	VOA	F9	CONTINUING	3/23/87	10:21			
						CARBON DISULFIDE		30.0
						1,1,2,2-TETRACHLOROETHANE	0.030	
5	BNA	F12	INITIAL	4/8/87	13:46			
						3,3'-DICHLOROBENZIDINE	0.010	22946.0
6	BNA	F15	INITIAL	4/8/87	19:56			
						PHENOL		33.0
						3,3'-DICHLOROBENZIDINE	0.010	
7	BNA	F8	CONTINUING	4/21/87	11:23			
						N-NITROSO-DIPROPYLAMINE		32.6
						2-NITROANILINE		48.0
						3-NITROANILINE		48.1
						2,4-DINITROTOLUENE		64.7
						DIETHYLPHTHALATE		28.4
						4-NITROANILINE		77.1
						ANTHRACENE		62.2
						3,3'-DICHLOROBENZIDINE	0.010	99.8
						BENZO(G,H,I)PERYLENE		79.9
8	BNA	F12	CONTINUING	4/21/87	20:33			
						N-NITROSO-DIPROPYLAMINE		27.4
						2-NITROANILINE		32.5
						2,4-DINITROTOLUENE		41.7
						HEXACHLOROBENZENE		31.8
						3,3'-DICHLOROBENZIDINE	0.010	
9	BNA	F12	CONTINUING	4/22/87	7:43			
						2,4-DINITROTOLUENE		35.8
						HEXACHLOROBENZENE		33.2
						3,3'-DICHLOROBENZIDINE	0.010	
10	BNA	F15	CONTINUING	4/21/87	8:05			
						3,3'-DICHLOROBENZIDINE	0.010	
11	BNA	F15	CONTINUING	4/21/87	20:10			
						3,3'-DICHLOROBENZIDINE	0.010	
12	BNA	F8	INITIAL	4/20/87	23:42			
						4-CHLOROANILINE		35.0
						3-NITROANILINE		40.0
						3,3'-DICHLOROBENZIDINE	0.010	
						BENZO(G,H,I)PERYLENE		37.0

Table 2. Instrument calibration information listing.

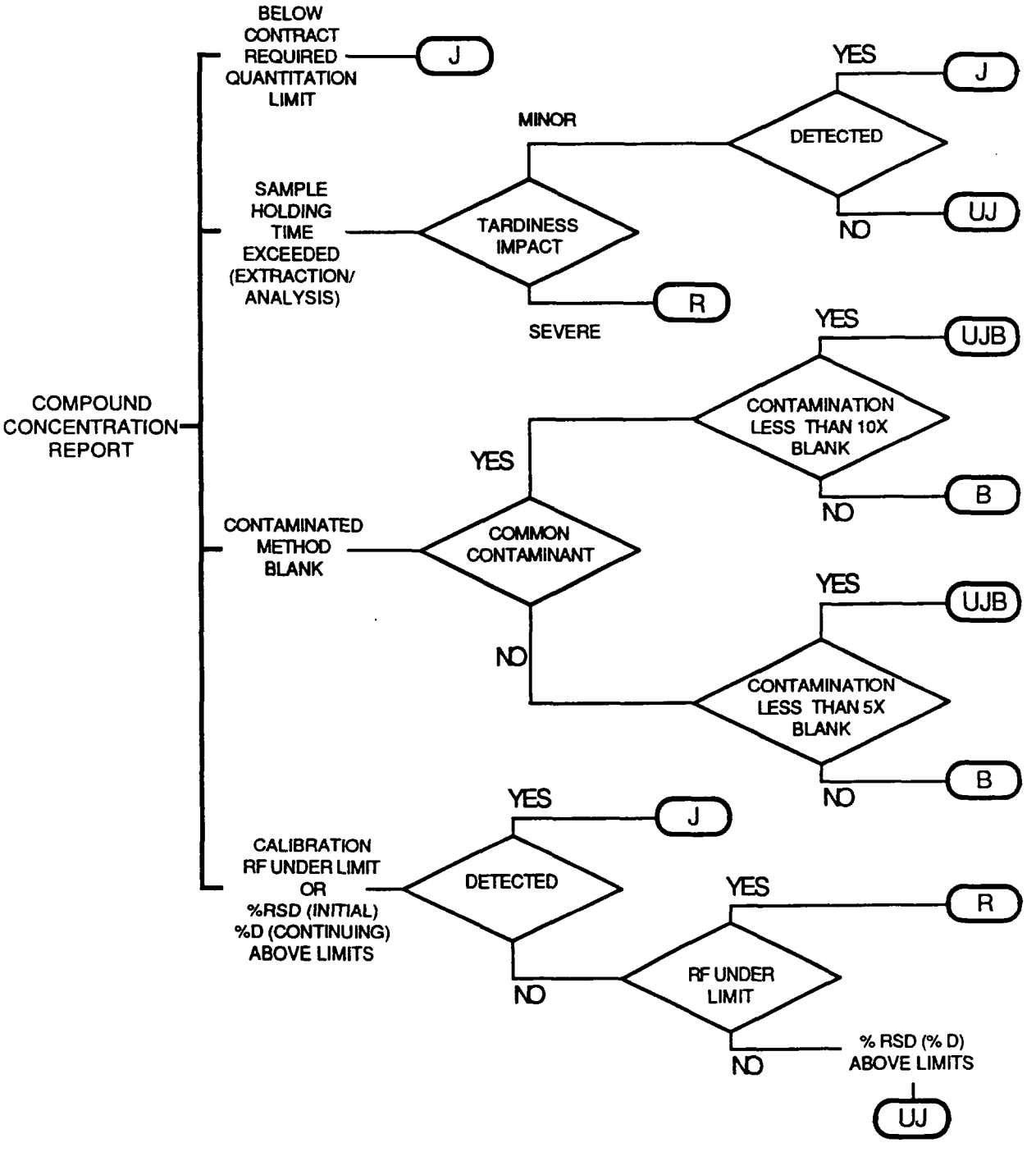

Figure 1. Compound concentration flagging system for data usability.

TCL SPREADSHEET

CASE NO: 8780
SITE: FEINSTONE -2B
SITE ID: FS-2B

SITE LOCATION: FEINSTONE, TEXAS
SITE DESCRIPTION: HAZARDOUS WASTE DRUM DUMP
ON-SITE GROUND WATER

SAMPLE NO:	VMB2954		SMB7304F15		KB342		KB345		KB347		KB348		KB349		KB350	
SAMPLE LOCATION:					SURFACE				SURFACE		GROUND		GROUND		LEACHATE	
SAMPLE TYPE:	METHOD BLANK		METHOD BLANK		FIELD DUP 1		FIELD BLANK 1		FIELD DUP 1							
MATRIX/ANALYSIS:	WATER/LOW		WATER/LOW		WATER/LOW		WATER/LOW		WATER/LOW		WATER/LOW		WATER/LOW		WATER/LOW	
DILUTION FACTOR:	1		1		1		1		1		1		1		1	
VOA			NOT ANALYZED													
METHYLENE CHLORIDE	10				4	UJB	6	UJB	2	UJB	80	UJB	110	B	5	UJ
ACETONE	5	J			4	UJB	7	UJB	2	UJB	45	UJB	60	B	50	JB
CARBON DISULFIDE	5	UJ			5	UJ	5	UJ	5	UJ	15	J	5	UJ	4	J
2-BUTANONE	8	J			10	UJB	11	UJB	8	UJB		R	10	UJB	12	UJB
1,1,2,2-TETRACHLOROETHANE		R			2	J		R	3	J		R		R		R
TOLUENE	2	J			2	UJB	2	UJB	1	UJB	10	UJB			25	JB
CHLOROBENZENE											6		10		5	UJ
ETHYL BENZENE	2	J			8	UJB			11	UJB					5	UJ
STYRENE	2	J													5	UJ
XYLENES (TOTAL)	2	J					1	UJB							5	UJ
BNA	NOT ANALYZED						NOT ANALYZED								NONE DETECTED	
PHENOL									8	J	20	J	12	J		R
3,3'-DICHLOROBENZIDINE				R		R				R		R		R		R
BIS(2-ETHYLHEXYL)PHTHALATE			5		2	UJB			3	UJB	88	B	45	UJB		R
PES	NOT ANALYZED		NOT ANALYZED		NONE DETECTED		NOT ANALYZED		NONE DETECTED		NONE DETECTED		NONE DETECTED		NONE DETECTED	

PAGE 1 ALL WATER MATRIX VALUES ARE REPORTED AS UG/L. ALL SOIL MATRIX VALUES ARE REPORTED AS UG/KG.

Table 3. Example case data spreadsheet.

usable for limited purposes only. Values associated with severe analytical deficiencies are flagged as unusable (R). In this case non-detects do not necessarily indicate the absence of the chemical compound.

Sample holding time between sampling, extraction (not applicable to volatiles), and analysis is checked (Table 4). When the prescribed holding times are exceeded, the reported concentrations of the affected analytes are flagged according to the degree of delay and its impact on data quality [14].

Validity is also evaluated based on instrument calibration information such as compound relative response factor, relative response factor linearity criteria, and frequency of calibration. Reports for compounds with an average relative response factor (RF) under the acceptable threshold are flagged, as well as reports for compounds with a high relative standard deviation on the calculated relative response factors for initial calibration (%RSD) or a high percent difference between continuing calibration and the corresponding initial calibration (%D).

Compound concentration levels which could be a result of contamination found in the associated blank [15] are appropriately flagged (B and UJB). Special criteria are used for commonly occurring volatile and semi-volatile contaminants.

Compounds reported below the contract required quantitation limit (by matrices and analysis levels) are automatically flagged. When non-detect reports are associated with deficiencies such as calibration problems or exceeded holding time the sample quantitation limit for that compound is flagged as estimated (UJ).

Data entry, file management and maintenance, and automated report generation functions are provided by the system. A case data tabular report (spreadsheet), individual detailed reports listing each of the items checked, and an optional narrative cover letter are produced.

RADAS IMPLEMENTATION

The program was developed on an IBM PS/2 Model 50 microcomputer and will run on any truly IBM PC-compatible machine. The minimum hardware requirements are 256 KB of available RAM and two floppy disk drives or a hard disk drive. The use of a larger memory is recommended to allow handling of larger data sets. Both color and monochrome monitors are supported and no graphics card or math coprocessor are required. RADAS has built-in support for many common printer models, allowing the selection to be made from inside the program. The program may also be used with other printers.

SAMPLE TIMELINESS SPREADSHEET

SAMPLE NUMBER	DATE OF RECEIPT	VOA ANALYSIS		BNA EXTRACTION		BNA ANALYSIS		PES EXTRACTION		PES ANALYSIS	
		DATE	DAYS LATE	DATE	DAYS LATE	DATE	DAYS LATE	DATE	DAYS LATE	DATE	DAYS LATE
VMB9238	03/16/87	03/23/87									
VMB2954	03/16/87	03/23/87									
SMB7304F8				03/18/87		04/21/87					
SMB7304F12				03/18/87		04/21/87					
SMB7304F15				03/18/87		04/21/87					
SMB7334F12				03/18/87		04/22/87					
PMB7304								03/18/87		04/11/87	
KB342	03/16/87	03/23/87		03/18/87		04/21/87		03/18/87		04/11/87	
KB343	03/16/87	03/23/87		03/18/87		04/21/87		03/18/87		04/12/87	
KB344	03/16/87	03/23/87									
KB345	03/16/87	03/23/87									
KB346	03/16/87	03/23/87		03/18/87		04/21/87		03/18/87		04/11/87	
KB347	03/16/87	03/23/87		03/18/87		04/21/87		03/18/87		04/11/87	
KB347MS	03/16/87			03/18/87		04/21/87					
KB347MSD	03/16/87			03/18/87		04/21/87					
KB348	03/17/87	03/23/87		03/18/87		04/21/87		03/18/87		04/11/87	
KB349	03/17/87	03/23/87		03/18/87		04/21/87		03/18/87		04/11/87	
KB350	03/16/87	03/25/87	2	03/30/87	9	04/21/87		03/18/87		04/11/87	
KB350MS	03/16/87							03/18/87		04/12/87	
KB350MSD	03/16/87							03/18/87		04/12/87	
KB351	03/16/87	03/23/87		03/18/87		04/22/87		03/18/87		04/12/87	
KB352	03/17/87	03/23/87		03/18/87		04/21/87					
KB352-PES	03/17/87							03/18/87		04/18/87	
KB353	03/17/87	03/23/87		03/18/87		04/22/87					
KB353-PES	03/17/87							03/18/87		04/18/87	
KB354	03/17/87	03/23/87		03/18/87		04/22/87		03/18/87		04/12/87	
KB355	03/17/87	03/23/87									
KB356	03/16/87	03/23/87		03/18/87		04/22/87		03/18/87		04/12/87	
KB356MS	03/16/87	03/23/87									
KB356MSD	03/16/87	03/23/87									
KB357	03/16/87	03/23/87		03/18/87		04/22/87		03/18/87		04/12/87	

Table 4. Sample holding time information listing.

RADAS was developed using the Turbo Pascal [16] compiler. The system was designed to be easy to use without extensive training (user friendly). Its user interface is based on "pull-down menus" and "pop-up windows". Error-handling and file integrity checking routines are included in the program.

SYSTEM PERFORMANCE AND VALIDATION

The current version of RADAS can evaluate results from organic volatile, base-neutral/acid (semi-volatile), and pesticide analyses performed by U.S. EPA Contract Laboratory Program Organic Methods [3], based on the modified Federal Register Methods 624, 625, and 608. The sample matrices included are water and soil, at "low" and "medium" concentration levels.

The analytical results and pertinent QA/QC information are received for review in "hard copy" format: thus, a data entry step is required until electronically delivered data becomes available. Once the necessary data is in the computer, the QA/QC review can be quickly performed automatically for the entire sample batch. The corresponding detailed reports can be printed in a delivery-ready format. In effect, work that was previously performed manually and required several people with varying degree of skills for weeks, can now be accomplished by fewer staff, in a more complete way, and in a matter of days or hours, depending on the required data entry.

The automated generation of final reports eliminates the word processing cycle (with the inherent possibility of introducing mistakes). This represents a considerable savings in writing and review time of both data auditing and clerical staff.

The total cost per sample for manual review and reporting is estimated to average over $25. The use of RADAS for data entry, evaluation, and reporting has reduced this cost by about 50 percent. The system has proven effective in reducing the turn-around time for final reports, increasing the completeness of the audits, and reducing error frequency in both the data validation determination and the report preparation.

Test cases were devised for assessing the performance of the program during its development. The system's preliminary validation consisted of an exhaustive auditing of a real sample batch concurrently utilizing the conventional manual methodology and RADAS automated auditing. The results obtained from RADAS for this case were found to be in complete agreement with the findings of the auditing staff. Other validation exercises are being conducted in-house before outside testing (beta testing), including validating a 900-sample study. Upon conclusion of this development phase, a final third party testing and validation will be conducted, and the system documentation will be completed.

DISCUSSION AND FUTURE DEVELOPMENTS

Simply meeting the QA/QC criteria does not imply that the analysis method employed is appropriate for the sample types and that the data is usable. An in-depth evaluation and a comparison of the data produced against the data quality objectives of the project are necessary. A system that can be adapted for use with different review guidelines represents a valuable tool to readily evaluate the appropriateness of data for its intended use, particularly given the expansion of Superfund work, the resultant sample load, and the short turn-around time required for data evaluation.

RADAS serves as a depository of knowledge on data validation. One of the design concepts was to make the information on variable data validation parameters external to the actual program's code. This allows one to readily make changes in the system's review procedures without extensive reprogramming, and furthermore, minimizes the staff retraining that accompanies changes in manual procedures. Methodology, format, and completeness of the auditing are consistent from case to case since they do not depend on circumstantial factors such as evaluator's skills and time availability.

Manually evaluating the quality assurance data is a very time-consuming process. RADAS provides improved speed, completeness, and consistency, as well as better documentation. Another potential benefit derived from the use of the system is easy retrieval of the case QA/QC information, which serves as a basis for the production of ad hoc reports (e.g. trends) as desired.

Development of the system as described in this paper required four equivalent man-months of programming effort. Work continues to expand RADAS to include additional QA/QC checks, as well as other types of analyses. Following completion of the validation and documentation for the current phase, RADAS will be made available to the Program Offices for potential implementation in their respective data review activities.

ACKNOWLEDGMENTS

Although research described in this article has been funded wholly or in part by the United States Environmental Protection Agency under contract number 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 trade names or commercial products does not constitute Agency endorsement of the product.

The authors want to thank Kent M. Kitchingman (U.S. EPA Region 9) and K. Sujith Kumar (Lockheed EMSCO) for their support of the development and Graciela I. Martucci (Lockheed EMSCO) for the valuable help provided during in-house testing of the system.

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LAST - A SOFTWARE SYSTEM THAT INTEGRATES FIELD, LABORATORY, MANAGEMENT AND GRAPHICAL DATA

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ABSTRACT

A computer software system will be described that integrates field, analytical, management and graphical data. The software is written in FORTRAN and runs on an IBM computer. It is used to calculate unit pricing factors both in units of dollars and labor hours. These factors are available with and without overhead labor and indirect costs. The software may be used to print sample labels, field sheets, laboratory data sheets, quality control reports, and to provide data electronically to graphics equipment for charts, graphs and maps. Additional reports that may be obtained from the system include work schedules sorted by client, state, and project leader, lists of overdue activities, average turnaround times for various units of work, time and dollar costs for a project, approved sample containers and preservatives, approved analytical methods, detection limit, accuracy and precision for each of the over 2,000 methods currently in the system, and the fraction of each employee's time charged to direct labor, indirect (overhead) labor and superfund. The software also tracks the numbers of determinations, analyses and samples analyzed by each of several laboratories. It accepts data electronically from and sends data to portable computers at remote field locations via phone lines. Data entry is minimized by employing the one-time-data-entry concept and by adding intelligence to many numbers used by the system. Since LAST was developed with public funds it is available to the public.

LABORATORY INFORMATION MANAGEMENT SYSTEMS: A MODULAR APPROACH

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ABSTRACT

With the volume of information handled in today's laboratory, the need for computers is obvious. However, the approach to implementing the use of computers is not always so obvious. What is right for one laboratory may be totally impractical for another. Implemented incorrectly, the computer may bring more problems than solutions. It is also true that the laboratory needs of today may not match the needs of tomorrow. To deal with differing and changing needs, TELECATION ASSOCIATES has developed a series of modularized solutions to laboratory computerization.

Using this approach, laboratory computerization can grow from a basic data entry and report generation program to a full PC-based, networked Laboratory Information Management System by simply adding modules. All modules address specific laboratory problems: report generation, QC/QA management, instrument interfacing, invoicing, archiving, chain-of- custody maintenance, even use of multiple work stations.

Of these modules, SMARTLOG (R) is a laboratory software package designed to provide all the basic data collection functions of a laboratory data station. For participants in the Environmental Protection Agency's "Contract Laboratory Program" (CLP), the deliverable data package may be generated with the SMARTLOG Inorganic CLP Report Module. SMARTLAB (R) is a PC-based Laboratory Information Management System (LIMS). Running on any IBM AT or compatible computer, SMARTLAB performs all the functions often associated with large LIMS. Modules may be used separately or together. When used together, modules interact by passing data from one module to another.

This concept of using modular software packages as the building blocks of a customized Laboratory Information Management System will be presented. Techniques for interfacing laboratory instruments to a PC/LIMS network will be discussed. Low cost alternatives to networking for electronic data transfer will also be addressed.

INTRODUCTION

The laboratory manager of today is faced with a tough challenge: how to get more done with fewer resources in less time. Recent publications estimate that laboratory staff members may spend only half their time dealing with physical samples and the chemical

tests required, and the other half converting the data obtained into useful information for reporting. With the volume of information handled in today's laboratory, effective automation of data management in a computerized Laboratory Information System (LIMS) should result in substantial productivity gains and an increase in overall organization, control and accuracy.

However, the approach to implementing the use of computers with such a system is not always obvious. Each laboratory has its own special requirements, and a system which is right for one laboratory may be totally impractical for another. Ideally, a laboratory information management system should be a practical tool, tailored to the laboratory's individual needs. Flexibility, ease of use, incremental pricing, and simplicity of data flow are just a few of the parameters required of this type of system.

To deal with the differing and ever-changing requirements of the laboratory, TELECATION ASSOCIATES has developed a series of modularized solutions to laboratory computerization. Using this approach, computerized data management can grow from a basic data entry and report generation program to a full PC-based, networked Laboratory Information Management System by simply adding modules. All modules address specific laboratory problems: sample tracking and report generation, QC/QA management, instrument interfacing, invoicing, even use of multiple workstations. These modules are, by design, totally integrated and compatible with one another. In this way, the unique requirements of virtually any lab can be addressed easily and economically. When these modules are combined, a powerful Laboratory Information Management System (LIMS) is developed.

The basic software building blocks of this system, SMARTLOG (R) and SMARTLAB (R), were introduced at this meeting last year. Since that time, each module has been enhanced, capabilities have been expanded, and the number of options for data transfer throughout the laboratory have been increased. The advancements have served to extend the upper end application of the total interactive system, thereby significantly enhancing the growth potential of the modular approach. Before discussing the enhanced capabilities, let us review the individual software modules.

BASIC BUILDING BLOCKS

SMARTLOG (R) and SMARTLAB (R) are two laboratory data management programs written for the IBM PC-AT or compatible computers. Both programs store analytical results and offer quality control modules, which automate the treatment and reporting of QC data. SMARTLOG is designed to collect analytical data, perform simple calculations, and print reports. SMARTLAB, which maintains additional sample handling and business information, functions as a Laboratory Information Management System. The programs may be used separately or together, as indicated in Figure 1. To illustrate how these basic software building blocks integrate with the

various modules having other laboratory functions, a description of each program follows.

LABORATORY SOFTWARE FROM TELECATION ASSOCIATES

SMARTLOG: The PC Data Station
* Provide manual data entry via computer keyboard
* Collect data automatically through custom instrument interface
* Perform calculations for precision, sample weight, volume and dilution factor correction
* Print customizable reports
* Transfer data to another computer or LIMS system including automatic transfer to SMARTLAB

SMARTLAB: Laboratory Information Management System
* Login sample information and results
* Track sample status
* Generate work sheets and status reports
* Monitor data quality
* Report analytical results
* Archive results and chain-of-custody

SMARTLOG/SMARTLAB: Data Station/LIMS system combination
* Collect data with dedicated SMARTLOG System interfaced to analytical instrument
* Transfer data to SMARTLAB to merge with main data base, when convenient

---

Figure 1. Features of SMARTLOG and SMARTLAB systems.

COLLECTING DATA WITH SMARTLOG

The main purpose of SMARTLOG is to provide a means of collecting, storing, reviewing, and reporting the results of laboratory analyses. For many analytical instruments, data may be collected automatically by simple connection of the instrument serial output to the RS-232 input of the computer. Data from newer instruments now found in the laboratory may already exist in files on the instrument's data station. For such instruments, SMARTLOG's data collection interface can be customized to extract the data from the instrument's own data files and insert it directly into SMARTLOG. Or data may be entered manually through the computer keyboard. Once in a common file structure, all results, regardless of the source, may be grouped by test or by sample number, and selected data may be isolated for easy review. Figure 2 illustrates a SMARTLOG data base record and the information which can be maintained for a single analyte on a single sample.

```
================== GENERAL DATA ENTRY SCREEN ====================

     Test ID: Al          Lab #:70198        Matrix:WATER
   Sample ID: 27-2824-31                        Sequence No.  23
      Client: Ajax Corporation

RECEIVED: date-07/08/87  time-08:30:00A  by- LJR          ============
PREPARED: date-07/10/87  time-10:00:00A  by- DDB         |Report
ANALYZED: date-07/11/87  time-02:45:00P  by- L. Knotts   |Name:  Ajax

-------------------------------RAW DATA---------------------------------
234.67__     235.38__     233.70__     ________     ________  ________
________     ________     ________     ________     Average  234.5833__
Alternate                                          SD 0.8435_ CV 0.36_%
Data .......estimated ________  recalibrated _________ r-value _____

--------------------------- CALCULATIONS ----------------------------
weight 1.13_  volume .200_  dil 5___  %sol _____ decimals __ (4 max)

====== REPORTED RESULT ======  -----------QUALITY CONTROL-----------
|  207.59___  mg/kg__ units   |  trend _______ qc _______  std _____
================================ ------------COMMENT-----------------
_____________________________________________________________________
=====================================================================
      F2 list  F3 find    F5 prev   F7 prt record   F9  copy   HOME
               F4 update  F6 next   F8 prt report   F10 menu   END
```

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ORIGINAL INSTRUMENT DATA

replicate	code
234.67	
235.38	
233.70	

Figure 2. Data maintained in SMARTLOG record.

After data is entered, you may add additional information about the sample, such as sample identification, sample type, and who submitted the sample for analysis. Up to ten replicate determinations of an analytical measurement can be entered into the data base. Where replicate measurements are made, the average result, standard deviation, and coefficient of variation are automatically calculated. Additional mathematical manipulations can be made on each result, to correct for such things as sample weight variations, or dilution factor differences.

If data has been collected automatically from an instrument, the results will be shown under the "instrument" section of the screen. This data area is inaccessible to manual entry or change.

All functions of the software are controlled by selection of a computer function key, the functions for which are always labeled at the bottom of the screen (as shown in Figure 2). Therefore, operation is self-prompting and quick to learn. To print a report of the selected data, for instance, one would simply depress the F8 function key.

The format of a data report is completely customizable. A report generation utility guides the user through the creation of customized report formats, starting with previously existing formats, which can be used as examples or templates. Any number of customized report formats may be created and stored for later use.

For laboratories involved in the Environmental Protection Agency's "Contract Laboratory Program" or who wish to report data in the CLP format, a quality control report option known as the SMARTLOG Inorganic CLP Report Module, automatically generates the CLP deliverable data package including the report forms, cover page and required floppy diskette. This module automatically determines the flags to be appended to data and calculates the required quality control parameters, as well as generating all of the QC forms to be submitted to the EPA. At the time of writing of this paper, the current revision is up-to-date with SOW 7/87, Revision 12/87.

One of the most valuable functions of SMARTLOG is its application as an interim data collection station, for subsequent data transfer to a larger "Laboratory Information Management System" (LIMS). Using SMARTLOG and an appropriately configured personal computer, data from a laboratory instrument can be collected and stored, until it is convenient to transfer the data into the laboratory's main data base.

The instrument/personal computer interface requires only simple direct connection for serial data transfer. The PC/LIMS connection may be made through a PC network, RS-232, modem, or even by simple disk transfer. These various options for data transfer to LIMS will be discussed more fully later. While a built-in utility provides automatic transfer of SMARTLOG data to the

SMARTLAB PC LIMS system, data can be transferred to any computer capable of receiving standard ASCII information.

RUNNING THE LABORATORY WITH SMARTLAB

SMARTLAB is a laboratory information management system designed for the IBM PC family of computers. The SMARTLAB basic system addresses the many different tasks associated with automating laboratory data management. SMARTLAB tracks the completion status of every sample, from login to archiving of the results. SMARTLAB identifies what samples are in the laboratory; what preparations have to be performed; what analyses have to be run; and what samples have been completed. Automatic quality control features aid the QC Manager in evaluating results. When a sample is completed, reports may be automatically generated. Old sample data and complete chain-of-custody documentation can be archived on floppy disk and accessed as needed.

SMARTLAB: ENTRY OF SAMPLE INFORMATION

Many details must be entered into a sample entry log, if complete documentation of the sample is to be maintained. A listing of the types of information which may be entered as part of sample documentation is shown in Figure 3.

SAMPLE LOGIN INFORMATION

- Client Information:
  - Client Name, Address, Contact, Phone number
  - Account Number
- Billing Information:
  - Client Name, Address, Contact, Phone number
  - Purchase Order Number, Discount
- Sample Information:
  - Lab Number, File Name
  - Login Date and Time
  - Priority / Due Date
  - Sample ID
  - Matrix
  - Collection:
    - Date, Time, Location, By Whom
  - Number of containers
  - Preservation method
  - Storage:
    - Location, Keep time, Discard date
  - Disposal:
    - Method, Date, By Whom
  - Additional Comments
- Tests Series to be performed
- Container number for each test

Figure 3. Client & sample information stored in SMARTLAB.

The goal for using a computer for laboratory information management is to expedite the handling of data and information. This includes information entry, as well as information access. SMARTLAB provides several means for speeding the process of information entry. When multiple samples from the same client are logged in at the same time, one key stroke will copy information from a previous sample into the record for each additional sample. Or multiple samples can be entered as a batch, by entering the basic client and sample information only once. And where a regular client is concerned, login is even simpler. Special files, sometimes referred to as "dictionaries" can facilitate operation of a LIMS. These special files control the configuration information used by the system. They define the parameters and procedures by which a LIMS can be structured to perform the unique information processing required by each laboratory. To a large extent, it is the "dictionaries", modified and updated interactively by the laboratory as needed, that come close to customizing the system for the user. With SMARTLAB, by typing in the client's account number, all of the client details are instantly looked up in the client "dictionary", and automatically entered. Frequently encountered series of analyses can similarly be identified in the test "dictionary" and automatically entered into the log. Even client-specific pricing will be recalled and used at invoicing time, for users having the Accounting Module. The types of information which may be kept in the test "dictionary" are listed in Figure 4.

TEST SERIES "DICTIONARY"

Test Series Name / Tests comprising series
Test Information:
- Test ID
- Laboratory section
- Test Hold Time / Expire Date
- Test Method
- Instrument / Instrument Detection Limit
- Upper and Lower Limits

Figure 4. Test Series Information stored in SMARTLAB.

SMARTLAB: DETERMINING SAMPLE STATUS

One of the most powerful benefits of SMARTLAB is its ability to provide the laboratory manager with a set of critically important reports which offer operational and managerial information about the laboratory's status. SMARTLAB maintains cross-referenced sample indices, listing the samples in a variety of useful orders. Samples may be displayed in any of the menu selectable orders by simply selecting the desired report from the Progress Report menu. This mode may also be used to generate lists of work to be done in each analysis section of the laboratory. Examples of a couple of the progress report options are shown in Figures 5 and 6.

ANALYSIS BACKLOG BY TEST AND DUE DATE December 10, 1987

section	test	lab #	client	description	due date
organic	2,4_D	610116	Waterford Engineering Laboratory	ground water	12/06/87
		610117	Waterford Engineering Laboratory	ground water	12/06/87
metals	Ag	610103	Burrow Enterprises	ore	11/25/87
		610104	Burrow Enterprises	ore	11/25/87
		610122	Geophysical Exploration, Incorpo	sediment	12/07/87
		610123	Geophysical Exploration, Incorpo	sediment	12/07/87
		610124	Geophysical Exploration, Incorpo	sediment	12/07/87
		610126	Geophysical Exploration, Incorpo	sediment	12/10/87
metals	As	610116	Waterford Engineering Laboratory	ground water	12/06/87
		610117	Waterford Engineering Laboratory	ground water	12/06/87
		610118	Ajax Manufacturing Company	effluent	12/07/87
		610119	Ajax Manufacturing Company	effluent	12/07/87
		610122	Geophysical Exploration, Incorpo	sediment	12/07/87
		610123	Geophysical Exploration, Incorpo	sediment	12/07/87
		610124	Geophysical Exploration, Incorpo	sediment	12/07/87
		610126	Geophysical Exploration, Incorpo	sediment	12/10/87
		610128	Ajax Manufacturing Company	effluent	12/13/87
		610129	Ajax Manufacturing Company	effluent	12/13/87
		610130	Ajax Manufacturing Company	effluent	12/13/87
		610131	Ajax Manufacturing Company	effluent	12/13/87
metals	Au	610103	Burrow Enterprises	ore	11/25/87
		610104	Burrow Enterprises	ore	11/25/87
		610122	Geophysical Exploration, Incorpo	sediment	12/07/87
		610123	Geophysical Exploration, Incorpo	sediment	12/07/87
		610124	Geophysical Exploration, Incorpo	sediment	12/07/87
		610126	Geophysical Exploration, Incorpo	sediment	12/10/87
metals	B	610121	Brown Farms	soil	12/09/87
		610125	Brown Farms	soil	12/12/87
		610127	QC	soil	12/10/87
metals	Ca	610032	Western Utilities Company	boiler water	11/29/87
		610033	Western Utilities Company	boiler water	11/29/87
		610120	Western Utilities Company	boiler water	12/08/87
organic	chloroform	610118	Ajax Manufacturing Company	effluent	12/07/87
		610119	Ajax Manufacturing Company	effluent	12/07/87
		610128	Ajax Manufacturing Company	effluent	12/13/87
		610129	Ajax Manufacturing Company	effluent	12/13/87

Figure 5. List of incomplete samples sorted by test.

SAMPLE PREPARATION BACKLOG December 10, 1987

prep method	test	lab #	client	description	due date
acetate leach	B	610127	QC	soil	12/10/87
		610121	Brown Farms	soil	12/09/87
		610125	Brown Farms	soil	12/12/87
	Mn	610127	QC	soil	12/10/87
		610125	Brown Farms	soil	12/12/87
		610121	Brown Farms	soil	12/09/87
	Mo	610127	QC	soil	12/10/87
		610125	Brown Farms	soil	12/12/87
		610121	Brown Farms	soil	12/09/87
	NO3	610121	Brown Farms	soil	12/09/87
		610125	Brown Farms	soil	12/12/87
		610127	QC	soil	12/10/87
	P	610121	Brown Farms	soil	12/09/87
		610125	Brown Farms	soil	12/12/87
		610127	QC	soil	12/10/87
benzene extract	2,4_D	610117	Waterford Engineering Laboratory	ground water	12/06/87
		610116	Waterford Engineering Laboratory	ground water	12/06/87
	chloroform	610129	Ajax Manufacturing Company	effluent	12/13/87
		610119	Ajax Manufacturing Company	effluent	12/07/87
		610128	Ajax Manufacturing Company	effluent	12/13/87
		610118	Ajax Manufacturing Company	effluent	12/07/87
		610130	Ajax Manufacturing Company	effluent	12/13/87
		610131	Ajax Manufacturing Company	effluent	12/13/87
	endrin	610116	Waterford Engineering Laboratory	ground water	12/06/87
		610117	Waterford Engineering Laboratory	ground water	12/06/87
distillation	CN	610119	Ajax Manufacturing Company	effluent	12/07/87
		610128	Ajax Manufacturing Company	effluent	12/13/87
		610131	Ajax Manufacturing Company	effluent	12/13/87
		610129	Ajax Manufacturing Company	effluent	12/13/87
		610130	Ajax Manufacturing Company	effluent	12/13/87
		610118	Ajax Manufacturing Company	effluent	12/07/87
	F	610117	Waterford Engineering Laboratory	ground water	12/06/87
		610116	Waterford Engineering Laboratory	ground water	12/06/87
HNO3 digestion	Ag	610103	Burrow Enterprises	ore	11/25/87
		610104	Burrow Enterprises	ore	11/25/87
		610126	Geophysical Exploration, Incorpo	sediment	12/10/87
		610124	Geophysical Exploration, Incorpo	sediment	12/07/87
	As	610130	Ajax Manufacturing Company	effluent	12/13/87
		610118	Ajax Manufacturing Company	effluent	12/07/87

Figure 6. List of incomplete sample preparations.

SMARTLAB: CUSTOMIZABLE REPORTS / SAMPLE DOCUMENTATION

A sample analysis is of little use until the results are reported. SMARTLAB will automatically find completed samples and print out the results in a report. The form of the sample report is easily customizable to your preferred format. Any of the information maintained on the sample in SMARTLAB may be included.

The completion and reporting of a sample does not mean that there will never be a need to review the results. Further, for environmental and legal work, it is becoming more and more important to document the movement and treatment of every sample, with name, date, and treatment information ("chain-of-custody"). SMARTLAB saves all completed sample information on the system's hard disk storage, with long-term archiving possible on other media, such as floppy disk.

MAINTENANCE OF LABORATORY QC DOCUMENTATION

Organizations are under external and internal pressures to be able to verify the accuracy of information reported. In order to make informed decisions when approving analytical results, you must have access to pertinent criteria of quality control. Both SMARTLOG and SMARTLAB QC Modules offer similar QC capabilities. In SMARTLAB, QC information is automatically available at the time that sample results are approved, thus providing the necessary information to make an informed decision on the validity of the results. Quality control results are stored in the sample analysis data record, for permanent reference. Standard techniques of quality control addressed by SMARTLOG and SMARTLAB include: determination of spike recovery, repeatability of replicate analyses, and agreement of results with known or certified values. Tabulated and graphed quality control documentation can be stored for later reference and printed for distribution. Figures 7 and 8 present examples of printed output from the QC routines. SMARTLAB can keep historical data on a client's samples and display it graphically, as shown in Figure 9, for a clear indication of trend.

SPIKE RECOVERY

lab #	test	units	result	spike	Recovery	% Recovery
610032	Ca	ug/L	1000.0	0.0		
610033	Ca	ug/L	1090.0	100.0	90.0	90.0
610032	Cu	ug/L	20.0	0.0		
610033	Cu	ug/L	48.0	25.0	28.0	112.0
610032	Fe	ug/L	30.0	0.0		
610033	Fe	ug/L	74.0	50.0	44.0	88.0
610032	Mg	ug/L	2000.0	0.0		
610033	Mg	ug/L	2487.0	500.0	487.0	97.4

Figure 7. Spike recovery table.

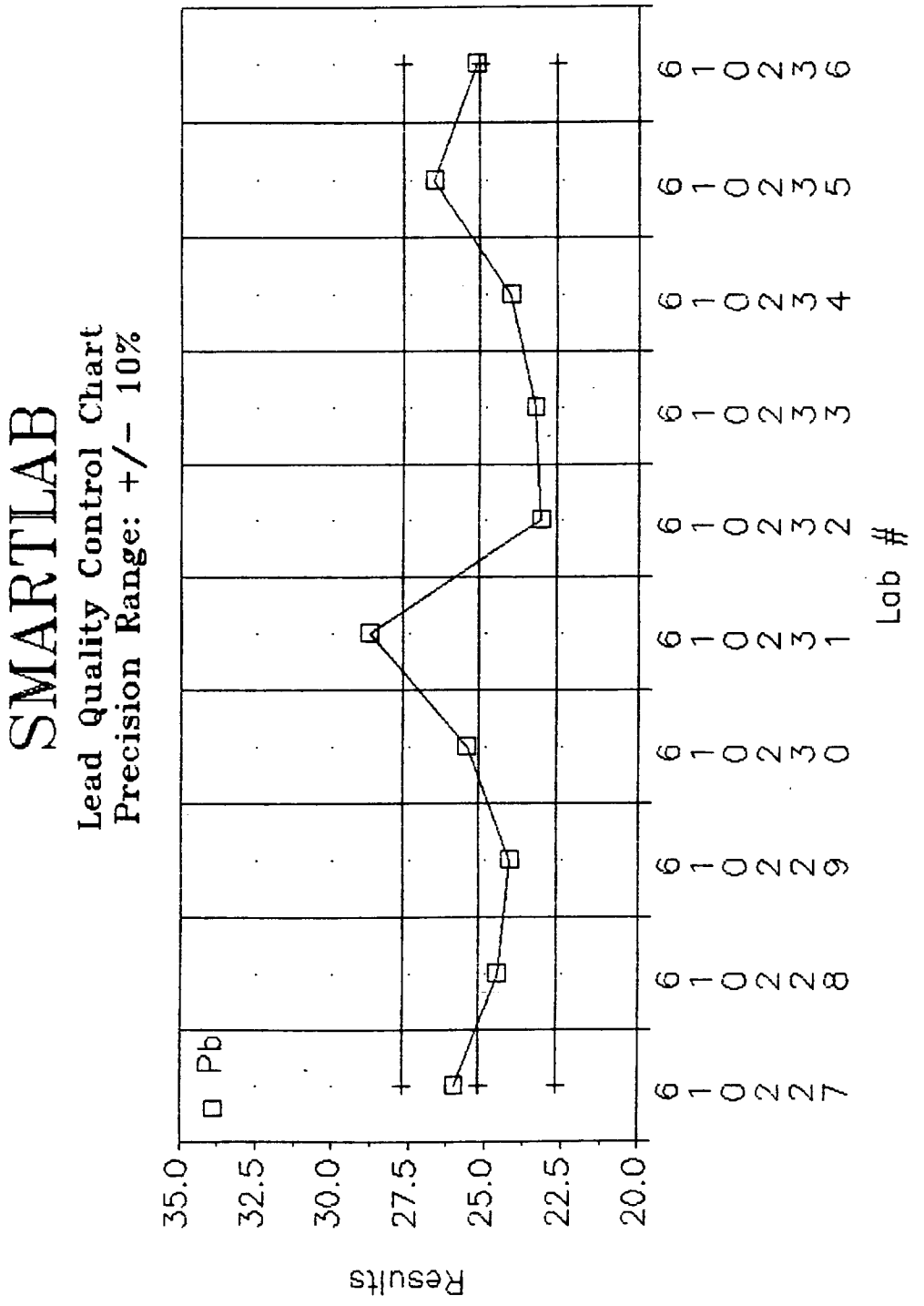


Figure 8. SMARTLAB quality control chart.

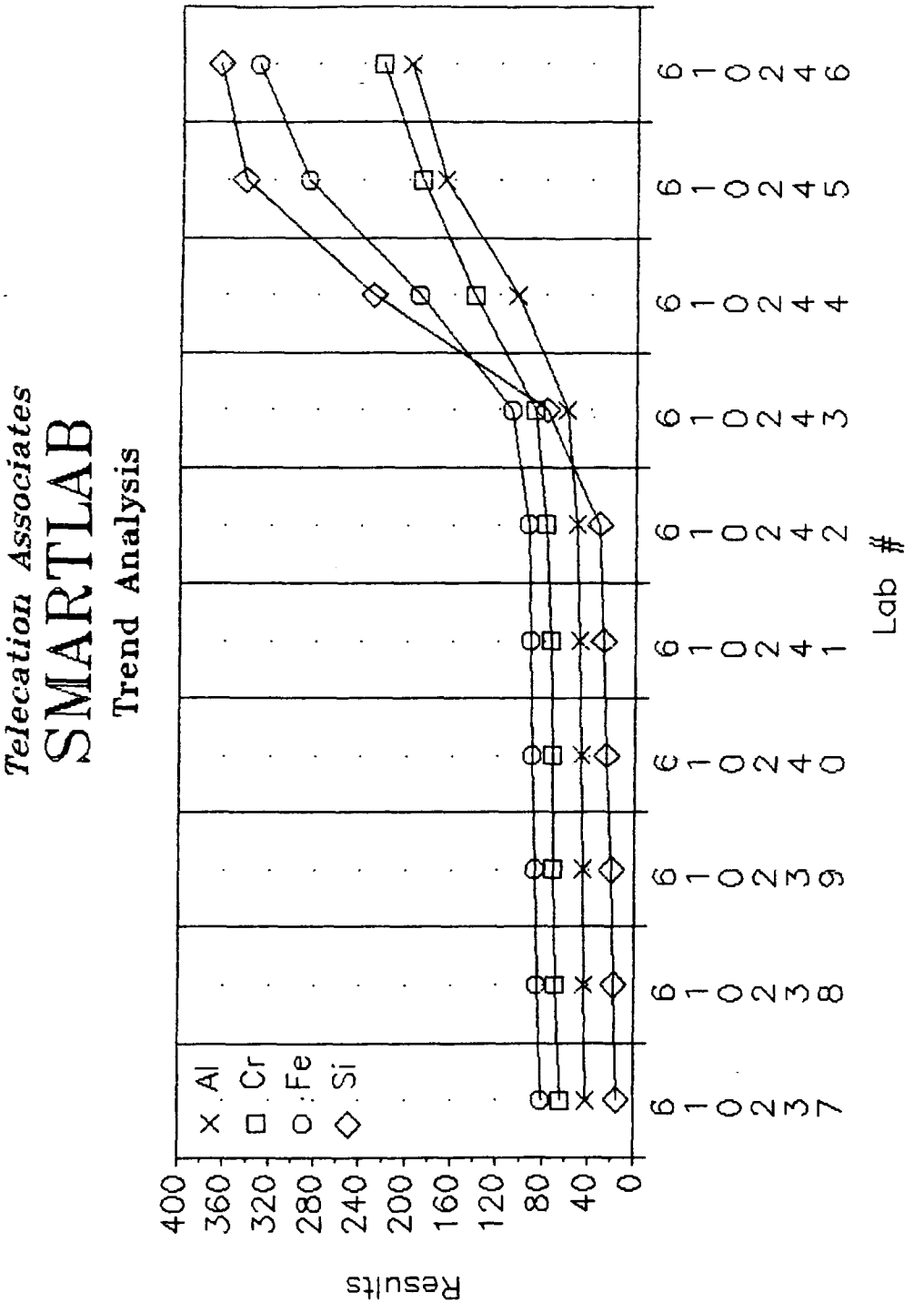


Figure 9. SMARTLAB trend analysis graph.

In addition to the standard QC displays, both tables and graphs are customizable to accomplish various additional graphing and tabulating functions, which might be required by a particular laboratory. A graph generation utility aides the user in designing custom graphs.

SMARTLAB: LABORATORY ACCOUNTABILITY

For laboratories who charge for their analytical services, SMARTLAB's Accounting Module can provide an integrated billing/invoice system for the lab. Charges for the tests may be automatically recalled from stored price schedules, pricing reviewed and modified according to the circumstances and invoices generated for completed samples. Two general formats for invoices are supplied, one for detailed analysis charges, another providing only sample totals. The exact form of each invoice format is customizable. An example of one of the standard invoices is shown in Figure 10.

GOLDEN ANALYTICAL SERVICES, INC.
P.O. Box 1486
Golden, Colorado 80043
phone (303) 555-2100

To: Burrow Enterprises
Route 2
Box 955
Cheyenne WY 89335

Invoice date 12/10/87
Attn: Accounts Payable Invoice no. 144207

- INVOICE -

Lab #	Submitted	Sample Identification	Charge
		Burrow Enterprises	
610101	11/19/87	sample 1	$47.96
610102	11/19/87	sample 2	$30.56
		Total	$78.52

Terms: net 30
Thank you.

Figure 10. SMARTLAB standard invoice.

In addition to the invoices which go out to your customers, SMARTLAB's Accounting Module can generate useful in-house accounting reports, such as the accounts receivable report, shown in Figure 11.

GOLDEN ANALYTICAL SERVICES, INC.
- ACCOUNTS RECEIVABLE -
Samples Completed and Billed

client	lab #	inv #	invoice date	charges
Ajax Manufacturing Company	610016	144204	11/27/87	$140.20
	610114	144205	11/28/87	$48.00
			Client Total	$188.20
Burrow Enterprises	610101	144207	12/10/87	$47.96
	610102	144207	12/10/87	$30.56
			Client Total	$78.52
Western Utilities Company	610113	144206	11/28/87	$32.00
	610110	144206	11/28/87	$32.00
	610112	144206	11/28/87	$32.00
			Client Total	$96.00
			Total Receivables	$362.72

Figure 11. SMARTLAB accounts receivable report.

NETWORKING PERSONAL COMPUTERS IN THE LABORATORY

Chemical analysis laboratories are well suited for using personal computers and PC-based, local area networks. Personal computers, located throughout the laboratory, provide low-cost local data acquisition and data reduction. Each PC in the laboratory is connected to a high disk capacity "file server", typically a 386 computer, for common access to a centralized data base.

From any PC in the network, data acquired can be reviewed, recalculated, reported or plotted by security authorized personnel. Combined reports of data from several laboratory

sections are easily generated, since all the data is stored in a single location. Results can be sent to other computers with the necessary communications software which is inherent with SMARTLAB, and the required hardware.

Networks provide a perfect growth path for your laboratory data handling needs. As your laboratory grows, or as you need to include additional instruments to your laboratory information system, simply add another PC with workstation software to the network.

DATA MANAGEMENT: A MODULAR APPROACH

Using the PC-based software modules described above, the laboratory can custom design and implement an approach to managing the information in the laboratory, based on their individual requirements and their personnel and budgetary constraints.

The ultimate in flexibility for a laboratory data management system is a LIMS where the various workstations are networked to the main data base. The use of a well-designed PC-based network offers many of the same capabilites as large LIMS, at a fraction of the cost. For many laboratories this approach will provide a low-cost way to leverage the PC resources already present in the laboratory. Such a comprehensive LIMS provides a cost-effective solution to the problem of automating laboratory record-keeping, testing and reporting.

Although a networked LIMS is the final goal for the automation of laboratory data management, TELECATION ASSOCIATES recognizes the need by some laboratories for a low risk "building block" approach. Using this strategy, the laboratory can add modules to the system as it sees a return. For laboratories in this type of situation, the following options exist.

A system can be as simple as a basic data entry and report generation system, SMARTLOG on a single user personal computer. That system can be enhanced through the use of instrument interfaces, which will provide the ability to automatically collect and process data generated by your analytical instruments.

For those whose requirements include the ability to login samples, track their progress in the lab, enter data, monitor QC, and generate analytical reports, the Basic SMARTLAB system provides the building block upon which to grow. For the smaller laboratory, all the basic capabilities of a larger LIMS are available for use on a single personal computer. The Accounting and extended QC modules can be added at any time to give their respective capabilities to the system.

SMARTLOG installed on a workstation can be added to this growing system, to add the capability of automatic data entry. The approach to this data transfer depends upon the laboratory's

goals. For the laboratory having just a single computer, both SMARTLOG and SMARTLAB may be installed on this computer. Samples are logged into SMARTLAB. Data is collected with SMARTLOG and then transferred, within the same computer, to the SMARTLAB data base. Any results not collected with SMARTLOG may be entered into SMARTLAB via the computer keyboard. The progress of samples may be monitored, analytical reports printed and data archived - all on a single computer.

For the laboratory wanting to use separate computers to act as workstations for data collection, the transfer of data from SMARTLOG may take one of several approaches, as outlined in Figure 12.

SMARTLOG-to-SMARTLAB DATA TRANSFER

* Disk transfer
* RS-232 cable linkage
* Communication via modem
* Communication via electronic mail services
* Local Area Network

Figure 12. Modes of data transfer from SMARTLOG to SMARTLAB

The simplest way is through a technique sometimes referred to as "sneaker-net". Data from SMARTLOG is transferred to a floppy disk. This floppy disk is then hand carried to the SMARTLAB computer, where a special utility transfers the information on the disk to the SMARTLAB data base. This lowest-cost alternative provides one-way data transfer capability without additional hardware and is easily implemented.

The SMARTLOG computer(s) may also be linked to the SMARTLAB computer through the use of RS-232 cables. Data transfer with this system is still one-way (SMARTLOG to SMARTLAB) and requires RS232 cables to be run between the computers. When SMARTLAB is ready to receive data from the SMARTLOG workstation, the SMARTLAB operator tells the SMARTLOG user to transfer the data via a serial communications port. When data transfer is complete, each user may continue using their respective computer.

If the laboratory does not desire to run RS-232 cables, the two computers may communicate via modem. SMARTLOG and SMARTLAB both have communications software within their systems for this use, however this option does require each computer to have its own modem and phone line. Essentially, when data transfer is to occur, the SMARTLOG computer "calls" the SMARTLAB computer; SMARTLAB "answers" establishing communiciation, and data is sent.

The use of RS232 cables and modems both require that the two computers be ready to communicate at the same time, and that their operators synchronize this action. A viable option which allows SMARTLOG to send its data when it is ready, and SMARTLAB to

capture this data at any later time, is through the use of an electronic mail service. By subscribing to one of these commercially available services, such as MCI Mail, Easylink, or Compuserve, the laboratory can provide a time-effective, inexpensive communication link between two computers. When the SMARTLOG user is ready to send his data to SMARTLAB, such as perhaps toward the end of the day, the SMART communications software is used to call up this electronic mail service. The data is then transferred to a password secured mailbox, which has been set up for SMARTLAB. When the SMARTLAB supervisor is ready to upload data from the SMARTLOG workstation, the mail service is called, the mail box is accessed, and data transfer is initiated. In this way, the electronic mailbox serves as a potentially large nterim storage device, which allows each computer in the lab to work more efficiently. This option does require a modem for each computer as well as subscription to the service.

However, if there are several staff members, each requiring access to the laboratory's data base at the same time, a networked Laboratory Information Management System, having full file and record-locking protection, is essential. Such a system is depicted in Figure 13. From any PC in the network, samples can be logged in and data entered, reviewed, recalculated, reported or plotted by personnel having security authorization for these functions. For example, one workstation could be used for sample login and the printing of sample labels. Other workstations could be placed throughout the lab for data entry. The QC Manager could monitor the quality of the analyses, generate QC charts and plot them from his office; and the Laboratory Manager could be using his workstation to check sample progress, generate worksheets, evaluate laboratory workloads, etc. Still other workstations could be used to print customer reports and invoices. With such a system, a LIMS can tie together all the instrumentation, resources, and personnel of the laboratory into a cohesive network.

TELECATION ASSOCIATES specializes in the design and development of practical software and computer solutions for the analytical lab. The modular approach has been developed as a means for easy and cost effective configuration to an individual laboratory's needs. Each module addressed a relevant laboratory application. Modules are selected, only as needed, thereby avoiding extra initial cost. An organized data handling system can be initiated with minimum capital, time and personnel. Then, as the needs expand, each modular addition to the system is a small incremental step. The ability to closely track expenditure with needs minimizes risk and maximizes output. When fully and correctly implemented, Laboratory Information Management Systems are bringing significant benefits to laboratory operations, including control and standardization within the laboratory, the reduction of paperwork-related errors, and providing business return on investment.

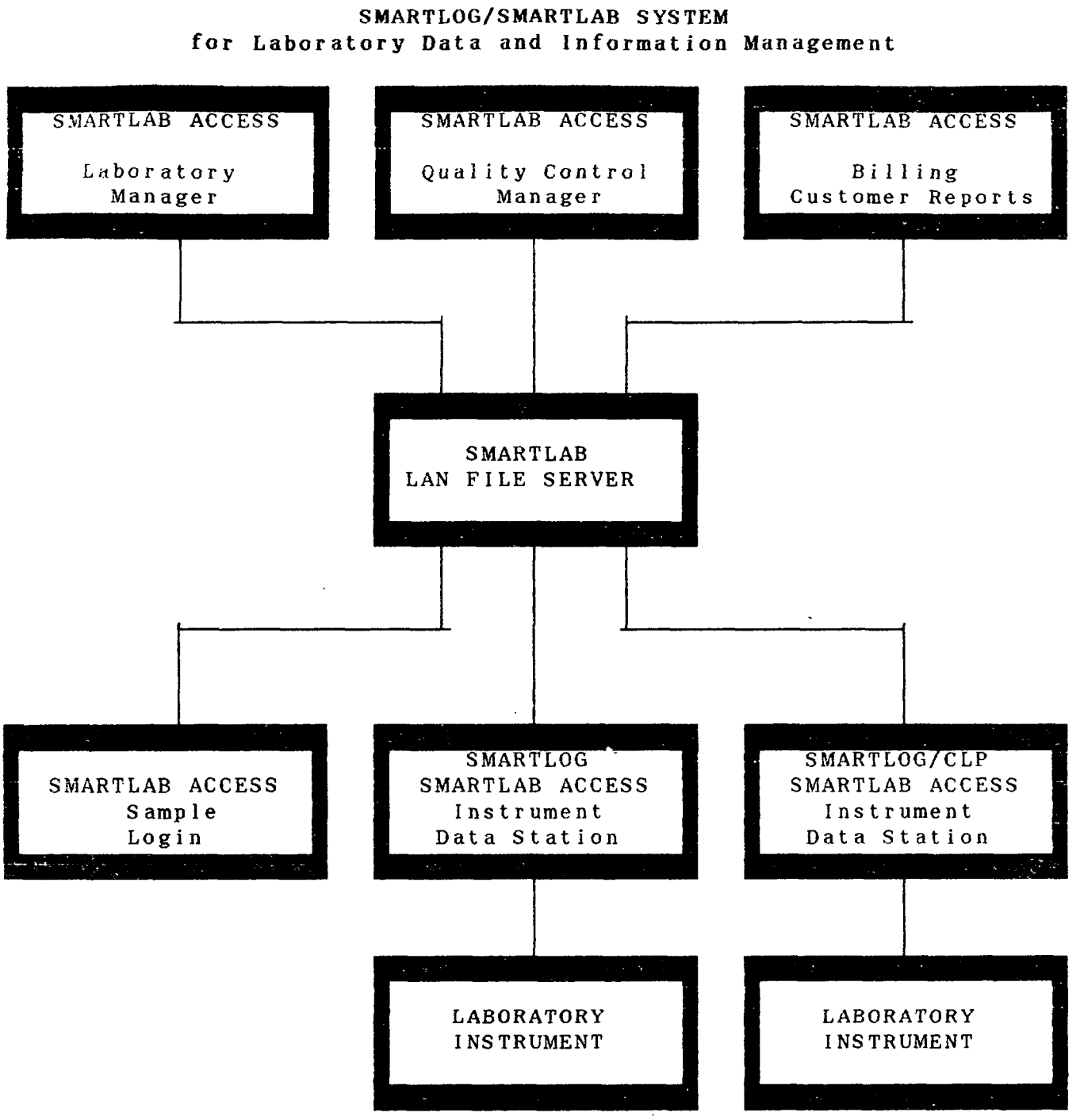


Figure 13. PC-networked LIMS.

DATA CONSOLIDATION AND REPORTING FOR THE ANALYTICAL LABORATORY

Norman Low, Scientific Instruments Division, Hewlett-Packard, Palo Alto, California 94546

ABSTRACT

Automation of laboratory instrumentation has significantly increased the rate at which samples could be analyzed. However, productivity in some other processes have not made the same gains due to the human decisions and operations involved. The personal computer, combined with the capabilities of the analytical data system, can be used in new ways to automate some of these processes. As a result, significant improvements can be made in throughput and quality of reports.

INTRODUCTION

Computers have changed the way we operate an analytical laboratory. Today, it is hard to find an instrument which does not have either microprocessors or an associated data system. These computers have given us the capability to extend our automation capabilities beyond what we thought possible only a few years ago.

Although data system-controlled batch acquisition and data reduction have enabled us to increase sample analyses and processing, the user-intensive operations of data review and report production have not changed as much. Oftentimes, data review consists of examining data scattered across several pages of reports. To evaluate a single sample, one may have one sheet for the sample, a second for its duplicate, a third for its matrix spike, and a fourth for the sample blank. Additional sheets are necessary if one has injections for a matrix spike duplicate and a travel blank. If a high concentration of a pesticide was found in a GC run, one may want to see if it was found in the GC/MS library search. In some cases one must verify results by examining the original data on two separate data systems. Data review is an essential part of the analytical process to ensure good quality data. It must be done, and done correctly, on a routine basis.

Another manual process which takes a considerable amount of time is report production. First, the chemist examines the data and edits his analytical results. Many of the steps taken in this phase, rounding numbers to the correct number of significant digits, calculating the detection limit for the sample, etc. are fairly

mechanical but gives great opportunity for error. The quality assurance officer of the lab must spend time making sure that everything is done correctly. Finally, a summary report is prepared for the typist who produces and proofreads the final customer report.

In this paper, we would like to discuss ways to improve productivity by considering the reports we need in the analytical process. In some cases, we want to view both the GC and GC/MS runs on a single data system. In other cases, we need to have a summary report in the form of a spreadsheet. Finally, we want to produce a customer report with the minimal amount of manual editing and transcription.

We examined what can be done using a personal computer in conjunction with an analytical data system. More specifically, for most of the cases, we used an HP RTE Mass Spectrometer Data System and an HP Vectra (IBM AT-compatible) personal computer. The Vectra PC, with the proper terminal emulation software, can be used as a terminal for the MS data system. Bidirectional file transfers between the RTE system and the PC allow great flexibility in examining various alternative processing methods.

PRODUCING REPORTS FROM SIMPLE ASCII FILES

For our first case, let us consider what we can do with the basic output from an integrator. Most data systems and integrators can produce simple ASCII files that include retention time, integrated area, and concentration data important for evaluating the analysis (Figure 1). A file like this can be transferred from an HP 3396 integrator to a personal computer via an RS-232 cable. When the uploaded file is imported into a spreadsheet, the entries for each line are placed into separate cells. Within a line, multiple fields are not separated. The spreadsheet does not know where a field begins or ends. To separate the fields on a line, we must use the data parse function of the spreadsheet. Once the file is parsed, individual fields may be easily added, deleted, or modified. The structure of a spreadsheet makes it easier to delete or add columns of data than a word processor.

After modifying the fields, one can perform any needed mathematical operations such as making dilution corrections, and rounding the results to the proper number of significant digits (see Figure 2). The columns showing intermediate data may be hidden and saved with a file, but not printed on the report . If a lab uses a well-designed, suitably protected spreadsheet, the time needed by the quality assurance department to check arithmetic is greatly decreased.

RUN # 8 AUG 27, 1987 08:54:27

SAMPLE NAME: NATGAS01 SAMPLE# 170

SIGNAL FILE: M:NATGAS.BNA
REPORT FILE: M:NATGAS.RPA

NATURAL GAS ANALYSIS

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#		MOL.%	NAME
1.866	BB	226327	0.109	34570	1		0.505	C6+
4.853	PB	1033411	0.125	137313	2		5.984	PROPANE
6.139	PB	594770	0.161	61446	3		3.000	ISO-BUTANE
7.100	PB	596342	0.186	53576	4		3.002	n-BUTANE
10.194	PB	210706	0.271	12961	5	R	0.997	ISO-PENTANE
11.570	PB	244834	0.298	13686	6		0.987	n-PENTANE
14.414	BB	134217	0.203	11008	7		0.990	CARBON DIOXIDE
16.820	PB	1299162	0.296	73238	8		9.041	ETHANE
18.153	VV	174179	0.319	9096	9		1.219	OXYGEN

Figure 1 - Integrator report

SAMPLE# 170
NATURAL GAS ANALYSIS

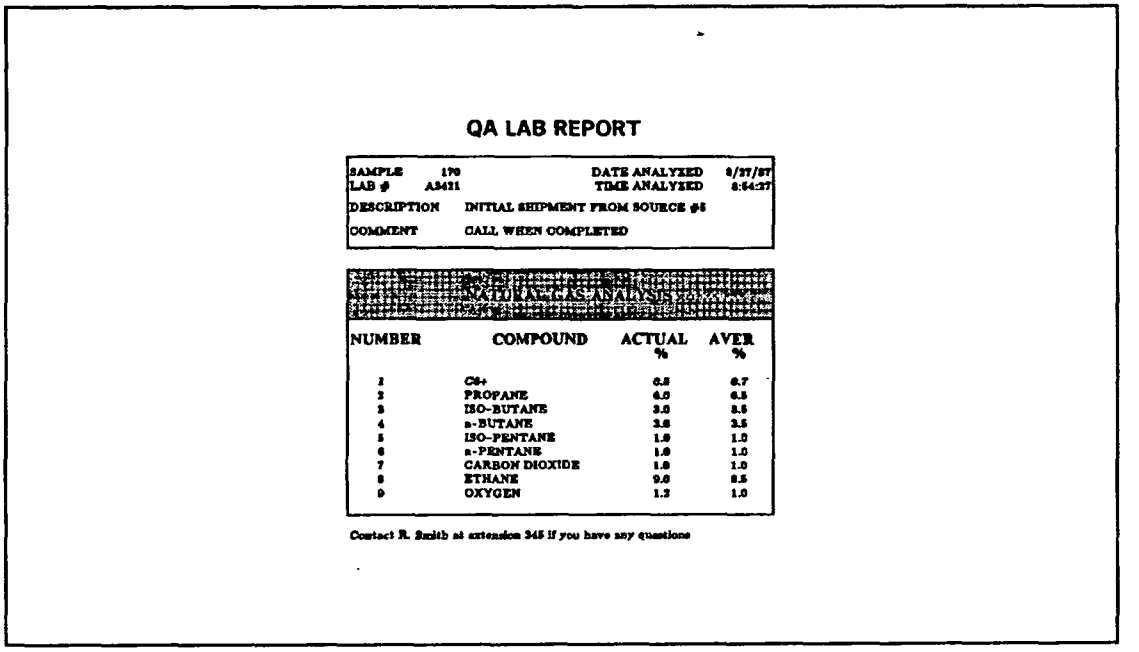
	NAME	MOL.%
1	C6+	0.5
2	PROPANE	6.0
3	ISO-BUTANE	3.0
4	n-BUTANE	3.0
5	ISO-PENTANE	1.0
6	n-PENTANE	1.0
7	CARBON DIOXIDE	1.0
8	ETHANE	9.0
9	OXYGEN	1.2

Figure 2 - Spreadsheet - modified integrator report

Some spreadsheets, such as Microsoft Excel, can be used for extensive report formatting. In Figure 3, we have used these capabilities to enhance the report by applying different fonts, line styles, and shadings. This report, which also incorporates some customer-required information, is significantly better than what is usually produced manually.

Parsing and formatting reports are best done by writing macros, batch procedures which repeat a sequence of steps. With some spreadsheets, these steps can be recorded and repeated with other sets of data. If this is the main method for producing reports, a lab would need to write macros for each data system that will be contributing result files. Macros also allow one to produce more complex reports, with multi-sample results placed in adjacent columns or the target compounds are placed in a different order.

We have shown how it is possible to produce a final customer report from a simple integrator report . All editing, calculations, and formatting can be automated through macros, thereby reducing the possibility of error and reducing the manual steps needed to produce a customer report. The hardware and software to perform these tasks are relatively common in laboratories and minimal training is required.



QA LAB REPORT

SAMPLE #	170	DATE ANALYZED	8/27/87
LAB #	A3421	TIME ANALYZED	8:54:27
DESCRIPTION	INITIAL SHIPMENT FROM SOURCE #5		
COMMENT	CALL WHEN COMPLETED		

NATURAL GAS ANALYSIS

NUMBER	COMPOUND	ACTUAL %	AVER %
1	C6+	0.8	0.7
2	PROPANE	6.0	6.5
3	ISO-BUTANE	3.0	3.5
4	n-BUTANE	3.8	3.5
5	ISO-PENTANE	1.0	1.0
6	n-PENTANE	1.0	1.0
7	CARBON DIOXIDE	1.0	1.0
8	ETHANE	9.0	8.5
9	OXYGEN	1.2	1.0

Contact R. Smith at extension 345 if you have any questions

Figure 3 - Integrator report enhanced by spreadsheet

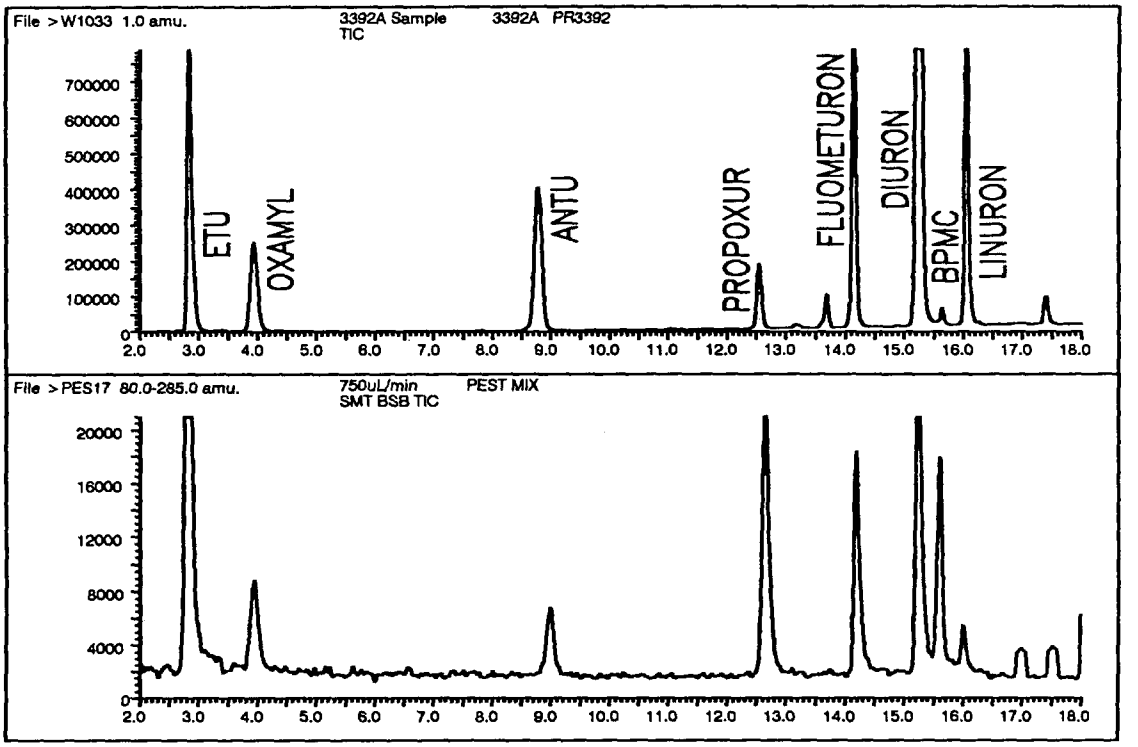


Figure 4 - Combined UV/Vis and Thermospray chromatograms

COMBINING GRAPHICS FROM MULTIPLE TECHNIQUES

When a sample has been run on multiple types of instruments, it is often desirable to compare their combined analytical results. A spreadsheet can be used to display the quantitative results, but there are times where graphics best describe the similarities and differences. Many instrument data systems allow plotting of multiple runs as long as they were acquired on the same type of data system. What can be done if one needed to compare LC or GC chromatograms from different data systems?

Consider the case of a methods development project where one wanted to compare HPLC chromatograms from both a UV/Visible detector and a thermospray mass spectrometer. Let us assume that we are using an HP 3392 integrator with the UV/Visible detector and a HP Pascal ChemStation for the HP 5988 Thermospray mass spectrometer. The RTE data system can convert raw data output of both the HP 3392 and the MS ChemStation into a suitable format for analysis, quantitation, and graphics. In Figure 4, we have a combined chromatogram drawn with the RTE MS Data System. The UV/Vis data was from an HP 1090 HPLC, but could have been any instrument connected to an HP 3392 integrator or the Hewlett-Packard Laboratory Automation System. This

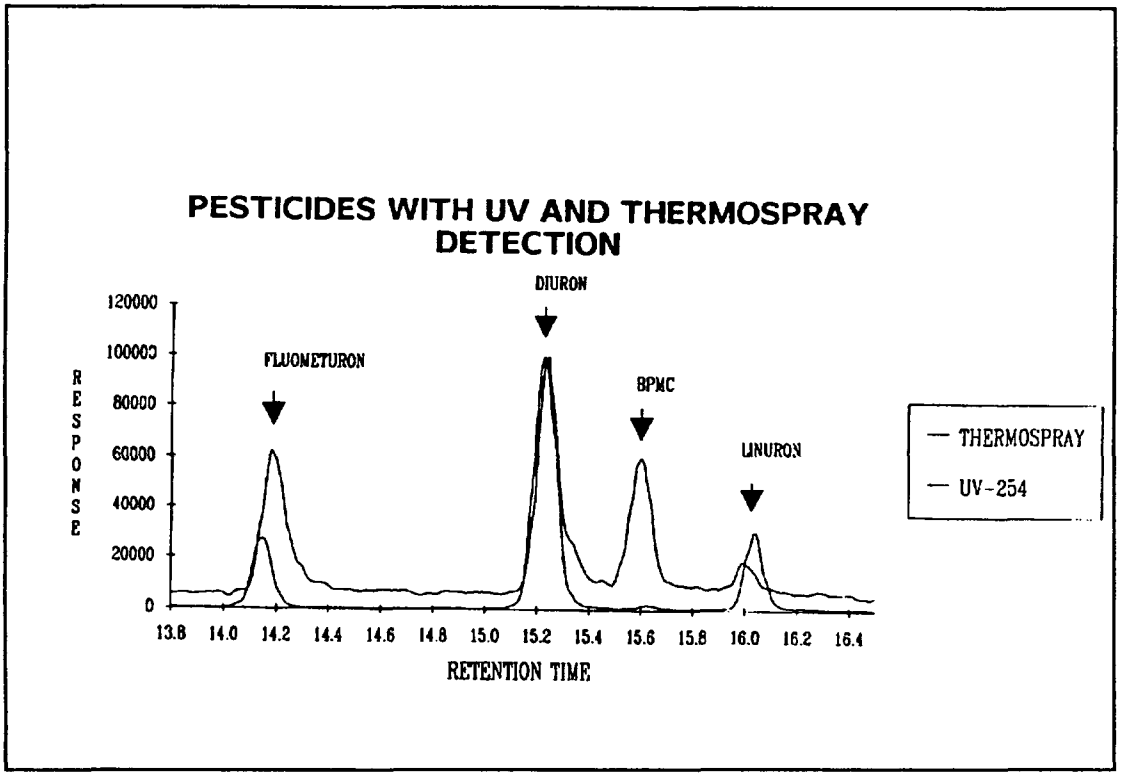


Figure 5 - Chromatogram drawn with spreadsheet graphics

flexibility allows comparison of many types of GC and LC detector output with MS data.

Displaying multiple chromatograms allows one to easily compare the relative difference in response between the different detectors. If desired, the two chromatograms may be superimposed. One chromatogram may be divided by the other to plot the ratio of response. These and other techniques are available for a variety of output from the integrator or the LAS.

For data systems which cannot accept integrator or other data system output, it may still be possible to combine multiple techniques data. If a data system can tabulate a chromatogram into constant time and abundance pairs, it is possible to draw chromatogramms with a spreadsheet. After tabulating the chromatograms with the RTE MS Data System, we can plot the chromatograms with Microsoft Excel (Figure 5). Although this method of drawing chromatograms is not recommended for routine operation, it can be used in situations where the graphics capability of a spreadsheet exceeds one's analytical data system.

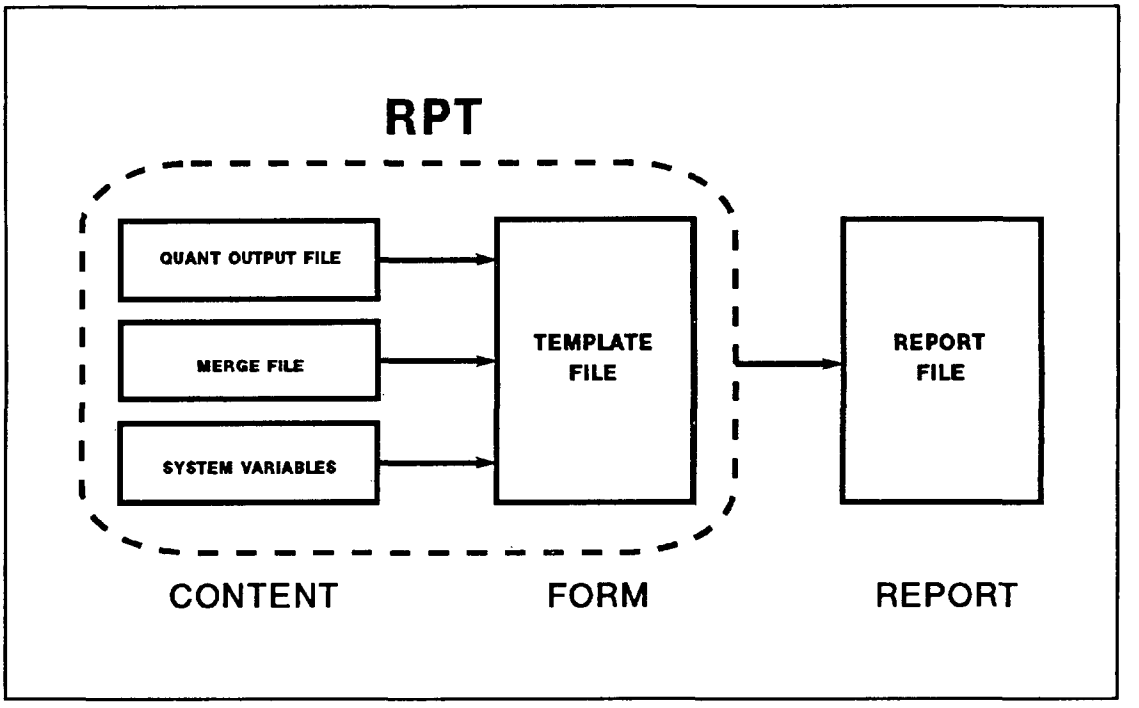


Figure 6 - Operation of Report Writer

DIRECTLY PRODUCING CUSTOM REPORTS

Using the same data system for a variety of techniques gives a uniform appearance to the final report. In addition to graphical reports, the RTE MS Data System can provide custom output of textual reports. The Report Writer can obtain data from a number of sources related to the data file, user input, and the system (see Figure 6). The typical report shown in Figure 7 has information from the customer as well as the analytical results. Reports may be produced automatically with a batch run and saved as a file for final review or final printing. More sophisticated reports with different fonts, lines, and other features (see Figure 8) can be produced using some of the capabilities of the HP LaserJet. To produce this enhanced report, codes were inserted in the report template to add the extra features. Again, this process can be scheduled to run automatically for multiple samples.

One would run into limitations with most report writers if very extensive formatting is required. For instance, proportional fonts, if used on columnar data, would would not line up correctly without decimal tabs. It is possible, but difficult, to do selective highlighting of various fields. These applications are better suited for a full-feature word processor.

CENTRAL ANALYTICAL LABORATORY
1000 MAIN STREET
KANSAS CITY, MO 64110

Customer: General Metals Company
Facility: Kansas City
Location: Well number 5

Customer number: A342
Lab number: C9826
Date sampled: 4/8/88

GC/MS ANALYSIS

Compound	Concentration
Chloromethane	0.000 ug/L
Bromomethane	0.000 ug/L
Vinyl Chloride	0.000 ug/L
Chloroethane	0.000 ug/L
Methylene Chloride	14.455 ug/L
Acetone	210.756 ug/L
Carbon Disulfide	0.000 ug/L
1,1-Dichloroethene	0.000 ug/L

Figure 7 - Customer report from RTE MS Data System

CENTRAL ANALYTICAL LAB
1000 MAIN STREET
KANSAS CITY, MO 64110

Customer:	General Metals Company	Customer number:	A342
Sample #:	ALZ5433	Lab number:	C982
Site:	Palo Alto	Date sampled:	4/8/88

GC/MS ANALYSIS

Compound	Concentration
Chloromethane	ND ug/L
Bromomethane	ND ug/L
Vinyl Chloride	ND ug/L
Chloroethane	ND ug/L
Methylene Chloride	14 ug/L
Acetone	210 ug/L
Carbon Disulfide	ND ug/L
1,1-Dichloroethene	ND ug/L
1,1-Dichloroethane	ND ug/L
1,2-Dichloroethene (total)	ND ug/L

Figure 8 - Enhanced report from RTE MS Data System

PREPARING FILES FOR PC APPLICATIONS

Although analytical data systems excel in processing raw data from instrumention, personal computers offer a far wider choice of general software such as spreadsheets, graphics, etc. A file created by an instrument data system can often be formatted to work in the PC environment. The exact format required by PC software depends on the application. Word processors generally are able to import ASCII text files directly. Spreadsheets can accept ASCII files, but must parse them before selective editing can be done. Database packages usually expect a properly formatted, consistently-structured file.

Both spreadsheets and database applications can import a comma and quote delimited file. In this format, all fields are separated by commas and text fields are placed between quotation marks. Preferably, these modification should be done programatically for existing reports. EDIT/1000 on the RTE systems allow users to write command files that add the proper delimiters to any desired column and row. The basic commands are quite simple, consisting of only two lines for each delimiter. This command file can be used repeatedly for reports which have the same structure.

A better way to prepare delimited files is to create them directly. Figure 9 shows a Report Writer template for a delimited file. When the command to create the report is issued, the compound entries are added (see Figure 10) to create a file that can be brought directly into a spreadsheet without parsing. More complicated report templates can be used to round off quantitation results or to produce multi-sample reports.

```
{Q_CNTL_DO ALL}
    "{Q NAME                          }",  {Q CONC}
{Q_CNTL_ENDDO}
```

Figure 9 - Template for delimited file

```
"*Bromochloromethane          ",  50.000
" Chloromethane               ",  21.613
" Bromomethane                ",  22.027
" Vinyl Chloride              ",  21.186
```

Figure 10 - Report from delimited template

PREPARING CUSTOMER-QUALITY REPORTS

Personal computer desktop publishing is commonly used in many businesses to produce professional quality reports. The near-typeset quality of the results are easily achieved with typical office computers and laser printers. In spite of this popularity, desktop publishing has not been used extensively in the laboratory because its interactive nature is unsuitable for high volume reporting.

Ventura Publisher, one of the most popular desktop publishing applications, uses style sheets to describe the appearance of every feature in a document. Each feature is assigned a tag which specifies such items as font, size, spacing, etc. These tags are simple ASCII strings such as "@LAB =". The tags can be added by using the application software interactively or by incoporating the tag in the source document. In creating the report template, one could easily include these tags with very little effort. Figure 11 shows a report with inserted tags. Once the template is finished, it can be used repeatedly for other data files. This preformatting feature allows one to automate report production with Ventura Publisher.

When a pretagged file is loaded into Ventura Publisher, it is ready for printing without any further formatting. The printed report shown in Figure 12 even has a logo drawn with HP's Drawing Gallery software for the PC. Although this report uses a proportional Times

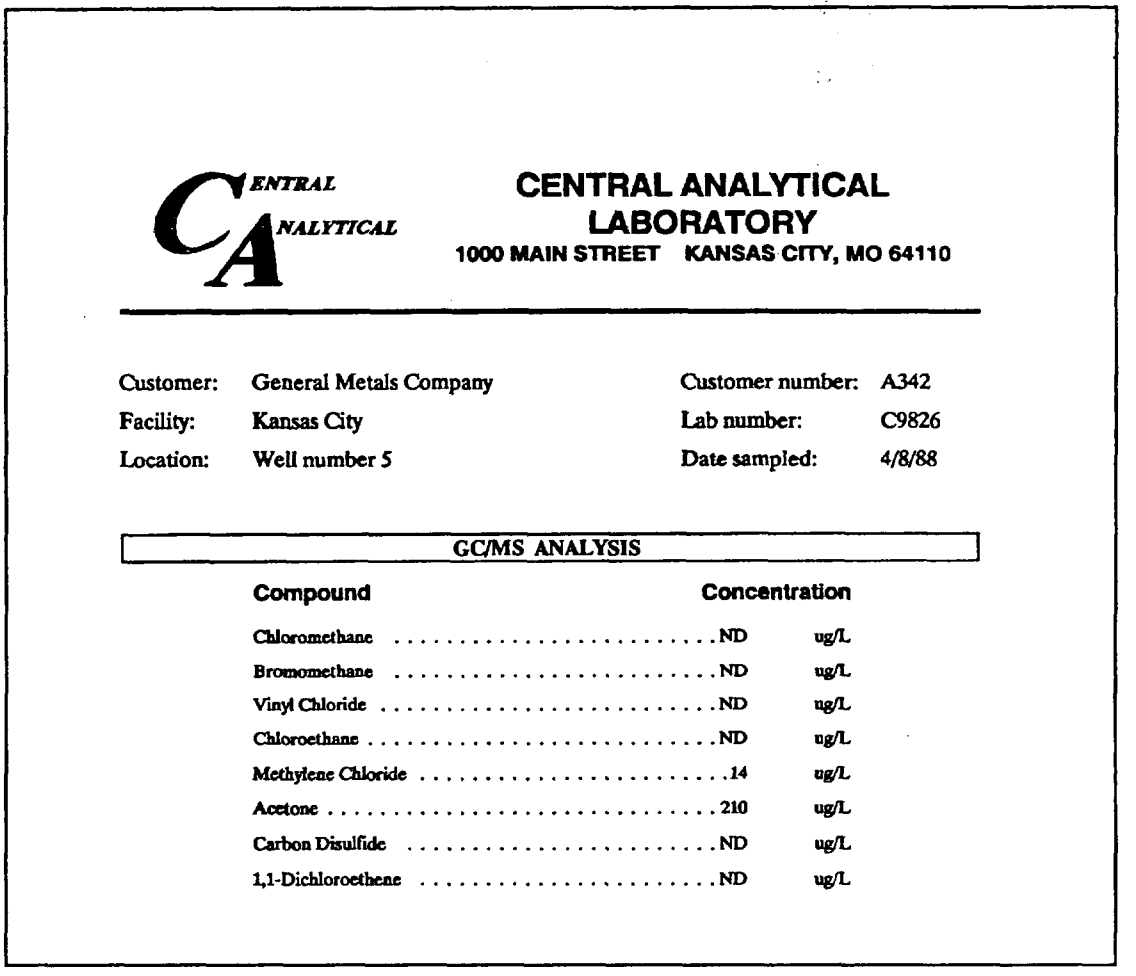
```
@LAB = CENTRAL ANALYTICAL LABORATORY
@ADDRESS = 1000 MAIN STREET
@CITY = KANSAS CITY, MO 64110

@HEAD1 = Customer: {u custo              }
@HEAD2 = Customer number: {u cnumb}
@HEAD1 = Facility: {u facil              }
@HEAD2 = Lab number: {u lnumb}
@HEAD1 = Location: {u locat              }
@HEAD2 = Date sampled: {u dsamp}

@TITLE = GC/MS ANALYSIS

@COL1 = Compound
@COL2 = Concentration
        Chloromethane                    ND
        Bromomethane                     ND
        Vinyl Chloride                   ND
```

Figure 11 - Preformatted file for desktop publishing



CENTRAL ANALYTICAL LABORATORY
1000 MAIN STREET KANSAS CITY, MO 64110

Customer:	General Metals Company	Customer number:	A342
Facility:	Kansas City	Lab number:	C9826
Location:	Well number 5	Date sampled:	4/8/88

GC/MS ANALYSIS

Compound	Concentration	
Chloromethane	ND	ug/L
Bromomethane	ND	ug/L
Vinyl Chloride	ND	ug/L
Chloroethane	ND	ug/L
Methylene Chloride	14	ug/L
Acetone	210	ug/L
Carbon Disulfide	ND	ug/L
1,1-Dichloroethene	ND	ug/L

Figure 12 - Customer report printed with Ventura Publisher

Roman font, the numerical data is still decimal tabbed. This alignment was made possible by inserting horizontal tabs (which were not printed in this reproduction) in the original template. Using the Monitor communications protocol in AdvanceLink, these tabs were retained during the transfer. If the file had been transferred by screen capture, this information would be lost and the columns would not print correctly.

Other features of desktop publishing are quite useful in automating routine work. Ventura Publisher can automatically number all pages and produce a table of contents with entries for each sample. The location of each sample report is retrieved automatically by searching for entries with a specific tag. In the same way, one can generate an index of the reports.

There are several advantanges in using desktop publishing software. First, this report obviously looks better than the average

laboratory report. Second, reports are produced automatically with minimal human transcription, thereby saving time and reducing errors. Third, desktop-published reports provide a common format for multiple techniques.

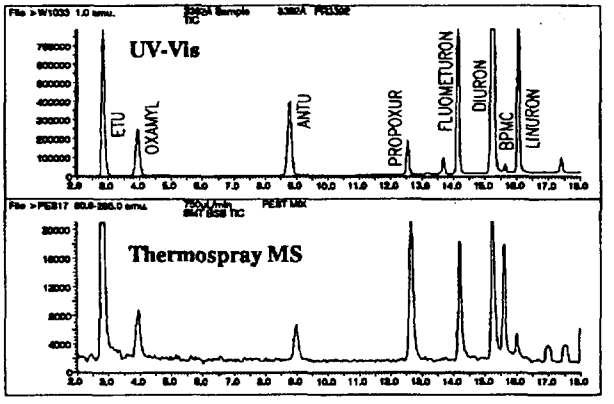
COMBINING TEXT AND GRAPHICS

Let us now consider combining both text and graphics reports on one page. The usual procedure is to prepare the text with pasting of photocopied graphics. A more direct way to incorporate graphics is to use a file format supported by desktop publishing software. Most of the accepted file formats are based on specific PC software. However, one format, HPGL (Hewlett-Packard Graphics Language), is commonly found on analytical data systems which have plotter capability. HPGL files are composed of plotter instructions for drawing an image.

A spool file is used to collect the HPGL instructions for PC applications. The RTE MS Data System allows one to send graphics output to a permanent file (called a spool file) as though it were going to a plotter. Ordinarily, the plot is produced when the file is closed, but the command can be specified so that the plot is never made on the plotter. After the spool file is transferred to a PC, it can be used by several major applications including Ventura Publisher, WordPerfect (version 5.0), and ChemText. Figure 13 shows the combined UV/Vis and Thermospray MS plot supplemented with some explanatory text. Thus, we are able to combine our graphical results with our textual results in one document.

Graphics transfer using an HPGL file offer several advantages. Once saved, the image can be resized and used in multiple software applications. The HPGL file used for a customer report may, at a later date, be used to make an overhead slide on a plotter. For multiple revisions of a group report, it is easier to keep the images stored as files rather than to recopy all the original graphics and paste them into each revision. All the files for an electronic report can be stored on floppy disks or magnetic tape for easy retrieval of all the component parts.

In summary, desktop publishing software can be used by the laboratory to produce very comprehensive reports of exceptionally high quality. The RTE MS Data System allows the automatic production of textual and graphics files for this application.



MERGED TEXT AND GRAPHICS REPORTS

The RTE MS Data System offers great flexiblity in combining data from various instruments and data systems. The data shown here was collected on an HP 1090 HPLC with a UV/Vis detector and an HP 3392 integrator. The Thermospray/MS data was collected with a Chem-Station. Both files were uploaded to the RTE system for processing.

Once on the HP 1000, the files can be processed with the full capabilities of the RTE program, including AQUARIUS and RPN graphics. The data files were converted and drawn in two windows along with user-entered names for the peaks. The software even allows for a selection of different fonts. The graphics were then drawn to a permanent spool file as plotter output. This spool file, which contains the HP Graphics Language (HPGL) instructions for the plotter, was then transferred to an HP Vectra Personal Computer with AdvanceLink terminal emulation/communication software.

Figure 13 - Combined text and graphics file

USING PC DATA IN ANALYTICAL DATA SYSTEMS

So far, we have discussed how PC appplication software can help produce reports from analytical data systems. Now let us consider what we can do with data uploaded from the PC to the instruments computer.

The environmental lab is as much a production site as a factory. Samples come in and reports go out. It is not unusual for a lab to find, after running several days of work, that some of the results are unusable because the mass spectrometer tune or the surrogate recoveries were out of specifications. You do not want to wait until the final report is prepared to know that something went wrong. Thus, there is a necessity for near-real-time QA - checking the results as soon as they are available.

There is a practical problem with near-real-time QA. During a regular shift it is possible to have a chemist examine the results after each run. However, what does one do when the autosampler is running overnight and no one checks the results until the next day?

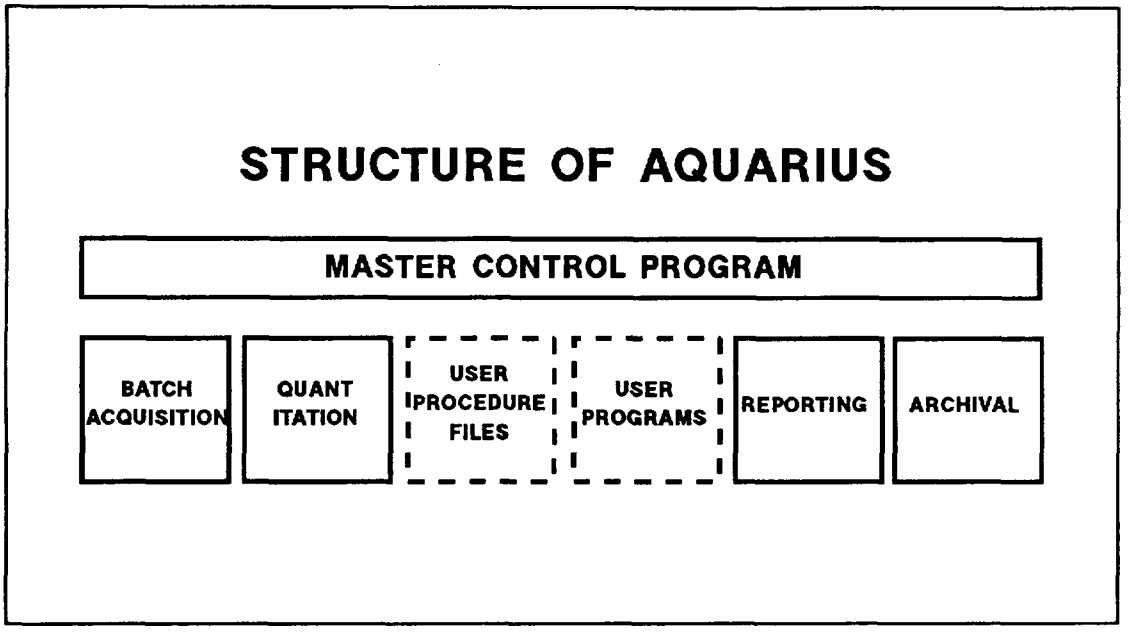


Figure 14 - AQUARIUS modules

If the results are not checked in the morning, several hours can be lost before the problem is recognized.

Some recent work by Charles Koch (Hewlett-Packard) and John DeWald (S-Cubed) may provide an answer to this problem of near-real-time QA for GC/MS analyses. In order to understand their solution, one must understand the component modules of the AQUARIUS automation software (Figure 14). Most laboratories use the batch acquisition, quantitation, and archival modules in automated operation. The other two modules allow one to schedule user-written procedure files and programs. Some labs use the procedure file scheduler to run a non-target compound library search and quantitation routine. Users can developed specialized reports (Figure 15) to display the surrogate recoveries for each run. Since the RTE MS Data System was designed for multiple users and terminals, one can display the QA report in the chemists' office rather than the lab. This brief report will help the chemist keep up with what is going on in the automated analyses.

The procedure file developed at S-Cubed goes beyond simply producing a report with the procedure file scheduler - it is a feedback control of the batch acquisition process. The flow chart in Figure 16 shows the decisions that are made. Once a run is completed, the procedure file checks to make sure that the DFTPP specifications are passed. It will look at various scans and combinations of scans to find a spectrum that passes the tuning criteria. If no scan passes, it suspends the sample injection sequence, goes to a designated vial

SURROGATE REPORT

INSTRUMENT: 1
FILE NAME: A3214::QT
INJECT TIME: 851029 17:44

COMPOUND	ID FILE RT	MEASURED RT	CALIB RF	MEASURED RF
2-Fluorophenol	8.506	8.452	.8935	.89022
Phenol-d6	11.440	11.425	1.3827	1.36431
Nitrobenzene-d5	13.823	13.757	.4147	.41322
2-Fluorobiphenyl	19.500	19.429	1.3643	1.44731
2,4,6-Tribromophenol	23.932	23.930	.2603	.26975
Terphenyl-d14	31.232	31.217	1.0130	1.07163

Figure 15 - Surrogate recovery form for QA

with DFTPP, injects the tuning compound, and re-evaluates the tune. If the tuning check still fails, the injection sequence is stopped. If the tuning check passes, a one-point calibration is made. If the calibration is out of specifications, the sequence is stopped. If the calibration passes, the first shift samples are run. The same process is repeated for the second shift samples - all automatically under AQUARIUS scheduling. The total time to run through the checking process is only one to three minutes, less time than it takes the GC oven to cool. This automated checking process frees the chemist from constantly monitoring the results as each sample is completed.

This type of checking can be extended for other applications. A procedure file could be set up to compare the target compound concentration with the linear range of calibration. If the concentration exceeds the linear range, a more dilute sample may be injected. This type of smart injection allows great flexibility to control processing depending on what was found in the sample.

With these capabilities in mind, let us consider what we can do with files uploaded from a personal computer. Suppose we are making a long term study of a site. We have a database with historical records of what compounds and levels were found in all the wells at this site. For the purposes of our study, we would like to reanalyze any sample with a component which exceeds the historical maximum at

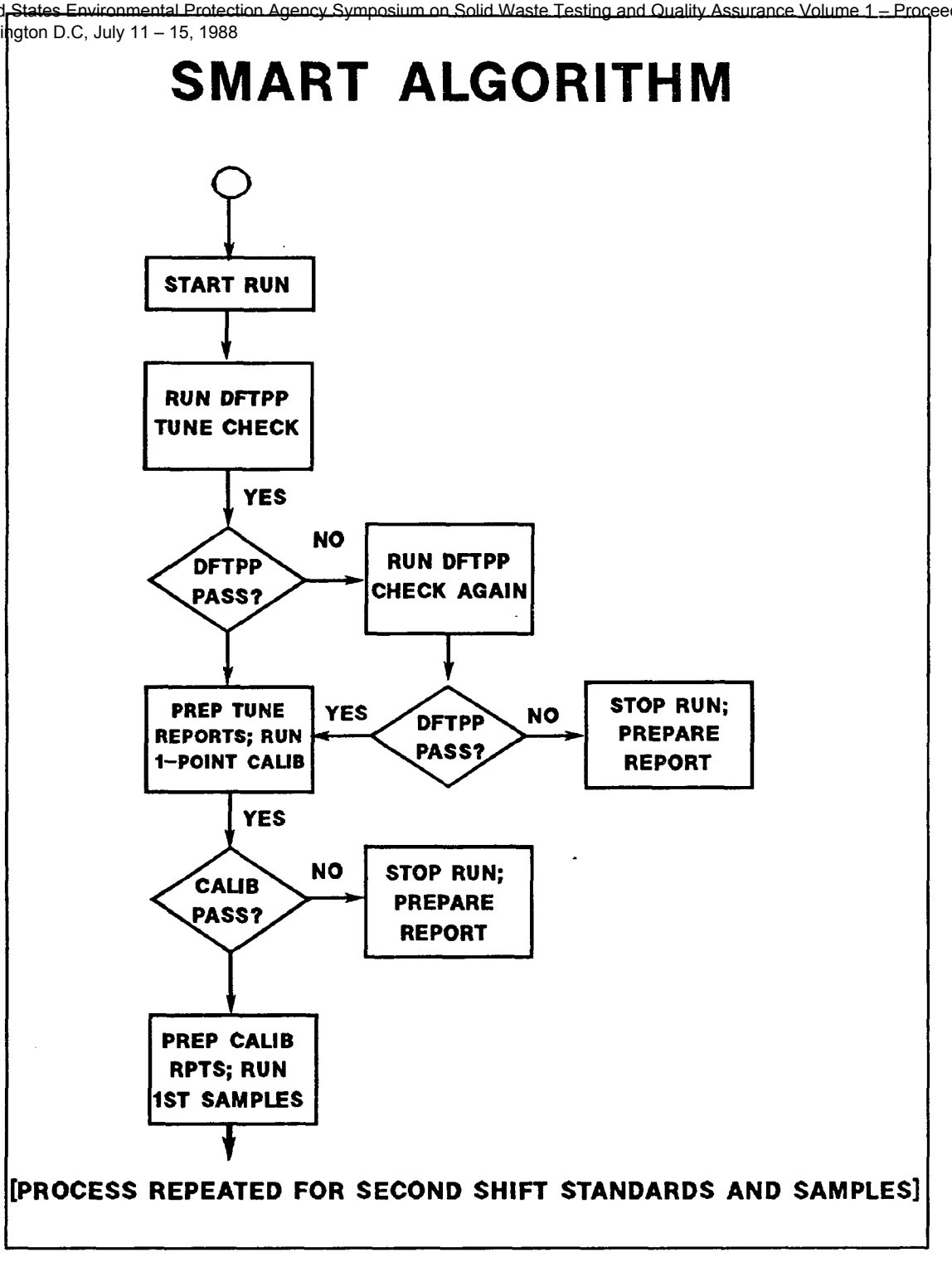


Figure 16 - Flow chart for intelligent autosampler

that well. From a database package, we can easily prepare a maximum concentration list for each well. This list is transferred to the RTE data system and used as part of the report template. At the completion of each injection, the results are quantitated and compared with the historical maximums. If a compound exceeds the

value, the sampling sequence can be paused and the same vial (or another vial from the same well) can be injected before the sequence resumes.

Bidirectional file transfers on the RTE MS Data System allows one to incoporate data found in other software. Sampling data, preparation data, anything stored in a text file can be retrieved, formatted, and added to the mass spectrometer report or a procedure file that controls acquisition.

NETWORKING DATA SYSTEMS

As we have shown in the preceding examples, file transfers between computers allow one to use the unique software capabilities of specific computers. So far, we have been dealing with relatively simple file transfers with serial connections or magnetic media. Let us now consider some more sophisticated applications of local area networking of analytical data systems.

We will begin with a discussion of HP 1000 networks using NS/1000 software. With this network, one can logon to another RTE system and issue select RTE system commands. The MS Data System can transfer files to and from the Laboratory Automation System, the Chromatographic Worksystem, the LABSAM Laboratory Information Management System, the new series of MS ChemStations (HP-UX), the HP 3000 business computers, and DEC VAX/VMS systems. Programs exist for converting raw MS, GC, and LC data files from the HP computers for use with the RTE MS Data System. NS/1000 also allows users to control devices, such as printers, located on remote HP 1000 computers. Considering what we have discussed of multiple technique graphics and text integration for customer reports, this network offers tremendous opportunities for laboratories.

The latest generation of multi-tasking HP Unix ChemStations (HP-UX series) offers even more capabilities. These data systems come standard with the needed networking hardware and software. To establish a network, one only needs to connect the ChemStations together with a LAN cable. If a lab has several of these data systems on a network, a user on one HP-UX ChemStation can retrieve a file from another HP-UX ChemStation, while both mass spectrometers are operating and the other user is performing interactive tasks. Copying, moving, deleting, and renaming files to, from, or on remote computers are as easy as performing the same task on one's own computer. A user at one data system can use a mass spectrometer, printer, or other peripheral on another HP-UX ChemStation. A mass spectrometer operated by a remote computer may have the display and printout on a local computer. One can monitor the progress of a run in one window of the screen while performing other tasks on an HP/DEC/IBM computer in another window.

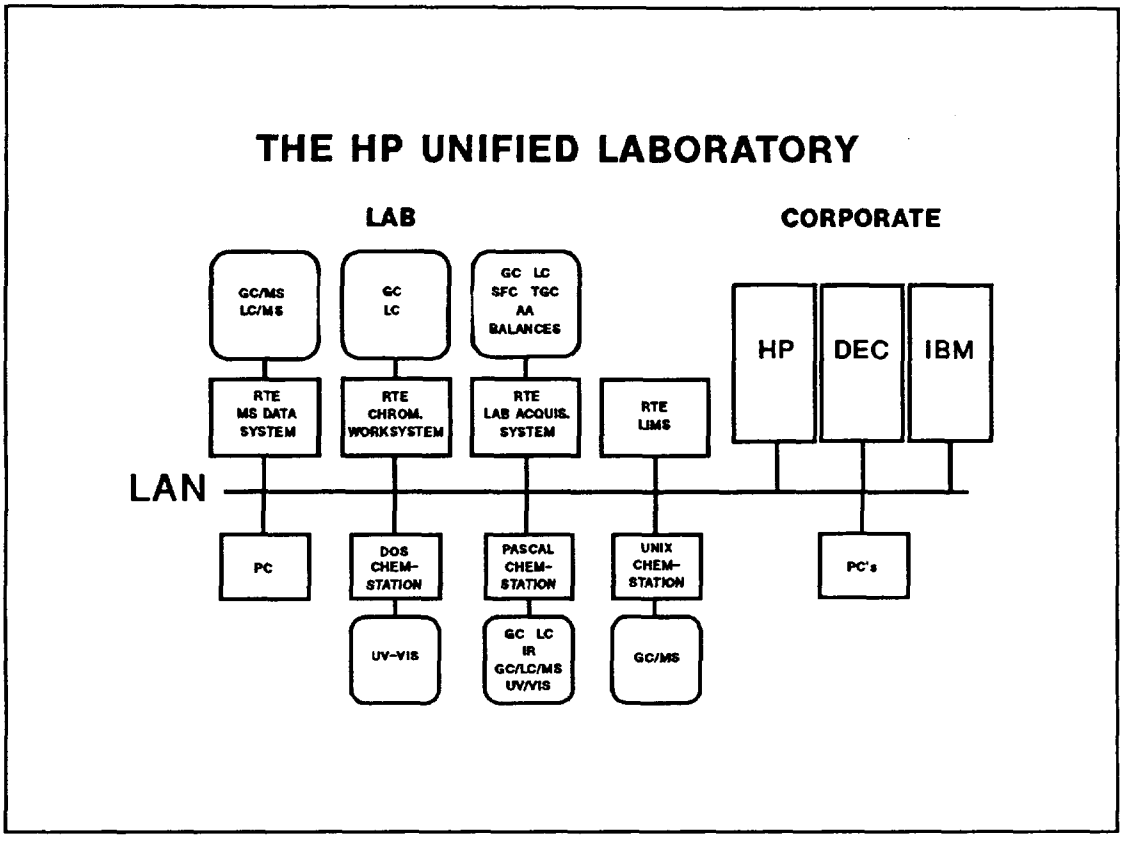


Figure 17 - Lab and Corporate Data Systems on a Network

How do we bring together different types of computers? As a major computer manufacturer, we have committed ourselves to industry standards. The diagram in Figure 17 shows a network based on IEEE 802.3. This networking standard, supported by many computer companies, is compatible with EtherNet (e.g. DECnet). All of our latest generation of analytical data systems can connect directly to this network. Older systems (such as the RTE-6 MS, LAS, and Labsam systems) with a DS connection to an RTE-A system can participate on the network. By the end of the year, the Pascal ChemStation will have the software needed be on this network. Using ThinLan and ThickLAN cables one can connect computers in the largest laboratories. The network can extend beyond the laboratories to corporate Management Information Systems to access services such as electronic mail to send and receive reports and correspondence. These networking capabilities will alter the way laboratories operate just as much as autosamplers have in the past.

For companies with laboratories in multiple locations, a wide area network offers some of the same capabilites as we have discussed. Local area networks can be integrated into a wide area network so that raw data and report files may be transmitted between

laboratories. Imagine the case where samples from a site were analyzed in two locations. One lab may want to see the raw data file from a sample previously analyzed at the other location. The raw data file, along with a copy of the original report, can be sent from one lab to the other for further study.

CONCLUSION

Our discussion began with what we can do with an integrator report and ended with networking in a corporation. The intent of this talk was to stimulate your thinking on creative applications of current technology to increase laboratory productivity. The combination of the personal computer with an analytical data system offers much greater capability than either one alone. This enhanced capability can be used to increase laboratory throughput by automating some steps of quality assurance and in report production. Using a personal computer with one's analytical data system is only the first step. Further productivity increases will occur as laboratory and corporate computer systems from multiple vendors are networked using industry standards.

REFERENCES

(1) C. A. Koch and J.DeWald, *LC*GC* 6,150 (1988)

NOTES

The following names are trademarks of the respective companies:

ChemText	Molecular Design
Excel	Microsoft
Ventura Publisher	Xerox
WordPerfect	WordPerfect

This article was produced using Ventura Publisher. Figures 3,5,8,12, and 13 were scanned from the original reports produced by the specified software and hardware combinations. No manual cutting and pasting was necessary to incorporate the graphics into the text. The entire text and graphics (original and converted) files of this report fits on a 1.2 Mb floppy disc.

LABORATORY INFORMATION MANAGEMENT OF ANALYTICAL AND QUALITY ASSURANCE DATA - A FLEXIBLE DATA MODEL

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ABSTRACT

Assuring the quality of analytical data requires timely and detailed results during all stages of sample processing. In addition, Quality Assurance/Quality Control (QA/QC) results need to be clearly linked with the corresponding analytical data. Flexible mechanisms are also needed for comprehensive data reduction, review, validation, and reporting. In conjunction with improving the quality assurance for both laboratory and project analytical results, Radian has developed a data model that defines the data relationships and attributes that are needed to provide such flexibility in analysis and reporting. The data model has been implemented using a relational database management system on a SUN computer and steps have been taken to provide similar capability as an extension to Sam®, Radian's PC-based Laboratory Information Management System.

The data model addresses several aspects of the problems involved in performing QA/QC on analytical data including the enforcement of data integrity, integration of detection and control limits with data capture and reporting, maintenance of links between QA/QC results and analytical results, and the categorization of QA/QC data. Data integrity is enforced by the use of validation tables of information for such attributes as labs, instruments, methods, and analytes. Whenever these attributes are input in the system, they are checked against these validation tables to assure consistency of values. There are other tables of information for defining the detection, control, and method specification limits. The data model allows these limits to change over time while still assuring the use of the correct ones for analysis and reporting. Links between the QA/QC results and analytical results are maintained with the analytical run as the fundamental link. An analytical run is identified by lab, instrument, method, matrix, date, and time. A run consists of calibration data along with both sample and QA/QC results. This makes it possible to look at results by any combination of these identifiers to narrow the search space for potential analytical problems. The data model divides the QA/QC data into six categories: Calibrations, Control Samples, Replicates, Spikes, Blanks, and Audits. The model supports user defined types within each of the categories to distinguish further the QA/QC data. For example, the model allows the user to distinguish among different types of spikes such as sample, matrix, surrogate, extract, and reagent spikes.

The current SUN implementation of the data model has improved the process of performing QA/QC on analytical data. The flexibility of the data model enables analysts to review data in ways that were previously time-consuming and prone to error. Steps have been taken to add a similar flexibility in Sam®, Radian's Laboratory Information Management System. This will allow for QA/QC at both the laboratory and project levels.

LIMS
THE EPA EXPERIENCE

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ABSTRACT

As early as 1974, the US EPA had begun a program to automate information management in its Regional Laboratories. The development of the Sample File Control system over the next 10 years gave the Agency valuable experience in overcoming the problems of integrating laboratory information to provide management control of laboratory data and resources. The knowledge gained provided EPA with the basis for the specifications necessary to procure competitively a commercial LIMS system for its Regional Laboratories in 1987.

In its efforts to automate laboratory information management in the Agency's Regional Laboratories, EPA has placed primary responsibility on the laboratories themselves to determine the information needed and its most efficient flow to the users. The EPA Baseline LIMS reflects these requirements, and it provides for evolution of the system to encompass future needs.

A laboratory prepares for LIMS by first developing a self portrait to describe both the volume of information to be managed and the flow of the data through the laboratory. This self portrait is the key to successful implementation, and it provides a way of contrasting perceived needs with the EPA Baseline LIMS.

The benefits of LIMS implementation are first to laboratory management and laboratory clients. Management can more efficiently make use of scarce resources, determine bottlenecks in production, and improve the quality of data by implementing a LIMS. Clients may benefit from decreased response time and the ability to receive historical reports not easily accessed through a paper based system. EPA can benefit from LIMS through standardization of procedures and the decreased costs resulting from the centralized development of new applications.

MOBILITY METHODS

DEVELOPMENT OF A PROCEDURE TO ASSESS ACID FORMATION POTENTIAL OF WASTES

Robert Hoye, Project Manager, Dr. Michael Taylor, Project Research Director, PEI Associates, Inc., 11499 Chester Road, Cincinnati, Ohio 45246

ABSTRACT

Currently, there is no standard procedure that can be used to assess the inherent potential of a waste to produce acidic leachates. The EPA is concerned that without such a procedure, the potential environmental impact of wastes that form acid will be underestimated. The objective of this project is to develop a laboratory test procedure which can be employed to obtain data indicative of the potential of a solid waste to, under certain condictions, give rise to an acidic leachate. The procedure should be applicable to a variety of wastes (such as mining wastes, electroplating wastes, tanning wastes, paper pulp wastes, and municipal sewage sludge) which are known to have the potential to give rise to acidic leachates.

The approach being used to develop the procedure entails performance of a thorough review of literature on the chemistry of acid leachate formation; development of test protocols; completion of column leaching studies to generate data that will allow assessment of parameters affecting or controlling acid formation; documentation of the effort with recommendations for further research that will lead to development of a lab scale test that can be incorporated into SW-846.

This paper discusses the following aspects of this project: Background and Scope; Literature Review; Experimental Design and Statistical Plan; Quality Assurance; Sample Collection; Column Leaching Studies; and Future Plans and Recommendations. The project was initiated on January 7, 1988 with submital of a Work Plan and initial project meetings. Column leaching studies will be underway at the time of the symposium.

DEVELOPMENT OF A LEACHING PROCEDURE FOR MONOFILLS

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ABSTRACT

The U.S. EPA's existing leachate quality estimation test procedures (e.g., TCLP, EP) are based on the waste being managed in a sanitary landfill. The Agency has a need to be able to estimate leachate quality under other disposal scenarios, considering both specific site conditions and management practices. To expand the Agency's ability to model other scenarios, development of a new test that will permit modeling of the "monofill" disposal scenario may be necessary.

The purpose of this paper is to present the status of a project designed to develop a leaching test for monofilled wastes. Pertinent information from literature and industry surveys is discussed along with plans for developing and evaluating a test method.

The first part of this study was a literature search to determine what environmental factors, such as weathering and biotransformation, affect the fate and transport of monofill-type wastes. The literature search concentrated on laboratory studies and modeling schemes that have been developed. This information was then used to design a study for the development of a monofill leach test.

The test method development study will consist of the following:

Sample Selection. From among the types of wastes being monofilled, samples will be selected for use in method development. Coordination with industry will be necessary in collection of these wastes. Included, at a minimum, will be the following wastes: municipal incinerator ashes, foundry wastes, electroplating wastes (with cyanide and without cyanide), utility wastes, and coal cleaning residues.

Column Design and Fabrication. A series of leaching columns containing selected wastes will be designed, fabricated, and assembled. Based on the literature search and a technical evaluation, the most appropriate leaching fluid (e.g., distilled water vs. acid precipitation) will be determined. Leachate fractions will be collected and analyzed for a variety of target constituents.

Leachate Comparisons. Results of the analyses of leachates from the columns will be compared with those from actual monofills containing the types of waste being tested. These results will also be compared with data from other available and potential monofill leaching methods.

Draft Test Method. A draft monofill leaching test will be developed based on the results from the columns test.

Reference Standards. Reference standards for use in collaborative testing of the draft monofill method will be developed.

PERFORMANCE OF A TCLP CAGE MODIFICATION TO PREPARE SOLDIFIED WASTES

Paul Marsden and Richard Phillips, S-CUBED, P.O. Box 1620, La Jolla, CA 92038; Llewellyn R. Williams, Deputy Director, Exposure Assessment Division, U.S. Environmental Project Agency, 944 E. Harmon, Las Vegas, NV 89114; Gail Hansen, Chemist, Office of Solid Waste, U.S. Environmental Protection Agency, 401 M Street SW, Washington, DC 20460

ABSTRACT

Method 1311, the Toxicity Characteristic Leaching Procedure (TCLP) currently requires that all samples be passed through a 9.5 mm screen before leaching. This requirement is probably not appropriate for wastes that have been stabilized (solidified) to withstand the environmental stresses encountered in a landfill. Unfortunately, no suitable single test exists to determine the stability of a waste.

This paper presents the results of the laboratory validation of a short term test to evaluate the stability or instability of waste. The test requires tumbling a solid sample inside of a stainless steel cage that is inside of the TCLP extraction jar containing the appropriate leaching buffer. After tumbling, the well stabilized wastes remain more or less intact and the poorly stabilized wastes are significantly degraded. This technique is well suited for use as a module to the TCLP because the leaching fluid generated during the stability testing is used for the analytical determinations for metals, GC/MS organics, pesticides, and herbicides; no additional size reduction of samples is required.

The initial method development work examined representative wastes solidified by five different techniques using three physical tests (wet/dry resistance, freeze/thaw resistance, and unconfined compression). The wet/dry and freeze/thaw procedures provided useful characterization data, however, there was a lack of precision the results of compression tests. Reasonable correlation was observed between the results of the cage tumbling procedure and these physical tests. The method was further tested by using characterized solid waste provided by the Waterways Experiment Station (WES), U.S. Corps of Engineers, Vicksburg, Mississippi.

The performance of the cage tumbling procedure supported its suitability for hazardous waste samples. Work is continuing to test the procedure and other modifications to the TCLP in an ASTM collaborative study.

INTRODUCTION

Method 1311, the TCLP was proposed on June 13, 1986 as a test protocol to measure the toxicity characteristic of wastes. The method was promulgated on November 7, 1986 as part of the Land Disposal Restrictions Program. On May 24, 1988, the EPA proposed to modify

the method by incorporating a stainless steel cage in the bottle extractor, the use of the cage will allow elimination of the particle size reduction for certain materials. Particle size reduction is currently required for all samples that cannot pass through a 9.5-mm sieve or have a surface area of less than 3.1 cm^2/g.

Particle size reduction was initially required as a sample preparation step because the Agency believed, given the uncertainties concerning the long-term environmental stability of solidified wastes, that milling all samples was an environmentally conservative approach. Numerous public comments were received on the issue of wastes with reduced contact (and subsequent leaching) between water present in the disposal unit and the toxic species present in the waste. Commentators objected to the requirement to mill wastes that are solidified by stabilization or fixation processes and wastes may exist as rugged, monolithic materials not likely to be physically degraded in a landfill.

It was recognized that these comments had merit for wastes that are truly well stabilized and will not degrade after disposal. It was also recognized that many solid wastes are poorly stabilized and will degrade after disposal. It was therefore necessary to develop a short-term test to measure the stability of wastes under the stresses encountered in a landfill. These stresses include compression by vehicular traffic, freeze/thaw cycles, and wet/dry cycles. The test also had to be short-term test (hours or days) in order to ensure that samples could be processed through analytical laboratories without requiring long sample holding times.

The proposed cage modification to Method 1311 was developed and validated in response to these requirements. The use of the cage will allow elimination of the particle size reduction step in the determination of metals, semivolatiles, and pesticides. This modification to the method should have no significant impact on the time required for a laboratory to process samples.

MATERIALS AND METHODS

Selection of Wastes for Method Testing

The method development effort proceeded using several materials selected from the variety of wastes disposed in landfills. The selected wastes include:

- Polymer encapsulated waste 7.5 cm diameter, 11.5 cm length obtained from Environmental Protection Polymer Inc., Hawthorne, CA. The waste matrix was stabilized in polybutadiene and subsequently encapsulated in polyethylene.

- A vitrified municipal waste in the form of pellets, approximately 0.5 cm diameter, 1.5 cm length that was prepared in a high temperature incinerator, and supplied by Catrel Corporation, Geneva, Switzerland.

- A municipal fly ash provided by the project officer and stabilized by the addition of lime.
- A metal bearing waste, K028, collected from industry by S-CUBED as part of an OSW listing/delisting study and solidified by the addition of cement.
- An organic waste, F024, that was collected from industry by S-CUBED as part of an OSW listing/delisting study and solidified by the addition of cement.

The polymer encapsulated and the vitrified wastes were used without additional stabilization.

The fly ash samples were initially stabilized with lime (Hydrated Lime, Colton Lime and Stone Co.), however, it was found that lime alone is a poor stabilization agent. In order to enhance the solidification of the lime-fly ash mixture four percent cement by weight was added. Sufficient water was mixed into each sample mixture to produce a slurry that was poured into 100-mL paper cup molds. The dimensions of the paper cups are shown in Figure 1. The waste to lime ratios are presented in Table 1.

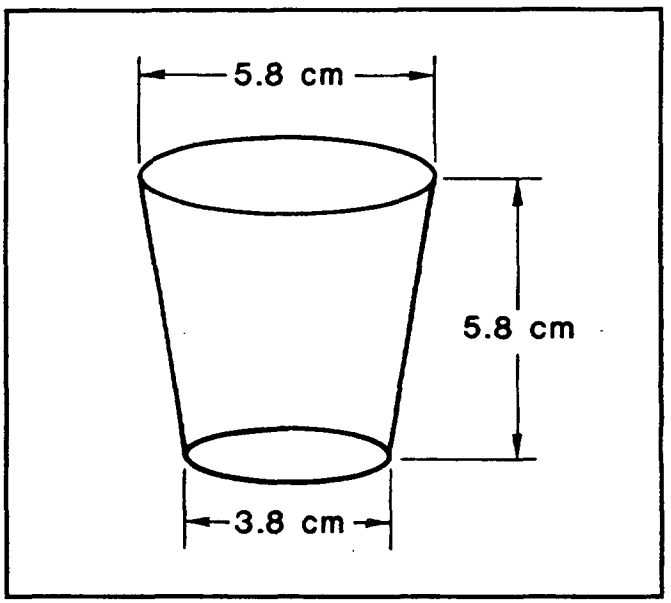

Figure 1. Paper cup mold used for solidified waste mixtures.

The two remaining wastes (K028 and F024) were stabilized by the addition of cement (Type 1/11 Portland Cement, Colton Lime and Stone Co., Colton, CA) at the waste to stabilizer ratios presented in Table 1. The cement stabilized waste samples were prepared on both a volume and weight ratio basis to yield test samples which varied in their durability. Because the hardness of cement increases with age, the durability of all samples prepared with cement increased with time. These samples were prepared in molds pictured in Figure 1. They weighed 150 to 160 g each.

A set of eight additional stabilized samples were prepared from four waste types at WES. The stability of these samples had previously been characterized in a cooperative program with the USEPA, industry, and EPA of Canada (Province of Alberta) to investigate test methods for solidified wastes. Although the wastes themselves and the proprietary solidification techniques could not be provided to S-CUBED

TABLE 1. COMPOSITION OF LIME AND CEMENT TEST SAMPLES

1. Cement Waste Mixtures vol/vol

Ratio	Waste (fl oz)	Cement (fl oz)	Water (mL)	
			F024	K028
5:1	2.7	13.3	240	236
4:1	3.2	12.8	348	310
3:1	4.0	12.0	212	350
2:1	5.3	10.7	200	365

2. Cement Mixtures wt/wt

Ratio	Waste (g)	Cement (g)	Water (mL)
F024 5:1	540	2,700	1,300
F024 2:1	591	1,182	600
K028 5:1	300	1,500	2,300
K028 1:1	909	909	1,400

3. Lime and Fly Ash Mixtures wt/wt

Ratio	Fly Ash (g)	Lime (g)	Water (mL)	Cement (g)
2:1	267	133	80	17
1:1	295	295	100	25

because of confidentiality agreements between the manufacturers and WES, the source of each waste, its identifier code and primary hazardous constituents is provided in Table 2.

TABLE 2. WATERWAYS EXPERIMENTAL STATION SOLIDIFIED WASTES

Waste Type	Contamination of Interest	Sample ID
WES Synthetic	Cd,Cr, Ni, Hg	KIAK AIEI
WTC Synthetic	As, Cd, Cr, Pb, Phenol	BAHE AAKC
Wood Protection	As, Pb, PCP, PAH, Hg	BKDE IAHE
Plating Waste	As, Al, Cr, Pb, Tl, CN	GFDG ADAC

Weathering Tests

The solidified waste mixtures were subjected to modifications of the draft ASTM wet/dry and freeze/thaw resistance tests developed by Peter Hannack. The wet/dry resistance test was conducted by placing a sample in an oven at 60° C for 24 hours, removing the sample, allowing 1-hour for temperature equilibration at 20°C and then placing the sample under distilled water for 23 hours; the sample was cycled through this sequence 28 times. The freeze/thaw resistance test was conducted by placing a sample in a freezer at -20°C for 24 hours and then placing under distilled water for 24 hours; the freeze/thaw cycle was also repeated 28 times. Similar ASTM procedures require only 11 cycles but the number of cycles was increased in this study to obtain a more conservative criteria for the environmental stability of these wastes. Any samples that passed the initial freeze/thaw resistance test were subjected to a wet/dry resistance test and those that passed the initial wet/dry resistance test were subjected to a freeze/thaw resistance test. The multiple resistance tests allowed for the determination of the effect of sequential weathering stresses.

The compression testing of the solidified waste samples was attempted using the ASTM Method C 109-80. Samples were placed in a 10 ton press and a steady pressure was added until visible cracks developed on the surface of the sample or until the sample disintegrated. Unfortunately, the molded samples prepared for this study did not have the plane parallel surfaces which are necessary for accurate determination of compressive strength. A leather square (3 x 3 x 0.25 inches) placed between the sample and each face of the press gave

some improvement in the reproducibility for samples with irregular surfaces.

Analytical Procedures

Although the primary focus of this project was the physical testing of solidified wastes, a limited amount of chemical analysis was completed. These analyses were completed using the appropriate SW-846 methods including Methods 6010 and 8270.

Leaching/Tumbling Procedures

Solidified samples were tumbled in glass jars according to the extractable section of Method 1311 and in the zero head space extractor (ZHE) according to the volatiles section of Method 1311. In order to prevent damage to the jars, three possible modifications to the jar procedure of Method 1311 were investigated:

- Placing Teflon sheets inside the jar as cushions.
- Placing a 0.25-inch stainless steel woven mesh of No. 18 gauge wire inside the jar as a liner.
- Placing a stainless steel cage constructed of a 0.25-inch woven mesh of No. 18 gauge stainless steel wire inside the jar.

Small (25 to 30 g) aliquots of the S-CUBED waste/cement mixtures were cast in paper cups in order to investigate a volatiles procedure for solids. Unfortunately, the design of the ZHE precludes the use of a liner and when these 25-g cement/waste samples was tumbled in the ZHE some damage to the cylinder walls were noted. Because of the design of the device it was not possible to use a liner in the ZHE. Associated Design and Manufacturing Company (Alexandria, VA) was contacted and they provided a ZHE that was hardened by a nitriding process that was used for additional tests.

RESULTS AND DISCUSSION

An initial characterization of the stability of wastes prepared at S-CUBED was performed using sequential application of the wet/dry and freeze/thaw tests. The results of this study are provided in Table 3. Those samples that lost less than 30 percent of their initial weight passed the test and are so indicated with a "P." Those samples that lost more than 30 percent of initial weight are indicated with "F", the cycle number when they failed is listed in parentheses. Only the encapsulated waste and a concrete stabilized F028 passed sequential application of the freeze/thaw and wet/dry tests.

TABLE 3. RESULTS OF INITIAL WEATHERING TESTS ON F024, K028, AND VITRIFIED WASTE MIXTURES

Waste		Weathering Test			
		Wet/Dry	2nd	Freeze/Thaw	2nd
F024 (w/w)	2:1	P	F (5)	P	P
	5:1	P	F (6)	P	P/F
K028 (w/w)	1:1	F (3)		F (2)	
	5:1	F (9)		P	F (5)
Lime/fly ash	1:1	F (3)			
	2:1	F (5)		F (1)	
Vitrified		P	ND	P	ND
Encapsulated		P	P	P	P

Although these tests were reproducible and semiquantitative, the labor and the time required to perform them makes these tests unsuitable for the preanalytical characterization of waste samples. Adopting a criterion that a sample could pass a freeze/thaw and a wet/dry test in lieu of size reduction prior to leaching would add weeks to the time required for sample preparation by Method 1311.

Compression testing of additional wastes aliquots demonstrated that this test gives highly variable results when applied to solids that do not have plane parallel surfaces. Even when leather inserts were used, method variability was unacceptable. The variability of the results (Table 4) and the lack of generally accepted criteria for what constitutes a stable material preclude adoption of compression testing as a sample characterization procedure.

TABLE 4. COMPRESSIVE STRENGTH OF MOLDED CEMENT SPECIMENS

Age (days)	Compressive Strength of Cement Samples (lbs/sq. in)								Average	RSD (%)
15	4,200	2,500	3,250	2,900	2,500				3,070	23
24	5,100	4,200	7,100	6,900	8,200	7,800			6,500	24
28	9,500	9,900	13,100	12,200	12,800	9,800	7,000	12,300	10,825	20

Sample tumbling was investigated as a potential stability characterization procedure. An initial investigation was made using concrete, and those data are reported in Table 5. As the concrete sample cured during the first 30 days and became harder, samples lost less weight during tumbling and broke extraction jars during leaching. In the course of preforming this study, samples were run in unlined jars, jars lined with teflon, jars lines with stainless steel screen, and jars with stainless steel cages. The decision to go to a cage from the screen was made at day 45 on the basis of damage to the jars and to screens during tumbling. The decrease in final weights between day 45 (screen) and 48 (cage) demonstrates that tumbling in the cage is a somewhat more aggressive procedure than is tumbling with a screen. However, the data for day 1 concretes demonstrate that it is not too aggressive for this application.

TABLE 5. INITIAL TCLP SAMPLE TUMBLING RESULTS

Sample Type	Age (Days)	Liner	Initial Weight (g)	Final Weight (g)	% Remaining
Cement	1	Cage	168	80	48
	1	Cage	170	83	49
	5	None	162	54	44
	8	None	156	80	51
	14	None	156	B/J	
	14	None	159	B/J	
	16	None	166	B/J	
	30	Teflon	161	142	88
	30	Teflon	166	159	96
	33	Teflon	170	B/J	
	33	Teflon	163	B/J	
	42	Screen	162	161	100
	42	Screen	160	160	100
	43	Screen	158	150	95
	45	Screen	161	132	82
	48	Cage	161	109	68
	49	Cage	164	98	60

B/J - Broken jar during the 18-hour tumbling period.

Samples previously characterized by the wet/dry and freeze/thaw resistance tests were subjected to the cage tumbling procedure to further investigate this technique A comparison of the wet/dry resistance, the freeze/thaw resistance, and the TCLP tumbling procedure for solid waste samples is given in Table 6. The sample integrity results in the wire cage tumbling process were similar to the results from the weathering tests. Both tests completely degraded poorly-stabilized pozzolonic (lime/fly ash) material and the poorly-stabilized K028 concrete mixture. Neither test caused significant degradation of the vitrified, polymer encapsulated, or well-stabilized F024 concrete

wastes. The cage procedure was most variable for the 5:1 K028 samples in that it removed 13 to 37 percent of the original sample weight. This same type of sample showed the most variation in the freeze/thaw and the wet/dry tests. Less than six percent of the original sample weight for five samples of K028/concrete was lost after 28 cycles of freeze/thaw testing, but rapid deterioration of K028 samples was observed in the wet/dry test with degradation of all samples after the 16th cycle.

TABLE 6. COMPARISON OF WET/DRY, FREEZE/THAW AND TCLP TUMBLING FOR SOLID WASTE SAMPLES

Sample	Wet/Dry*	Freeze/Thaw*	Average Compression Strength (psi)	Tumbling 1	Tumbling 2
KIAK	F	F	14	0	0
AIEI	P	P	1663	63	67
BAHE	F	F	107	0	0
AAKC	P	P	771	8	16
BKDE	P	P	13	75	84
IAHE	P	P	624	48	60
GFDG	P	P	117	6	5
ADAC	P	P	1026	60	54

*Data supplied by wastewater experiment station.

The results of the TCLP tumbling of duplicate WES wastes are less straightforward and are presented in Table 7. Four of the samples retained more than 50 percent of their original weight after tumbling (AIEI, BKDE, IAHE, and ADAC). Each of these four samples passed wet/dry and freeze/thaw but only AIEI, IAHE, and ADAC could tolerate >500 psi in the compressive strength test, BKDE cracked after application of only 13 psi of comprehension. Samples KIAK, BAHE,and GFDG were almost completely degraded by tumbling in the cage and each had compressive strengths of <200 psi. KAIK and BAHE also failed both wet/dry and freeze/thaw but GFDG passed both. Sample AAKC was degraded by TCLP but passed wet/dry, freeze/thaw, and showed a compressive strength of 771 psi. Thus six of eight WES samples show a good correlation between passing wet/dry and freeze/thaw and stability in the cage tumbling procedure (AIEI, BKDE, IAHE, ADAC, KAIK, and BAHE). The other two (GFDG and AAKC) show an inconclusive correlation. A number of method variables are expected that contribute to the degree of sample degradation during tumbling, and they will be refined during collaborative testing of Method 1311. These include sample size and cage dimensions because a sample that rotates freely within the cage is

degraded more completely than one with little room to move within the cage.

TABLE 7. COMPARISON OF WET/DRY, FREEZE/THAW AND TCLP TUMBLING STRESS TO SOLID WASTE SAMPLES

Sample	Wet/Dry	Freeze/Thaw	Tumbling 1	Tumbling 2	Tumbling 3
F024 2:1	P 73	P 77	61	80	-
F024 5:1	P 84	P 91	92	85	-
K028 1:1	F (3)	F (17)	0	0	0
K028 5:1	F 18	P 90	87	65	63
Lime/fly ash 1:1	F (1)	F (1)	0	0	0
Lime/fly ash 2:1	F (2)	F (2)	0	0	0
Polymer encapsulated	P 100	P 100	100	-	-
Vitrified*	P 97	P 89	86	-	-

*1-L jar used due to small quantity of waste available.

In a test of the volatiles portion of Method 1311, solid samples tumbled in a ZHE underwent some size reduction but they damaged the 316 stainless steel cylinder walls of the device. Because the design of the ZHE incorporates a moving piston, it was not possible to use a cage or similar liner. Therefore, an alternate approach using a hardended ZHE was tested. It was found that while solidified samples could be tumbled without damage to the cylinder, the filter holder was damaged by the sample during the tumbling by the sample. Work is in progress at Associated Design to produce a ZHE modification that will prevent the sample from contacting the filter holder during tumbling and will be compatible with fillering oily samples. At this time, solid samples require size reduction prior to leaching.

Sample Analysis

A limited number of samples were prepared by Method 1311 and by modified Method 1311 (cage) and analyzed. The results of ICP analysis (Method 6010) of triplicate determinations of leachates prepared from a 5:1 concrete/K028 waste mixture by three slightly different leaching procedures are reported in Table 8. One leachate was produced from a milled sample (unmodified Method 1311), one was produced by a 150-g solid sample tumbled in a stainless steel cage (modified Method 1311), and the other was produced by leaching the solid residue that remained after an 18-hour cage tumbling (Modified 1311 - retumbled). The

observed concentrations are very similar for all of the metals measured. Slightly more chromium and nickel were detected in the first leachings with the cage but those values were both lower than the second cage leaching.

TABLE 8. METAL ANALYSIS OF CEMENT/K028

Sample	Metal Concentration (mg/L)							
	Ba	Cr	Co	Cu	Pb	Ni	Zn	Al
Unmodified 1311								
1	0.76	0.10	-	0.09	0.02	0.14	0.21	1.76
2	0.81	0.04	-	0.07	-	0.08	0.19	1.87
3	0.79	0.04	-	0.06	0.04	0.06	0.17	1.93
Modified 1311								
1	0.78	0.07	-	0.07	-	0.25	0.29	1.77
2	1.14	0.18	0.04	0.15	-	0.39	0.66	4.38
3	0.57	0.12	-	0.06	0.05	0.12	0.33	1.55
Modified 1311-Retumbled								
1	0.61	0.09	-	0.05	-	0.07	0.13	1.66
2	0.70	0.04	-	0.05	-	0.21	0.30	1.67
3	0.58	0.06	-	0.06	0.03	0.07	0.14	1.97

If those differences are real, it may be the result of metals leaching from the cage. However, the evaluated values for chromium and nickel were not observed in subsequent analyses. We believe that these elevated values were the result of the fact that this set of analyses was performed with a new set of cages. The potential for chromium and nickel from the cage to contribute to TCLP background levels is in investigation as part of an ASTM collaboration study of modified Method 1311.

An analysis for organic semivolatile (Method 8270) was performed on aliquots of the same leachates and is reported in Table 9. Four compounds were observed in the leachates, two (benzoic acid and 2,6-dinitrotoluene) gave spectral matches with the N.B.S. library and the other two were unknowns but appear to be chlorinated ethylene dimers.

Unlike the inorganic results, each of the leachates had somewhat different concentrations of organic constituents. The highest concentrations were observed in the milled sample and the lowest in the second tumbling of the solid samples. The relative concentration of the analytes corresponds to the amount of milled solid in the leaching buffer (150 g for unmodified 1311, 60 g for the first cage leaching, and 35 g for the second leaching).

TABLE 9. ORGANIC ANALYSIS OF CEMENT/K028

Sample	Benzoic Acid (μg/L)	2,6-Dinitrotoluene (μg/L)	TIC 1* (μg/L)	TIC 2* (μg/L)
Unmodified 1311				
1	17	3.2	115	177
2	18	3.8	114	187
3	20	4.6	98	170
Modified 1311				
1	-	2.6	29	47
2	13	1.9	11	21
3	12	3.2	3	17
Modified 1311-Retumbled				
1	8	1.3	45	83
2	7	1.0	27	46
3	8	1.6	87	144

*Based on a response factor of 1.

The fact that only the organic analytes show this dependence is probably due to the fact that concrete is a highly alkaline matrix. The final pH of all three of these leachates were greater than 9 which limited the solubility of metals but not of organic analytes.

CONCLUSION

The results of this study demonstrate that tumbling solid waste samples in a rigid stainless steel cage during the TCLP rotation is a sufficient test of the integrity of the sample to establish it as well or poorly stabilized. Those samples which are not degraded during tumbling do not require size reduction; those wastes which are degraded are sufficiently size reduced to allow the TCLP to be performed without additional sample milling, and the leaching fluid produced by tumbling the solid can be used for chemical analysis. Thirteen samples representing the spectrum of state-of-the-art solidification technologies were used to evaluate the milling process. Reasonable correlation was seen between the tumbling results and the pass/fail criteria of the classical wet/dry resistance, freeze/thaw resistance and unconfined compressive strength tests. Samples that failed all three classical tests were completely size reduced by the TCLP tumbling period. Samples that passed all three classical tests generally maintained their integrity and most of their mass. Samples that gave inconclusive give results in the classical tests varied in the amount of degradation during the TCLP tumbling process. These data demonstrate that this proposed modification to the TCLP can be used for a variety of waste samples and that the method modification should be tested in collaborative study.

THE IMPACT OF PARTICLE SIZE ON TCLP EXTRACTION OF CEMENT-STABILIZED METALLIC WASTES

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ABSTRACT

The Hazardous and Solid Waste Amendments of 1984 (HSWA) mandated EPA to change the existing RCRA Extraction Procedure (EP) to ensure that it accurately predicts leaching from landfilled wastes. Subsequently, EPA introduced the Toxicity Characteristic Leaching Procedure (TCLP) in proposed rulemakings associated with HSWA. Of particular interest is the proposed use of the TCLP in a revised RCRA toxicity characteristic proposed in the Federal Register on June 13, 1986.

The TCLP has been compared to the EP in a number of EPA and industry studies and is found to be generally comparable for the extraction of toxic metals. Limited EPA ruggedness testing has evaluated the method's sensitivity to variations in operating conditions such as liquid/solid ratio, extraction time, headspace, extraction fluid acidity, extraction bottle material, filter pre-wash, and filter type. However, the effect of waste particle size on TCLP extraction has not yet been formally evaluated.

To ensure uniformity in testing, the TCLP method requires that waste samples must have a surface area greater than 3.1 cm^2/gm or must pass through a 9.5 mm (0.375 in.) standard sieve. For wastes such as those stabilized with cement to immobilize hazardous constituents, this generally requires size reduction. However, the TCLP method only specifies a maximum particle size (9.5 mm sieve opening) and sets no lower limit. Thus for a given sample it is possible to conduct TCLP extractions on particle size extremes ranging from 9.5 mm down to a finely pulverized powder.

The authors conducted experiments to evaluate the effect of particle size on the extraction of arsenic from a waste which had been stabilized with Portland cement. Five discrete particle size fractions ranging from material which passed through a 9.5 mm sieve but was retained on an 8.0 mm sieve down to a size fraction which passed through a 0.5 mm sieve were extracted by the TCLP procedure. Extraction fluid pH was monitored during the first 2 hours of the extractions. It was found that arsenic leached much more readily from the larger particles. The arsenic leachate concentration from the largest particles (8.0-9.5 mm) averaged 2.2 mg/l while that from the smallest particles (<0.5 mm) was 0.03 mg/l. The pH of the extraction fluid during the first 2 hours showed that for the larger particles pH stayed acidic while for the smaller particles pH quickly became basic. Final pH for the extracts after the 18 hour extraction ranged from 11.0 for the largest particles up to 11.9 for the smallest particles. A similar experiment using a chromium waste solidified with Portland cement was also conducted which yielded trend results consistent with the earlier arsenic waste work.

The results of this work indicate that particle size has a strong influence on TCLP extraction results for the tested waste(s). This suggests that the particle size variable needs tighter controls than presently specified in the TCLP method to ensure reproducible results for at least some wastes.

INTRODUCTION

Congress, in the Hazardous and Solid Waste Amendment of 1984 (HSWA) mandated that the U.S. Environmental Protection Agency (EPA) revise the existing Resource Conservation and Recovery Act (RCRA) Extraction Procedure (EP). Specifically, the Administrator was given 28 months from the date of enactment (November 8, 1984), the "examine the deficiencies of the extraction procedure toxicity characteristic as a predictor of the leaching potential of wastes and make changes in the extraction procedure toxicity characteristic, including changes in the leaching media, as are necessary to insure that is accurately predicts the leaching potential of wastes..." (1). In a subsequent rulemaking proposal dated January 14, 1986, EPA formally introduced the Toxicity Characteristic Leaching Procedure (TCLP) as an integral part of a land disposal restriction affecting certain dioxin-containing and solvent-containing wastes (2). On November 7, 1986, this proposed rulemaking was promulgated and established the TCLP in Appendix I to Part 268 of RCRA (3).

More directly related to the congressional mandate under HWSA, on June 13, 1986, EPA proposed an expanded RCRA Toxicity Characteristic which also incorporated the TCLP extraction method (4). Under this proposal, the present EP Toxicity Characteristic based on 8 metals and 6 organic pesticides will be replaced by a new Toxicity Characteristic incorporating 38 additional organic compounds.

In the extraction of metals, the TCLP has been compared to the EP in several EPA and industry studies and is found to be generally comparable (5, 6). EPA has also evaluated the method's sensitivity to variations in operating conditions which could be expected under routine use. These ruggedness tests have studied the influence of liquid/solid ratio, extraction time, headspace, extraction fluid acidity, extraction bottle material, filter pre-wash, and filter type (7). Each of these parameters was varied outside the TCLP method's specifications to evaluate their impact on the resulting TCLP extract. However, to the best of our knowledge, there have been no such ruggedness evaluations on the effect of waste particle size on TCLP extraction.

To ensure uniformity in testing, the TCLP method requires that waste samples must have a surface area greater than 3.1 cm^2/gm or must pass through a 9.5 mm (0.375 in.) standard sieve. The existing EP has a similar requirement, although the EP offers the option of the Structural Integrity Procedure for monolithic wastes as an alternative to mandatory particle size reduction (8). Thus the TCLP, as well as the EP, fixes no minimum particle size and for a given sample it is possible to perform extractions on particle sizes ranging from 9.5 mm down to an infinitely fine pulverized powder. correspondingly the surface area of the waste sample could range from about 3 cm^2/gm up to many thousands of square centimeters per gram of waste (9).

Monsanto's Environmental Sciences Center became fully equipped to perform TCLP extractions in early 1986. Extractions were performed on a variety of Monsanto waste streams in anticipation of water disposal regulations which would be based on TCLP extracts. By mid-to-late 1987, sufficient commercial laboratory TCLP capacity was available to meet routine plant needs. The focus of our internal TCLP capability is now on the non-routine, research-oriented application of the method. One aspect of this has been test work on stabilized inorganic waste materials containing two of the metals (arsenic and chromium) which are addressed by the present and proposed future RCRA toxicity characteristic.

A number of monolithic waste materials which are generated as monoliths or which have been made into monoliths by solidification processes have been examined. The potential importance of particle size on TCLP extraction was recognized and informal laboratory controls were practiced on this parameter. All particle size reductions were restrained to the best of our ability to yield particles which would just meet the requirement of passing through the required 9.5 mm standard sieve. However, no absolute, rigid control was placed on minimum particle size for our extraction work with these materials.

In 1987, experiments were designed and conducted to determine if there was a relationship between particle size and TCLP extraction of metals from waste matrices stabilized with Portland cement. The following two case studies present the results of these experiments.

METHODS

CASE STUDY I METHODS

Case I experiments were performed using a waste containing an inorganic arsenic compound. The waste had been solidified with Type 1 Portland cement at a ratio of 3 parts by weight dry cement to 1 part by weight waste (dry basis). The raw waste material was physically a moist solid with 66.7% solids content. On a wet basis, the arsenic content of the raw waste, as arsenic, was nominally 1.2%.

The initial experiment consisted of performing a set of duplicate TCLP extractions (extraction fluid #2) on the following nominal size fractions of the solidified waste material: 0.5 to 1 mm, 1 to 4 mm, 4 to 8 mm, and 8 to 9.5 mm. These fractions were obtained by breaking up the solidified waste monolith with a hammer and chisel and then screening them using 8 inch diameter U.S. Standard Sieves and a Tyler portable sieve shaker. The sample fraction nominally identified as 0.5 to 1 mm passed through a 1 mm standard sieve and was retained on the 0.5 mm sieve. Each of the other 3 particle size fractions similarly was collected on and passed through their respective nominal sieve designations.

Quality Assurance features were incorporated into the TCLP extraction work as follows:

a. The extraction work was performed in duplicate on separate days to evaluate day-to-day variability.

b. An extraction was performed on a virgin sample of clean sand and on a sample of the same sand which had been processed through the sieving equipment.

c. Each extraction fluid batch was analyzed and a TCLP method blank extraction was performed with each extraction fluid batch.

Quality Assurance features were also incorporated into the TCLP extract samples which were analyzed for arsenic as follows:

a. Two extract samples were divided and submitted as blind duplicates for analysis.

b. One sample was split, with one half being spiked at 0.5 mg/L As.

c. One sample was split, with one half being spiked at 2.0 mg/L As.

The second experiment on the Case I arsenic waste was designed to better define any arsenic extraction trends. Particle size fractions were prepared for TCLP extraction as previously described. In addition to the 4 fractions previously extracted, a "less than 0.5 mm" fraction was included. Observation of extract pH was expanded beyond the final pH to include the end of the first and second hour of tumbling. Whole waste analysis for arsenic was also performed for each of the 5 particle size fractions.

Quality Assurance features were incorporated into the TCLP extraction work as follows:

a. The extraction work was performed in duplicate for 3 of the 5 samples on separate days to evaluate day-to-day variability.

b. An extraction was performed on sand of original known purity which had been processed through the sieving equipment.

c. Each extraction fluid batch was analyzed and a TCLP method blank extraction was performed with each extraction fluid batch.

Quality Assurance features were also incorporated into the TCLP extract samples which were analyzed for arsenic as follows:

a. Two extract samples were divided and submitted as blind duplicates for analysis.

b. One sample was split, with one half being spiked at 0.5 mg/L As.

c. One sample was split, with one half being spiked at 2.0 mg/L As.

Quality Assurance in the whole waste analysis for arsenic consisted of submitting one blind duplicate sample.

CASE STUDY II METHODS

Case II TCLP extraction experiments (extraction fluid #2) were performed using a waste containing chromium compounds, primarily calcium chromite. The waste had been solidified with Type I Portland cement at a ratio of 2.5 parts by weight dry cement to 1 part by weight waste (dry basis). The raw waste material was physically a wet solid, resembling mud, with 70% solids content.

The experiment to demonstrate any relationship of particle size to TCLP extraction of chromium from this waste was modeled closely after the final Case I arsenic experiment performed earlier. Duplicate TCLP extractions were performed on separate days on the following nominal size fractions of solidified waste material: less than 0.5 mm, 0.5 to 1 mm, 1 to 4 mm, 4 to 8 mm, and 8 to 9.5 mm. Besides final pH, measurement of TCLP extract pH was performed at the end of 15 minutes, 30 minutes, 60 minutes and then hourly through 4 hours of the 18 hour extraction cycle for one of the duplicate sets of extractions. Whole waste analysis for total chromium was also performed for each of the 5 particle size fractions.

Quality Assurance features were incorporated into the TCLP extraction and extract analytical work as in the second arsenic experiment cited above.

RESULTS

CASE STUDY I RESULTS

The experimental results of the first effort with the arsenic-bearing waste are shown on Table 1. While results for most of the quality assurance measures are not shown, they did affirm the validity of the report data.

TABLE 1. TCLP Extraction Results for an Arsenic Containing Waste

Sample	TCLP Extraction Date	Nominal Particle Size Range (mm)	TCLP Extract Arsenic Content (mg/L)	TCLP Extract Final pH
A	6/25/87	0.5 to 1	0.02	12.18
B	6/25/87	1 to 4	0.13, 0.13*	11.87
C	6/25/87	4 to 8	1.00	11.53
D	6/25/87	8 to 9.5	2.05	11.26
E	6/27/87	0.5 to 1	0.03	12.11
F	6/27/87	1 to 4	0.06	11.83
G	6/27/87	4 to 8	0.98	11.60
H	6/27/87	8 to 9.5	1.65, 1.64*	11.38

*Blind duplicate analysis

While preliminary trends were indicated, the experiment had not included whole waste arsenic analysis of the various size fractions of waste. Thus, it could not be determined if the trends were a TCLP phenomena or were reflections of some unexpected arsenic distribution related to waste particle size. Accordingly, additional experiments were performed which included several refinements in experimental design.

The experimental results of the second set of experiments with the arsenic-bearing waste are shown on Table 2. While results for most of the quality assurance measures are not shown, they did affirm the validity of the reported data.

CASE STUDY II RESULTS

Experimental results for the chromium-bearing waste are shown on Table 3. while results for most of the quality assurance measures are not shown, they did affirm the validity of the reported data.

TABLE 2. TCLP Extraction Results and Whole Waste Analysis for an Arsenic Containing Waste

Sample	TCLP Extract. Date	Nominal Particle Size Range (mm)	Arsenic Content		TCLP Extract pH		
			TCLP Extract (mg/L)	Whole Waste (wt. %)	1 hr.	2 hr.	Final (18 hr.)
A	8/27/87	<0.5	0.03	0.24	11.78	11.78	11.94
B	8/27/87	0.5 to 1	0.06, 0.05*	0.26	11.50	11.59	11.89
C	8/27/87	1 to 4	0.15	0.25	8.68	10.76	11.66
D	8/27/87	4 to 8	1.11	0.27, 0.26*	4.97	5.52	11.31
E	8/27/87	8 to 9.5	2.31, 2.33*	0.24	4.73	4.92	11.00
F	8/29/87	<0.5	0.03	0.24	--	--	11.92
G	8/29/87	1 to 4	0.18	0.25	--	--	11.52
H	8/29/87	8 to 9.5	2.65	0.24	--	--	10.82

*Blind duplicate analysis

TABLE 3. TCLP Extraction Results And Whole Waste Analysis for a Chromium Containing Waste

Sample	TCLP Extract Date	Nominal Particle Size Range (mm)	Total Chromium Content		TCLP Extract pH						
			TCLP Extract (mg/L)	Whole Waste (ppm)	.25 hr.	.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.	Final (18 hr.)
A	12/31/87	<0.5	0.027	1090	--	--	--	--	--	--	11.44
B	12/31/87	0.5 to 1	0.026	1080	--	--	--	--	--	--	11.51
C	12/31/87	1 to 4	0.027	1060	--	--	--	--	--	--	11.38
D	12/31/87	4 to 8	0.054	980	--	--	--	--	--	--	11.16
E	12/31/87	8 to 9.5	0.073	940, 960*	--	--	--	--	--	--	10.73
F	01/07/88	<0.5	0.039	1090	11.49	11.58	11.75	11.75	11.77	11.84	11.48
G	01/07/88	0.5 to 1	0.028	1080	10.29	11.23	11.41	11.68	11.74	11.76	11.51
H	01/07/88	1 to 4	0.026	1060	5.42	6.14	9.61	11.06	11.38	11.50	11.42
I	01/07/88	4 to 8	0.050, 0.051*	980	4.51	4.77	5.17	5.92	6.27	8.58	11.14
J	01/07/88	8 to 9.5	0.063, 0.065*	940, 960*	4.34	4.58	4.88	5.47	5.58	6.24	10.96

*Blind split analysis

DISCUSSION

Case I results for the initial particle size experiment evaluating effects on TCLP arsenic extraction, shown in Table 1, presented two apparent trends. Arsenic apparently leached more readily from the larger particle sized waste. Secondly, the final pH of the TCLP extract showed a definite downward trend with increasing waste particle size.

Results of the second Case I experiment to evaluate the effect of particle size on TCLP arsenic extraction, shown in Table 2, verified the trends seen earlier and demonstrated that these were TCLP phenomena and were not due to any segregation of arsenic by waste particle size. Thus both experiments with this cement stabilized arsenic waste clearly demonstrated that arsenic was more easily leached from the larger waste particles. The difference is about 2 orders of magnitude and thus is highly significant from a regulatory standpoint. Consistently over both Case I waste experiments, the smallest particles yielded TCLP arsenic concentrations of 0.02 to 0.03 ppm while the largest particles yielded TCLP arsenic concentrations between 1.64 and 2.65 mg/L. Furthermore, the arsenic contents by whole waste analysis of the various particle size fractions were essentially identical, ranging from 0.24 to 0.27 percent by weight arsenic in the cement stabilized arsenic-bearing waste.

The trend in final TCLP extract pH indicated in the first Case I experiment was also shown in the second experiment in that the final pH showed a definite downward trend with increasing waste particle size. Moreover, the more detailed pH measurements taken in the second Case I experiment give insight into the mechanism responsible for the leaching trend. For the smaller particle size fractions, the acidic TCLP extraction media (fluid #2, pH 2.88) was rendered basic in less than 1 hour. Conversely the extraction media for the larger particle fractions remained acidic through the first 2 hours of extraction and did not attain as high a pH value after 18 hours as with the smaller particle size fractions.

Results of the Case II experiment to evaluate the effect of particle size on TCLP chromium extraction (Table 3) show similar trends to those developed for the arsenic waste. The chromium was leached more easily from the larger waste particles. The difference is roughly a factor of 2 and would seem great enough to have regulatory significance. In the duplicate extractions, the smallest size fraction, <0.5 mm, yielded total chromium concentrations of 0.027 and 0.039 mg/L while the largest particle size fraction, 8.0 to 9.5 mm, yielded TCLP chromium concentrations of 0.073, 0.063, and 0.065 mg/L. The chromium content by whole waste analysis of the various particle size fractions was very similar, ranging from 940 to 1090 ppm total chromium in the cement solidified chromium-bearing waste.

The trend in final TCLP extract pH for the chromium waste is the same as that demonstrated earlier for the arsenic waste, i.e., the extracts from the smaller particles reach higher final pH values than do the extracts from larger particles. Likewise, the extracts from the small particles become basic much more quickly

than the extracts from larger particles. In fact the data for the smallest particle cut (<0.5 mm) indicated a very rapid neutralization reaction because the extract fluid pH after only 15 minutes had reached the value which was also noted at the end of the 18 hour extraction. Because, hydrated Portland cement is predominantly calcium hydroxide this neutralization should be expected (10). The neutralization reaction occurred much more rapidly with the smaller particle size waste which also should be expected. The unexpected consequence, however, is that metals (arsenic and chromium) were more effectively leached from large rather than small waste particles in the two tested Portland cement waste matrices.

In summary, experiments with two specific cement-solidified wastes demonstrated that arsenic and chromium were leached more effectively by the TCLP extraction method from large rather than small particles of waste. The particle sizes evaluated in this work ranged from the maximum allowed by the method, 9.5 mm, down to particles which passed through a 0.5 mm standard sieve. (Note that there is no lower limit placed on minimum particle size by the TCLP method when samples require size reduction). The enhanced metals leaching from the larger particles of the two tested cement stabilized wastes could have significance from a regulatory compliance perspective. For the arsenic waste, the difference was nearly 2 orders of magnitude. For the chromium waste, the difference was about a factor of 2.

Particle size, therefore, is a significant TCLP method variable which needs closer control specifications. Without closer control than is imposed by the presently promulgated TCLP method, wide variations in test results can be expected in cement stabilized metallic wastes as shown in the two examples. This phenomenon may well extend beyond metallic wastes to include any Portland cement stabilized waste or any waste whose hazardous constituents (metallic or non-metallic) depend on a chemically basic matrix for immobilization.

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MOBILIZATION OF VOLATILE TOXIC COMPONENTS FROM PETROLEUM PRODUCT-CONTAMINATED SOILS BY TCLP

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ABSTRACT

In order to determine if soils contaminated with petroleum products need to be defined as hazardous waste under the toxicity characteristic criteria proposed by the U.S. EPA Office of Solid Waste, two different types of soil contaminated with petroleum products were subjected to the toxic characteristic leaching procedure (TCLP). One soil (Kaw) was characterized by approximately equal proportions of sand and silt-sized particles, with 0.6% organic carbon content. The other (Action) had roughly equal proportions of silt and clay with a higher organic carbon content (2.3%). The soils were spiked with unleaded gasoline, No. 2 diesel fuel, and No. 6 fuel oil at five nominal concentration levels (10,000, 5,000, 1,000, 100, and 10 ppmw). The gasoline saturation level of both soils was also determined. The contaminated soils were subjected to the TCLP test, and the TCLP leachates were analyzed by GC/MS for a number of target volatile and semivolatile organic analytes. The neat fuels were also characterized by GC/MS.

The gasoline-contaminated soil leachates exhibited concentrations that were dependent on the soil type. When subjected to TCLP, the gasoline-contaminated Kaw soil released the volatile target analytes more readily than the Action soil. The Action soil system was controlled by the carrying capacity of the soil, while for the Kaw soil the leaching system was controlled by the carrying capacity of the leaching fluid. In the case of diesel- and fuel oil-contaminated soils, there were no significant differences between the concentrations of volatiles in the leachates of the two types of soil, possibly due the influence of more abundant and more hydrophobic semivolatile PAHs.

Since TCLP is an extraction procedure, the initial concentrations of each analyte from each fuel in each soil tested were estimated and compared to the leachate concentrations. The percent recovery of each analyte in the leachates was calculated. This recovery is indicative of the leaching efficiency of the TCLP test. The results indicate that there were significant differences in the leaching efficiency by TCLP which depended on soil characteristics and extent of contamination.

INTRODUCTION

It has been estimated that there are several million underground storage tanks (USTs) in the United States which are used to store motor fuels, chemicals, and wastes.[1] Leaking USTs have become a matter of national concern. Loss of materials stored in these tanks poses a risk of groundwater contamination, with an attendant potential risk to human health. A recent study estimated that approximately 35% of the nonfarm underground motor fuel storage tank systems do not pass tank tightness tests.[2] This test is designed to determine the airtightness of a storage tank system, and failure of the test may indicate that some part of the tank system may leak.

The U.S. Environmental Protection Agency recently developed a method whereby solid wastes are subjected to a leaching procedure whose major objective is to accurately model the mobility of toxic constituents from wastes under environmental conditions.[3] The toxic characteristic leaching procedure (TCLP) is a two-step procedure in which the liquid portion of the waste (filtrate) is separated from the solid part (filter catch) by high pressure filtration. The filter catch is then leached with a buffered acetic acid solution (pH 5) at a liquid-to-solid ratio of 20:1. This leaching step is carried out by rotating the vessel with the waste and fluid end-over-end for 18 hr. At the end of this period, the leachate is filtered. The filtrate and the leachate may be combined and analyzed together, or analyzed separately and the results combined mathematically. The TCLP extract concentration thus obtained is compared to threshold values for various analytes determined using theoretical considerations, and if the concentration in the TCLP extract exceeds the threshold value, the waste is defined to be a hazardous waste.

The purpose of this study was to determine if TCLP would mobilize significant amounts of organic analytes from soils artificially contaminated with petroleum products. This information can be used to determine if the contamination levels at which the soils were contaminated with fuels would be defined as hazardous wastes under the criteria selected by EPA/OSW using TCLP. It can also provide qualitative information on the TCLP leaching process by subjecting a material contaminated with known levels of contaminants to it.

EXPERIMENTAL

Soil Characterization

Two different types of soil were used to determine the mobility of several organic components from petroleum products mixed with them. The first soil was a light-colored, fine-grained, sandy-silty soil obtained from Kaw Sand Company (Lawrence, KS). A second soil was obtained from Action Topsoil Company (Olathe, KS). The Action soil was dark and loamy.

The particle size distribution of each soil was determined by wet-sieving a dispersed suspension of each soil through a 230-mesh sieve. The sieve catch was the sand-sized fraction, and a size analysis of the remaining particles in the suspension was performed using the pipet technique.[4] Soil total organic carbon content (TOC) was determined by oxidation with dichromate and back-titration of excess dichromate with ferrous sulfate (Walkley-Black method).[5]

Soil-Petroleum Products Mixing

Three different types of petroleum products were mixed with the two soils described above. Unleaded gasoline and diesel oil were obtained from a Fairway Oil gasoline station (Shawnee, KS) in June 1987. No. 6 fuel oil was obtained from Consolidated Fuel Oil Co. (Riverside, MO) in May 1987.

Each soil was spiked with each of the petroleum products at five nominal concentration levels. These concentrations were 10,000, 5,000, 1,000, 100, and 10 ppm (wt fuel/wt mix). Approximately 200 g of soil was supplemented with the appropriate amount of the petroleum products and mixed in sealed, 32-oz jars which had been modified so that portions of the glass wall were pushed like fingers into the cavity of each container. These glass fingers promoted more efficient mixing of the fuel and soil when the sealed jars were rotated in a tumbling table. This

procedure was adequate for the less viscous fuels (gasoline and diesel), but the more viscous No. 6 fuel oil did not mix well with the soil, resulting in clumping. An alternative mixing procedure for this fuel involved dispensing the fuel and soil into unmodified 32-oz jars and storing at -10°C for several hours. At the end of this period, three ceramic balls (1-in diameter) were placed in the jar, and the jar was rotated for 3 to 4 hr. This procedure broke the solidified clumps of fuel oil and effectively mixed them into the soil. At the end of the mixing period, the contaminated soils were transferred to smaller volume jars to minimize headspace and were stored at -10°C.

In order to determine the amount of gasoline necessary to fully saturate both types of soil used for this study, each soil was ground on a mortar and pestle, spiked with an excess amount of unleaded gasoline in a beaker, mixed by hand with a spatula, and allowed to stand for 5 min. After this period, the mixture was transferred to a medium-mesh conical paint filter placed on a powder funnel emptying into a graduated cylinder. The free liquid (gasoline) was allowed to drain out of the mix for at least 5 min, but not before the soil appeared to have no free liquid. The saturated soil was transferred to a preweighed beaker, weighed, and placed in a mechanical convection oven (65°C) overnight. The soil was then reweighed, and the difference between the initial and final weight was defined as the mass of gasoline in the saturated soil. These results were corrected for soil blanks.

Leaching of the Petroleum Product-Soil Mixtures

The contaminated soils were subjected to the toxic characteristic leaching procedure (TCLP) described in the Federal Register.[6] TCLP for volatile organic compounds was performed using a 90-mm diameter zero-headspace extractor (ZHE) described in the TCLP protocol. The samples (approximately 10 g) were added to the ZHE while still cold, and the sample was allowed to warm up inside the ZHE after the headspace had been removed by pressurizing the system. About 200 mL of the leaching fluid--an acetic acid buffer mix--was pumped into the ZHE from a graduated cylinder using a piston pump. Each ZHE was then placed into a rotating box (30 rpm) for 18 hr, after which the leachate was collected in glass, gastight syringes (50 mL). The first portion collected was used as a rinse and discarded, and the second and subsequent portions were dispensed into 40-mL vials to overflowing and sealed with PTFE-lined screwcaps. These vials were stored inverted in sealed cans containing charcoal at 4°C.

Volatile Organic Compound Analysis

Volatile organic compounds were analyzed by purge-trap-desorb (PTD) gas chromatography/mass spectrometry (GC/MS).[7] The volatile compounds selected as target analytes were benzene, toluene, ethylbenzene, and o-, *m*-, and *p*-xylene.

A Varian 3700 GC fitted with a purge and trap device was used with a 6-ft x 0.1 in i.d. glass column packed with 1% SP 1000 on Carbopack-B (60/80 mesh) and helium carrier gas (30 cm/min). Aqueous leachates were injected directly into the purging device, but the fuels were dispersed in tetraglyme, and an aliquot of the dispersion was added to organic-free water in the purging device. Also added to the purging device were 10 µL of an internal standards solution (bromochloromethane, D_4-1,2-dibromoethane, and D_5-chlorobenzene at 25 µg/mL). The purging tower was sparged with nitrogen at 40 mL/min. for 11 min (ambient temperature) into a trap filled with 3% OV-1 on Chromosorb-W, 60/80 mesh (1 cm), Tenax GC (15 cm), and

Davison grade 15 silica gel (8 cm). The trap was then back-flushed with helium (20-60 mL/min) at 180°C for 4 min, and the effluent directed into the GC injector port (200-225°C). After 3 min at 45°C, the column temperature was programmed from 45 to 220°C at 8°C/min. The column was taken to a Finnigan/ MAT CH4B single-focusing, magnetic sector mass spectrometer via jet separator (250-300°C), and the mass spectrometer was set to scan a mass range of m/z 35-260 at 3-sec intervals using the electron impact ionization mode (70 eV). The instrument was calibrated daily with PFK (perfluorokerosene) and BFB (bromofluorobenzene).

Not all of the TCLP leachates generated were analyzed. The leachates from the most contaminated soils were analyzed first, followed by the next lower level, and so on. Decreasing trends in the analyte concentrations were monitored, and if any particular level which followed the established trend showed analyte concentrations below the respective quantitation limits, the next lowest nominal concentration leachates were not analyzed.

RESULTS AND DISCUSSION

Table 1 presents the results of the soil characterization studies. The data indicate that the Kaw River soil was characterized by a very low clay content, with sand- and silt-sized particles occurring in approximately equal proportions. The Action topsoil had a very low sand content, with the bulk of the material split about equally between silt- and clay-sized particles. The Kaw soil had a low organic carbon content (0.6%), while the Action topsoil had 2.3% organic carbon. Both soils had between 10 and 15% water content. The gasoline saturation study indicated that both soils were saturated with about 15% (wt fuel/wt mix) of gasoline.

The results obtained from the analysis of the three fuels used to spike the individual soils are presented in Table 2. The unleaded gasoline was enriched in volatile aromatic hydrocarbons, with concentrations above 10,000 µg/g for all the volatile target analytes. The diesel fuel used in this study had volatile aromatic concentrations about two orders of magnitude lower than the gasoline (between 300 and 650 µg/g). No. 6 fuel oil had the lowest volatile aromatic concentrations of the fuels studied, with concentrations of less than 70 µg/g.

The results of the analyses of the TCLP leachates for both soils contaminated with unleaded gasoline are presented in Table 3. As can be seen from these results, the leachates from the contaminated Kaw soil were appreciably more concentrated than those for the corresponding Action soil for all of the volatile aromatic target analytes at all the spiking levels. In addition, the 10,000-ppm contaminated Action topsoil exceeded current TCLP regulatory threshold levels for benzene, as did the Kaw soil contaminated at all nominal contamination levels above 1,000 ppm. Furthermore, the toluene concentration of the 10,000-ppm contaminated Kaw soil was within 10% of the regulatory level for toluene.

Table 4 presents the results of the percent efficiency of TCLP in mobilizing volatile aromatics from the gasoline-contaminated soils. As can be seen, the efficiency of TCLP in mobilizing the target volatiles from Action topsoil was relatively low, but increased at the higher nominal contamination levels. Less than about 20% of the available target aromatics were mobilized by TCLP from the gasoline-contaminated Action soil. On the other hand, the gasoline-contaminated Kaw soil had generally high extraction efficiency (> 40%), but the values were

highest at the lower nominal contamination levels. This suggests a stronger affinity of the analytes for the Action soil than for the Kaw soil.

Isotherm plots[7] were drawn for these compounds in both types of soil used. However, since it was not known to what extent equilibrium is achieved using TCLP, the use of the isotherm plots to derive partition coefficients was thought to be inadequate. Therefore, all of the observations based on these plots are strictly qualitative. In addition, the isotherm lines drawn are estimates which are not based on least-squares fitting of the points.

Figure 1 suggests fundamental differences in the interactions between the two soils and the volatile components of the fuels used to test mobility by TCLP in this study. The gasoline-contaminated Action soil shows a soil-limiting system for all of the volatile aromatic target analytes. This is to be expected, given the relatively high organic carbon content of the soil.[8,9] Such a system readily sorbs the contaminating components and releases them into the leaching fluid at very slow rates. In this way, sorption of the contaminants onto the soil tends to buffer the concentration of the organic components in the aqueous phase, as suggested by Moore and Ramamoorthy.[10] When the carrying capacity of the soil is surpassed, the excess material is released into the leaching medium.

The gasoline-contaminated Kaw soil exhibited a different isotherm for the volatile analytes. This system is better described as being limited by the carrying capacity of the leaching fluid. This soil did not sorb the contaminants very strongly (as predictable from the low organic carbon content), thereby favoring their movement into the aqueous phase. The concentration of the leaching fluid increased linearly with increasing spiking levels of the soil up to the 5,000-ppm nominal spiking level. From that point, the carrying capacity of the leaching fluid was exceeded, as reflected by the upward dip in the isotherm. It is important to note that the scales of the ordinate in the Action and Kaw soil isotherm plots are different by as much as one order of magnitude, reflecting the extent of the difference in the mobilization of volatile components from both soils.

The results of the analyses of the TCLP leachates for diesel-contaminated soils are presented in Table 5. Contrary to what was observed in the gasoline-contaminated soils, differences between the two types of soil were not well reflected in the concentrations of the target analytes in the leachates from both diesel-contaminated soils. Table 6 summarizes the results of the extraction efficiency calculations for diesel-contaminated soils. The efficiency for benzene was not calculated because it was detected at a concentration below the reliable quantitation limit in the fuel. The percent extraction efficiencies of TCLP in the diesel contaminated soils were not appreciably different at the highest nominal contamination levels (5,000 and 10,000 ppm), while at the 1,000-ppm level, Kaw soil released significantly more volatiles than the Action soil.

The Action soil isotherms for volatile aromatics from diesel fuel (Figure 2) did not exhibit the strong retention by the soil that was observed in the gasoline-contaminated Action soil. This is reflected in the high linearity of the isotherms (with the exception of toluene) as compared to the more curvilinear isotherms observed in the gasoline-contaminated Action soil. One possible explanation for this phenomenon is that the diesel fuel had higher concentrations of PAHs than gasoline. Because of the higher hydrophobic character of PAHs, they would be preferentially sorbed on the soil, thereby resulting in minimal sorption

or exclusion of the volatiles.[10] Volatile organics leached from diesel-contaminated Kaw soil suggest that this soil system is controlled by the carrying capacity of the leaching fluid. This is similar to what was observed for gasoline-contaminated Kaw soil.

Table 7 presents the results for the No. 6 fuel oil-contaminated soils. As was the case for the diesel fuel, the differences in leachate concentration between the two types of soil were not significant. Table 8 presents the percent extraction efficiency of TCLP on fuel oil-contaminated soils. At both the 5,000- and 10,000-ppm nominal concentration levels, essentially all of the volatile aromatics were mobilized by TCLP, with minimal differences between the two soils. Figure 3 presents these data in isotherm plots, and as was the case for diesel contaminated soils, there were no major differences between the isotherms. No. 6 fuel oil had an abundance of PAHs, and they may have had the same effect on soil sorption suggested above in the diesel-contaminated soils.

CONCLUSIONS

Action soil had a stronger retentive effect on the volatile components of the spiked fuel than did the Kaw soil, which essentially released as much of the toxic components as the leaching medium could accommodate. The volatile components in gasoline were more effectively immobilized by the Action soil than the equivalent components in diesel fuel or No. 6 fuel oil. The reason for this is believed to be competition for the sorptive sites in the soil which favor the more hydrophobic PAHs. The fuel spiking levels used in this study were less than 10% of the soil saturation level for gasoline, and in all probability, about as much for the diesel. Even so, the 10,000-ppm gasoline-contaminated Action topsoil and all the leachates produced from Kaw soil contaminated at levels higher than 1,000 ppm exceeded TCLP regulatory threshold. The carrying capacity for volatile organics in the higher capacity Action soil was being approached, even at the relatively low spiking levels used in this study. For several of the analytes in the Kaw soil, the carrying capacity of the aqueous leaching medium was being approached.

Prediction of the behavior of fuel spills in the environment is, based on the information presented above, difficult at best. Each soil is going to present different conditions which may or may not affect mobility of contaminants. Some generalizations can be made, but they cannot be made quantitatively with any degree of accuracy. The nature of the fuel will also be of importance in determining the mobility of contaminants from fuel spills. Exclusion of volatile compounds from sorption sites by semivolatile components is a point in case.

TCLP as a method to mobilize potentially toxic components from contaminated soils depends to a great extent on the characteristics of the soil and the extent of contamination in the soil. Thus, for a soil that does not retain contaminants readily, TCLP is an effective extraction procedure at low contamination levels. On the other hand, soils that have a high retentive capacity for organic contaminants have higher extraction efficiencies at higher contamination levels. Furthermore, it is not known if TCLP is an equilibrium process. These potential biases may be reduced by subjecting the contaminated soil to several leaches until an equilibrium between the solid and aqueous phases has been attained. The sum of the contaminant concentration levels from the various leaches would be a better estimate of total mobility of contaminants in real-world conditions.

ACKNOWLEDGMENT

This study was supported by the U.S. Environmental Protection Agency under EPA Contract No. 68-01-7383. Helpful discussions with Mr. Mike Kaliwoski, Project Officer, Office of Underground Storage Tanks, EPA, Washington, D.C., and Ms. Janet Dean, Dr. John Coates, and Dr. Clarence Haile, Midwest Research Institute (MRI), are gratefully acknowledged. The GC/MS work was done at MRI's Mass Spectrometry Facility by Mrs. Audrey Zoog.

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TABLE 1

SOIL CHARACTERIZATION RESULTS FOR KAW RIVER SOIL AND ACTION TOPSOIL

	Kaw River Soil	Action Topsoil
Percent water	10.1	13.5
Percent sand	45	3.7
Percent silt	51	55
Percent clay	4.0	41
Percent total organic carbon	0.6	2.3

TABLE 2

VOLATILE ORGANIC TARGET ANALYTE CONCENTRATIONS IN UNLEADED GASOLINE, DIESEL FUEL AND NO. 6 FUEL OIL

	Gasoline (µg/g)	Diesel (µg/g)	No. 6 Fuel Oil (µg/g)
Benzene	13,800	< 125	10.0
Toluene	58,000	359	60.4
Ethylbenzene	12,500	312	21.7
m-Xylene	33,100	669	69.0
o- and *p*-Xylene	32,100	638	65.2

TABLE 3

TARGET ANALYTE CONCENTRATIONS IN TCLP LEACHATES (µg/L)
FROM UNLEADED GASOLINE-CONTAMINATED SOILS

Analyte	Soil	Contaminated Soil Nominal Concentration (ppm) 10	100	1,000	5,000	10,000
Benzene	Action	NA	ND	TR	TR	109[a]
	Kaw	TR	31.6	241[a]	1,660[a]	2,380[a]
Toluene	Action	NA	TR	7.62	417	2,620
	Kaw	15.9	209	1,480	8,420	13,100[a]
Ethylbenzene	Action	NA	ND	6.38	402	1,210
	Kaw	4.88	46.9	329	1,800	2,530
m-Xylene	Action	NA	ND	22.2	1,220	3,460
	Kaw	13.5	129	876	4,520	6,560
o- and *p*-Xylene	Action	NA	TR	28.6	1,340	3,670
	Kaw	12.7	131	899	4,600	6,720

NA = Not analyzed.
ND = Analyte not detected.
TR = Analyte detected at trace levels (below the quantitation limit).

[a] Analyte concentration in the TCLP extract exceeds threshold level for determination of hazardous wastes.

TABLE 4

EXTRACTION EFFICIENCY OF VOLATILE ORGANIC COMPOUNDS
FROM GASOLINE CONTAMINATED SOILS BY TCLP

Analyte	Soil	Contaminated Soil Nominal Concentration (ppm) 10 (%)	100 (%)	1,000 (%)	5,000 (%)	10,000 (%)
Benzene	Action	NA	0	0	0	1.6
	Kaw	0	45	33	49	35
Toluene	Action	NA	0	0.27	2.8	8.9
	Kaw	53	70	49	60	45
Ethylbenzene	Action	NA	0	1.0	13	19
	Kaw	76	73	50	59	41
m-Xylene	Action	NA	0	1.4	15	21
	Kaw	79	76	50	56	40
o- and *p*-Xylene	Action	NA	0	1.8	16	23
	Kaw	77	79	53	59	42

NA = Not analyzed.

TABLE 5

TARGET ANALYTE CONCENTRATIONS IN TCLP LEACHATES (µg/L) FROM DIESEL FUEL-CONTAMINATED SOILS

Analyte	Soil	Contaminated Soil Nominal Concentration (ppm) 100	1,000	5,000	10,000
Benzene	Action	NA	ND	TR	4.04
	Kaw	2.04	51.8[a]	7.45	42.9
Toluene	Action	NA	TR	11.7	62.2
	Kaw	2.09	51.6[a]	36.8	6.4
Ethylbenzene	Action	NA	TR	20.8	52.0
	Kaw	TR	15.0	31.3	47.4
m-Xylene	Action	NA	3.57	47.6	106
	Kaw	TR	32.6	64.5	99.1
o- and *p*-Xylene	Action	NA	4.30	51.0	110
	Kaw	TR	33.8	66.5	104

NA = Not analyzed.
ND = Analyte not detected.
TR = Analyte detected at trace levels (below the quantitation limit).

[a] Potentially inaccurate results.

TABLE 6

EXTRACTION EFFICIENCY OF VOLATILE ORGANIC COMPOUNDS
FROM DIESEL-CONTAMINATED SOILS BY TCLP

Analyte	Soil	Contaminated Soil Nominal Concentration (ppm) 100 (%)	1,000 (%)	5,000 (%)	10,000 (%)
Benzene[a]	Action	NA	NA	NA	NA
	Kaw	NA	NA	NA	NA
Toluene	Action	NA	0	13	35
	Kaw	120	300[b]	42	37
Ethylbenzene	Action	NA	0	27	33
	Kaw	0	100	41	30
m-Xylene	Action	NA	11	29	32
	Kaw	0	100	40	30
o- and *p*-Xylene	Action	NA	14	32	34
	Kaw	0	110	43	33

NA = Not analyzed.

a Benzene was detected in the fuel at a concentration lower than the quantitation limit.

b Recovery calculation based on potentially inaccurate leachate concentration (see Table 5).

TABLE 7

TARGET ANALYTE CONCENTRATIONS IN TCLP LEACHATES (µg/L) FROM No. 6 FUEL OIL-CONTAMINATED SOILS

Analyte	Soil	Contaminated Soil Nominal Concentration (ppm) 1,000	5,000	10,000
Benzene	Action	NA	115[a]	17.3
	Kaw	8.96	6.26	11.1
Toluene	Action	NA	52.9[a]	47.9
	Kaw	11.7	13.4	38.0
Ethylbenzene	Action	NA	5.44	13.8
	Kaw	2.60	5.57	10.2
m-Xylene	Action	NA	14.4	39.6
	Kaw	6.80	16.7	30.3
o- and *p*-Xylene	Action	NA	13.4	40.0
	Kaw	6.66	17.0	31.3

NA = Not analyzed.

[a] Potentially inaccurate results.

TABLE 8

EXTRACTION EFFICIENCY OF VOLATILE ORGANIC COMPOUNDS BY TCLP FROM DIESEL-CONTAMINATED SOILS

Analyte	Soil	Contaminated Soil Nominal Concentration (ppm) 5,000 (%)	10,000 (%)
Ethylbenzene	Action	94	130
	Kaw	97	92
m-Xylene	Action	78	110
	Kaw	91	86
o- and *p*-Xylene	Action	77	120
	Kaw	98	94

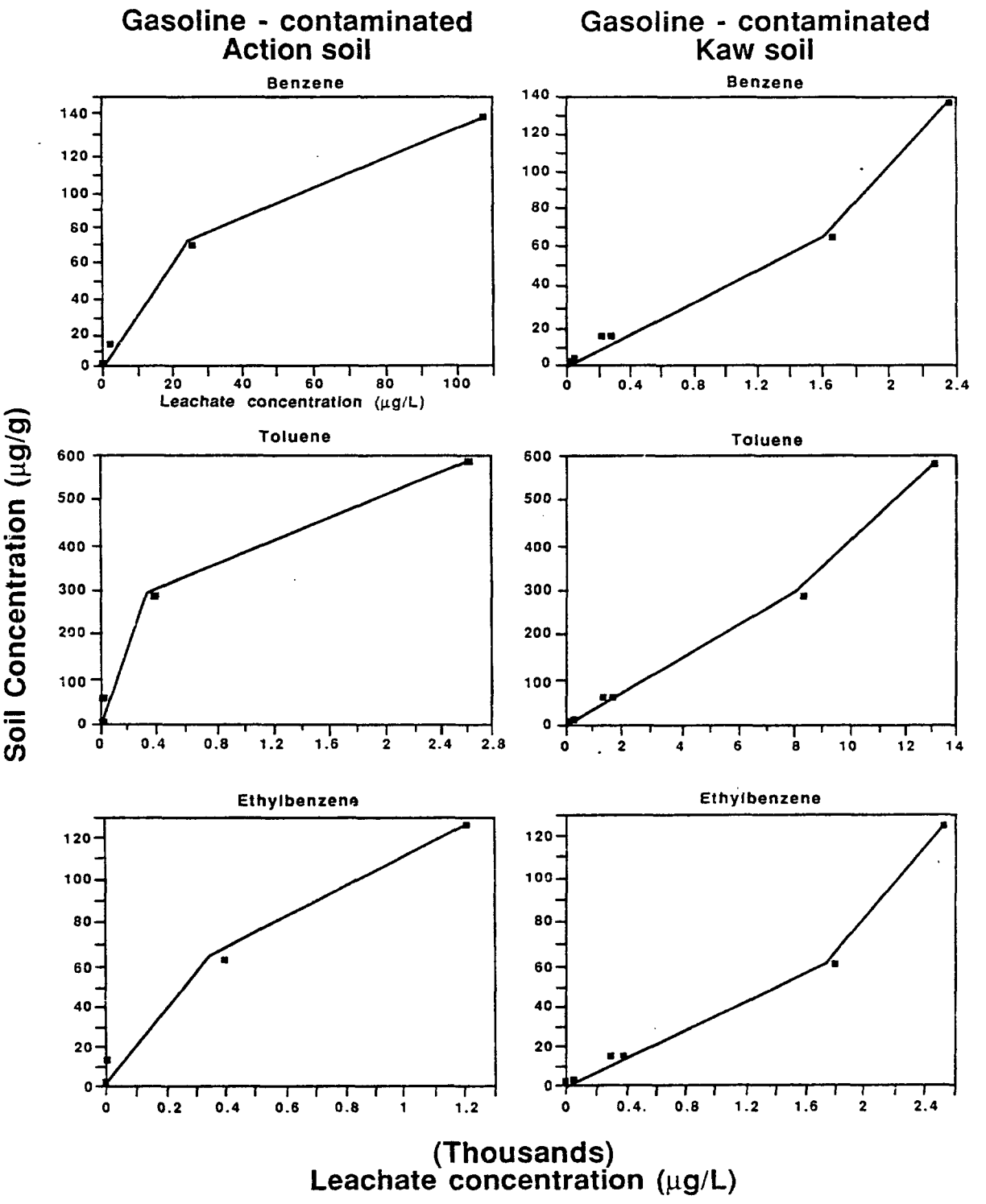

Figure 1. Isotherm plots for volatile organic compounds mobilized from gasoline-contaminated soils by TCLP.

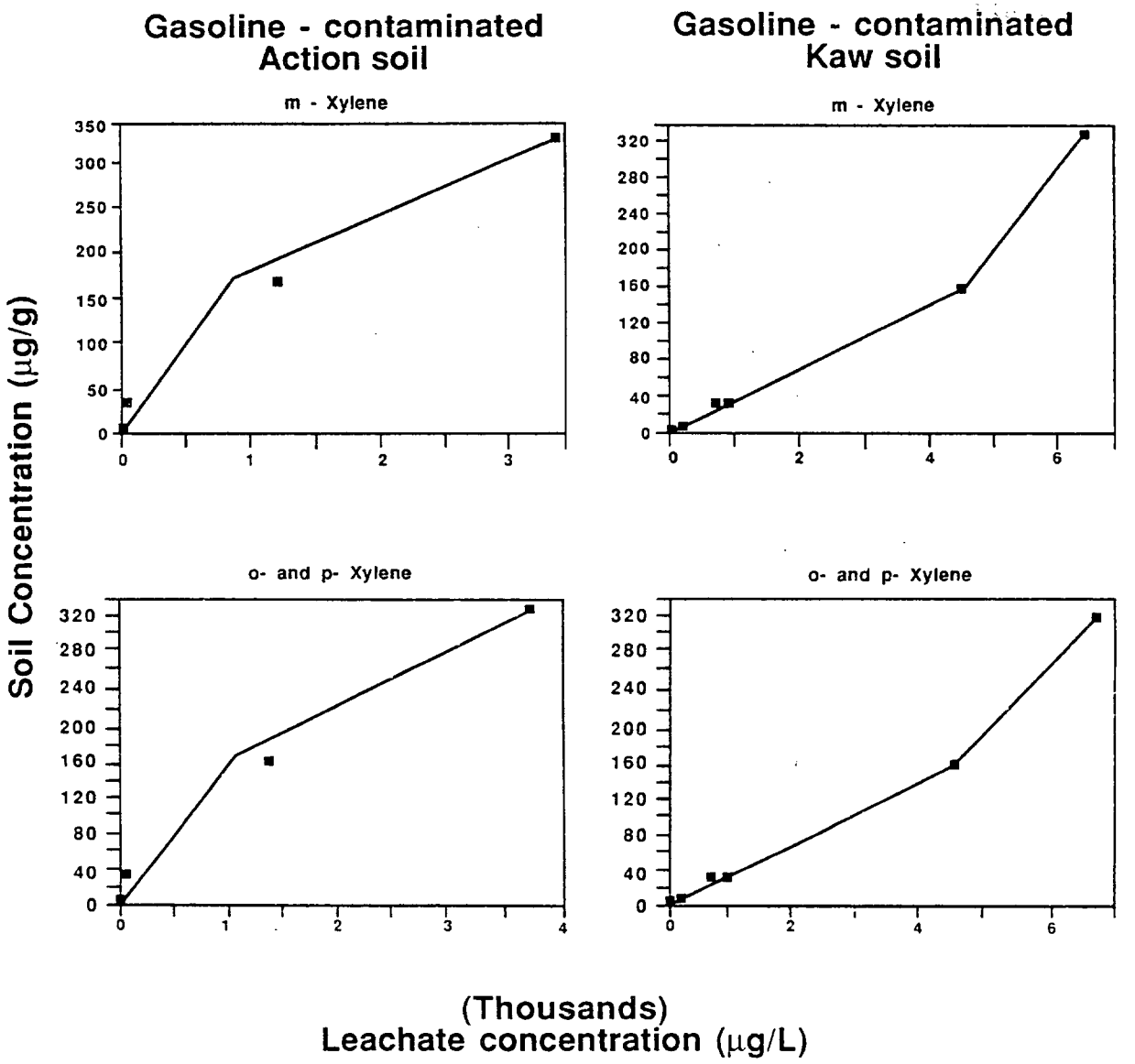

Figure 1 (continued)

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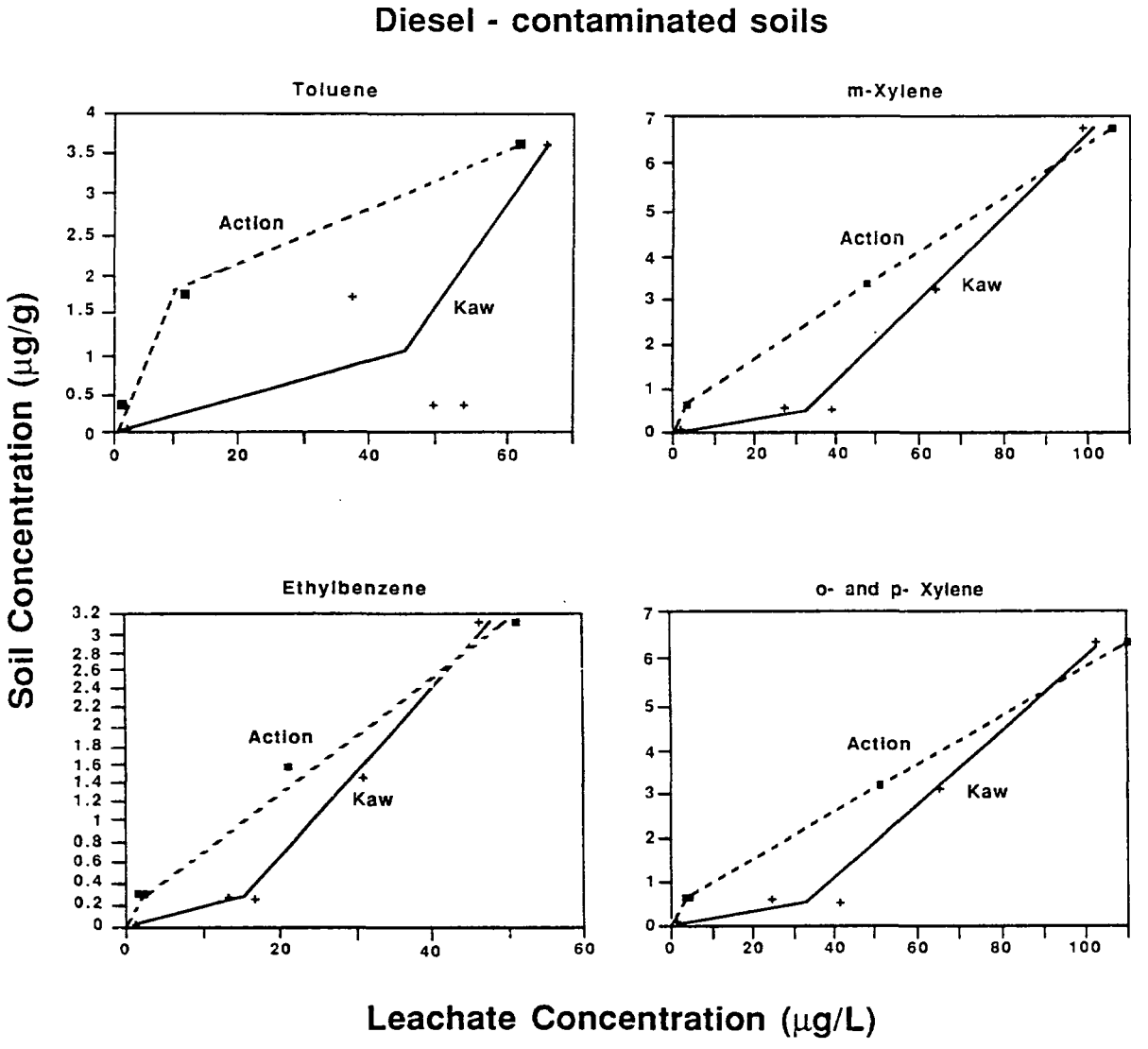

Figure 2. Isotherm plots for volatile organic compounds mobilized from diesel-contaminated soils by TCLP

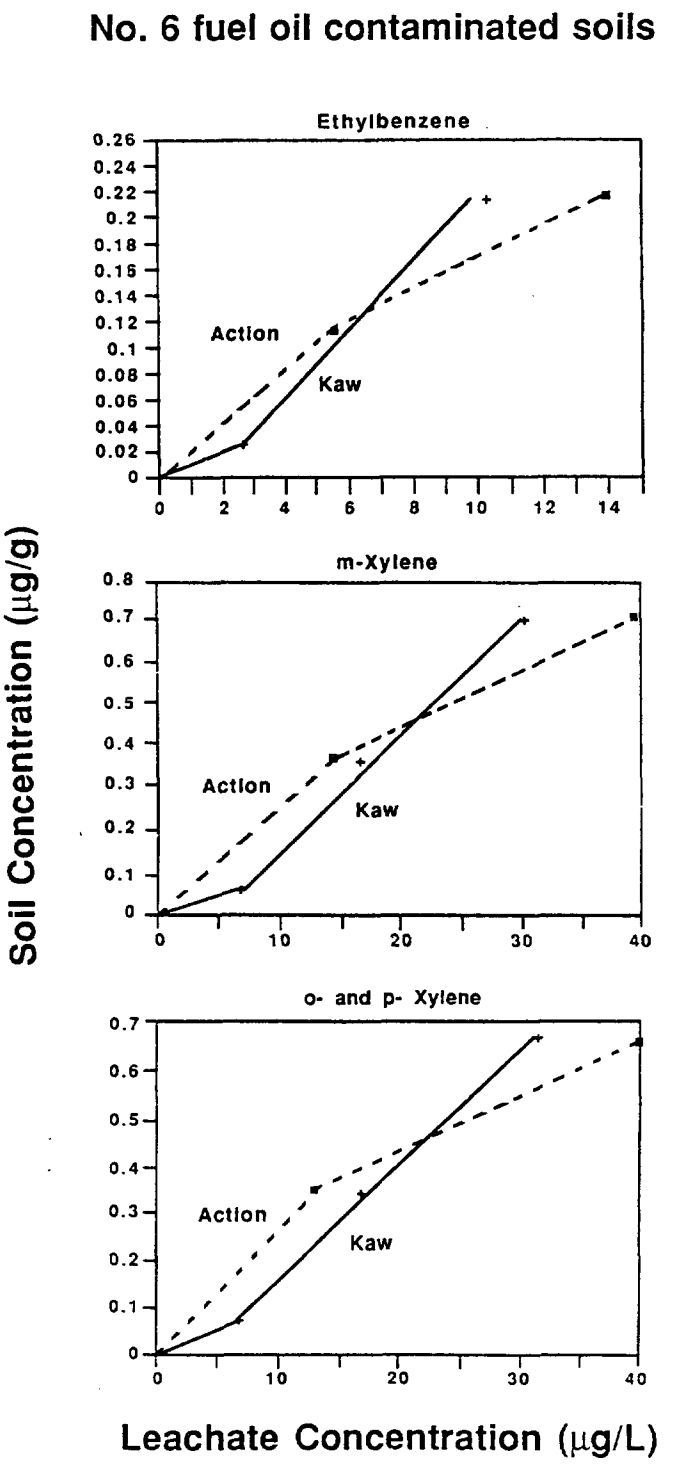

Figure 3. Isotherm plots for volatile organic compounds mobilized from No. 6 fuel oil-contaminated soils by TCLP

EVALUATION OF THE TCLP FOR DETERMINING THE RELEASE POTENTIAL OF OILY WASTES

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ABSTRACT

The Toxicity Characteristic Leaching Procedure (TCLP) was designed to model release of contaminants from a reasonable worst-case waste mismanagement scenario: codisposal of 5 percent industrial waste with 95 percent municipal refuse in an unlined sanitary landfill. The TCLP includes an initial filtration step that is intended to separate the fraction in a waste that will be released from the landfill as a separate liquid phase from the solid fraction that will remain behind and be leached in the landfill. During a previous work assignment, it was concluded that the TCLP, as currently proposed, is not suitable for certain oily wastes because filter clogging during the initial filtration step can result in over-estimation of the waste's percent solids content, thereby underestimating the release potential of the waste's liquid phase. Filtration step modifications investigated during this project to solve this problem included a Celite filter precoat, protective prefilters of Teflon felt or coarse glass fiber, and a sintered stainless steel filter. These filtration options were tested in triplicate using four oily wastes: API separator sludge, slop oil emulsion, creosote sludge, and waste motor oil. These wastes also were percolated through soil columns to determine the actual fraction of each waste that may be mobile in a landfill for comparison with the filtration results.

Of the four filtration modifications tested, the stainless steel filter was most successful in solving the filtration problems. The liquid portion of each waste passed easily through the steel filter in a reasonable amount of time, and it did not sorb or hold back waste components as did the Celite precoat. In addition, the waste solid fractions determined using the steel filters were very similar to the immobile fraction determinations in the column percolation studies, indicating that the steel filter is more accurate than the other filter alternatives tested. Stainless steel filters were therefore selected for further evaluation. Full TCLPs were conducted with four oily wastes to evaluate the single-laboratory precision of the modified procedure and to identify any procedural problems associated with testing oily wastes. Both TCLP test devices (extraction bottles and the ZHE) were evaluated. In addition, column leaching tests were conducted on the same four wastes to produce data on their leaching behavior for comparison with the TCLP results. These studies are necessary to show that the modified procedure is sufficiently precise and accurate to conduct a full collaborative study prior to proposal as a standard RCRA test method.

A FORMAL WAY TO MEASURE AND COMPARE WASTE COMPONENT MOBILITY. WASTES AND LEACHATES

A. D. Sauter, J. J. Downs, P. Marsden and L. R. Williams

The potential of toxic components from solid wastes to contaminate groundwater is of concern in a number of RCRA scenarios. Currently, various procedures such as the toxicity characteristic leaching procedure (TCLP) are employed to estimate this potential. In the TCLP, mobility is assessed by comparison of the concentrations of various waste components in the leachate to a set of published values. While "large" concentrations of a regulated material in the TCLP leachate can be clear evidence of the potential of a waste to contaminate ground water when improperly disposed, such evidence is less clear when leachate concentrations are of low to intermediate values. The relationships between wastes, waste components, and leachates from such wastes need better definition if the ultimate ability of a waste to contaminate ground water is to be accurately predicted. In this paper, a method is proposed that uses a slight modification of existing RCRA test procedures to classify the potentiial of a waste to contaminate ground water. The approach is based on simple, fundamental aspects of physical chemistry. It allows the behavior of waste components to be compared both in and among wastes and leachates that differ widely in physical and chemical characteristics.

NOTICE

Although the research described in this article has been supported by the United States Environmental Protection Agency, through contract 68-03-1958 to S-CUBED, 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.

IMPROVED ANALYSIS SCHEME FOR LEACHATES FROM HAZARDOUS WASTE LANDFILLS

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ABSTRACT

Science Applications International Corporation (SAIC) developed a rigorous analysis scheme for organics in hazardous waste landfill leachates, to support the development of a multi-compound hazardous waste synthetic leachate for landfill liner compatibility testing. Because a 1985 study using standard analytical methods identified only about 4 percent of the total organic carbon (TOC) content of leachates from 13 landfills, the more thorough scheme was developed and applied to fresh leachate from three of the same landfills.

The analytical scheme was designed to identify all organics, including very polar organics not usually detected by standard analyses. Higher molecular weight components (>500 daltons) were separated by gel filtration chromatography (GFC) and functional groups were characterized by ultravioletvisible spectrophotometry (UV-VIS), infrared spectrophotometry (IR), and carbon-13 nuclear magnetic resonance spectrometry (C-13 NMR). The <500 dalton base/neutrals were fractionated by column chromatography on silica gel into nonpolar, aromatic, and polar fractions to reduce interferences during subsequent gas chromatography/mass spectrometry (GC/MS) analysis. The aqueous fraction following solvent extraction was analyzed by GC on a water compatible column and also by high performance liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). TOC content was tracked throughout the analytical scheme to assure a reliable mass balance of the organic components of the leachates.

The major organic constituent of all three leachates was phthalic acid diamide; it was found in the aqueous fraction, which is not usually analyzed. From 9 to 52 percent of the TOC of the <500 daltons fraction was identified, a significant improvement over the previous standard analytical scheme.

The following categories of organics potentially damaging to landfill liners were found: organic bases (70 percent of the identified organic content), organic acids (23 percent), oxygenated and heteroatomic hydrocarbons (3.1 percent), aromatic hydrocarbons (2.3 percent), halogenated hydrocarbons (1.4 percent), and aliphatic hydrocarbons (much less than 1 percent).

The information in this document has been funded by the U.S. Environmental Protection Agency under Contract No. 68-01-7043, Work Assignments P-28, P1-3, P1-22, and P2-19, to Science Applications International Corporation. The document which this paper summarizes has been subject to the Agency's peer and administrative review, and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

INTRODUCTION

The Hazardous Waste Engineering Research Laboratory (HWERL) of the U.S. Environmental Protection Agency (USEPA) has been exploring the feasibility of formulating a synthetic hazardous waste leachate for testing containment liners for landfills and other hazardous waste storage, treatment, and disposal facilities. This project was to gather composition data on leachates from hazardous waste sites to use in assessing the feasibility of formulating a synthetic leachate from stock chemicals for liner testing.

A previous study characterized leachates from 13 hazardous waste landfills in 1985 was conducted by SAIC for HWERL.[1] The analyses, by EPA Methods 624 and 625, accounted for only about 4 percent of the organic fraction. Therefore, this follow-on study was initiated in 1986 to develop a more rigorous analytical method and analyze leachates from 3 of the original 13 facilities. A more thorough analytical procedure was developed by SAIC's Trace Environmental Chemistry Laboratory (TECL), San Diego, and used to characterize a fresh leachate sample from Site 8 of the original 13 sites. During the second phase of the project, minor improvements to the method were incorporated, and leachates from Sites 4 and 7 of the original study were characterized, including organic priority pollutants and also polar compounds not usually detected by standard procedures. Also, the method characterized higher molecular weight compounds by identification of functional groups. Total organic carbon (TOC) measurements conducted at critical steps allowed tracking the organic carbon mass, assignment of relative proportions of functional group classes, and estimation of the thoroughness of the analyses in organic compound identification.

The leachate analysis results were categorized by organic functional group class according to a scheme used frequently for reporting liner compatibility data. Representatives of six organic compound classes were recommended for a synthetic leachate formulation.

LEACHATE SAMPLING

Leachate sampling was performed by a team of two SAIC investigators experienced in proper field procedures and sampling techniques, and familiar with the characteristics of the landfill site. The sampling techniques and equipment were similar to those used for the 1985 study. The leachate samples were not chemically preserved.

Each sample was collected in three 1-gallon glass amber bottles with poly(tetrafluoroethylene) (PTFE)-lined caps, and at least three 40 mL glass volatile organic analysis (VOA) glass vials with PTFE-lined septa. The field blanks consisted of one 1-gallon glass amber bottle and one 40 mL VOA glass vial filled with double deionized (DDI) water. Leachate sample collection was performed in accordance with USEPA sample collection protocols. All sampling equipment was washed with a low residue detergent and rinsed with doubly deionized (DDI) water prior to each site visit. Each sample container was carefully packed in ice chests and maintained at 4° C with bagged or blue ice.

Four field tests, namely, temperature, pH, redox potential (Eh), and specific conductance, were performed on the leachate samples at the time of collection. Temperature, pH, and Eh were determined using a Hach Digital pH meter, Model 19000. Eh was determined using an Orion Research platinum redox

electrode, Model 97-78, in conjunction with the Hach meter. Specific conductance of the leachate samples was measured with a Hach Model 16300 Portable Conductivity Meter.

ANALYTICAL SCHEME

The analytical scheme that was developed is outlined in Figure 1. Initial characterization included screening tests for generic categories of substances likely to influence the liner compatibility of a leachate. These tests were made to provide other information on the chemical nature of the leachates: total organic carbon (TOC); total organic chloride (TOX); hydrogen ion concentration (pH); total (reduced) nitrogen content; methylene blue active substances (MBAS); and electrical conductivity (EC).

TOC measurements were made on the raw leachate and at various critical steps throughout the analytical process to track the mass balance at each step and to estimate the effectiveness of the analyses in identifying all of the organic compounds in the leachate. The TOX provides an estimate of the amount of chlorinated organics. The pH indicates the concentration of strong acids or strong alkalis which can affect landfill liner degradation rates. The reduced nitrogen content permits an assessment of the amount of unidentified amine compounds in the leachate. MBAS analysis allows for an estimation of the anionic surfactants or detergents which may not be chromatographable. The conductivity measurement provides an indication of the amount of dissolved, mostly inorganic, ions present in the leachate.

The next step was identification and quantitation of volatile compounds by gas chromatography/mass spectroscopy (GC/MS) purge and trap techniques. The purged samples were then subjected to a molecular weight (MW) fractionation using GFC. The >500 dalton fractions were analyzed for TOC and TOX for mass balance of those parameters, and by UV-VIS, IR, and C-13 NMR for functional group characterizations. After TOC analysis, the <500 dalton fractions were extracted (base and neutral), separated by silica gel chromatography of the extract into aliphatic, aromatic, and polar fractions, and subjected to GC/MS analysis. The aqueous remainder was then acidified and extracted. After GC/MS analysis, the extracts were derivatized with diazomethane and reanalyzed to verify compound identities. The aqueous residues after acidic extraction were analyzed by high performance liquid chromatography (HPLC), liquid chromatography/mass spectrometry/mass spectrometry (LS/MS/MS), aqueous compatible capillary column GC/MS and, finally, TOC. A detailed description of each step shown in Figure 1 follows.

Initial Leachate Characterization (Step A). TOC measurements were made using an O.I. Corporation Model 700 TOC analyzer, and followed the USEPA 600/4-79-020 Method 160.4.[2] TOX was measured on a Xertex/Dohrmann DX-20 Total Organic Halide Analyzer, following the USEPA Method 9020. The other nonspecific analyses were done following procedures outlined in Standard Methods for the Examination of Water and Wastewater.[3] The particular methods for each analyte are as follows: nitrogen by Method 420A; MBAS by Method 512B; EC by Method 205; and pH by Method 423, using an Altex 4500 pH meter with an Orion 8104 probe.

Volatiles Analysis (Step B). The raw leachates were analyzed by GC/MS for volatile organics according to USEPA Test Method 624, using internal and surrogate standards, and a 2.8 m x 2 mm glass column packed with one percent SP-1000 on Carbopak 3, 60/80 mesh. Compound identifications and quantitations

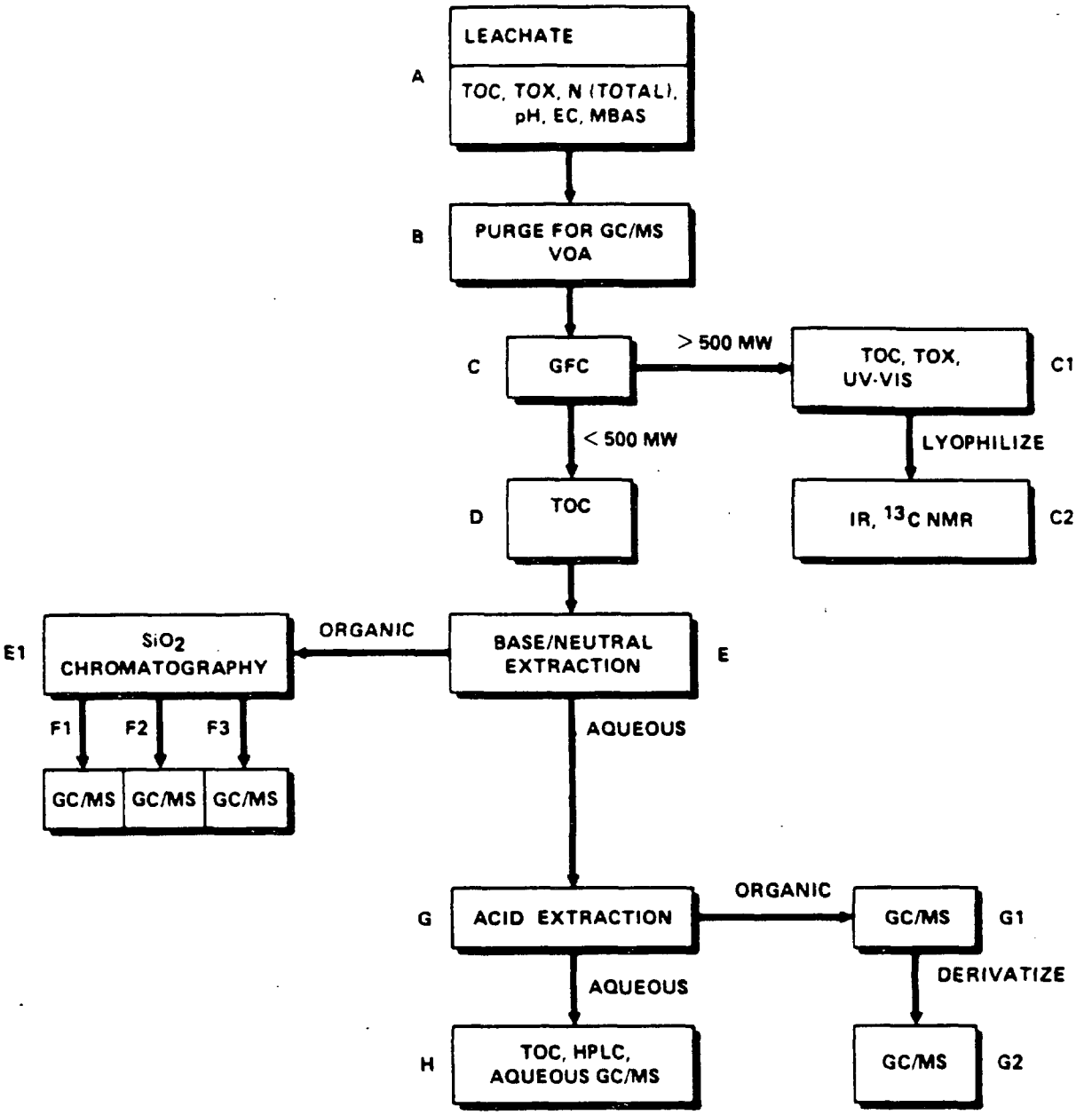

FIGURE 1. DIAGRAM OF ANALYTICAL STEPS

were done with a Finnegan 4021 quadrupole GC/MS equipped with a Nova 3 computer.

A 600 mL aliquot of each leachate sample was purged with ultrapure nitrogen for 24 hours at a rate of approximately 100 mL nitrogen/minute. Aliquots were taken and analyzed by GC/MS to assure total removal of volatile organic compounds. A 500 mL aliquot of the purged leachate was used in Step C for Site 8. Only 50 mL of purged leachate from Sites 4 and 7 were utilized in Step C in order to circumvent excessive time and logistical requirements.

Gel Filtration Chromatography (Step C). A molecular weight fractionation was completed using GFC with G-10-120 Sephadex. The GFC separations were calibrated by separation of compounds with known molecular weights, which were eluted and quantitated by GC/MS. Analyses by GC/MS for this step - as well as Steps E2, G1, and G2 - were accomplished on a Finnegan 4021 quadrupole GC/MS with a Nova 3 computer. The GC/MS analyses of 1 uL solvent extracts was carried out using a 30 mm x 0.32 mm ID DB5 fused silica capillary column directly inserted into the mass spectrometer ion source. A plot of molecular weight versus volume eluted was linear, indicating that all compounds >500 daltons were eluted in the first 34 mL, and all compounds were eluted in 142 mL. To fractionate 500 mL of sample, multiple columns were needed, with each column receiving multiple passes. For Site 8, 10 columns each received 5 loadings of 10 mL each. The dilution effects of GFC resulted in the volume increase of the <500 dalton fraction to 5.0 L, and the >500 dalton volume increased to 1.58 liters (L) from an initial volume of 0.48 L. For Sites 4 and 7, one column received five loadings of 10 mL each with a proportional volume increase due to dilution effects.

Because of the excessive TOC loss (29 percent) experienced for the Site 8 sample during the GFC step, a different gel was tested. An aliquot of Site 7 leachate was subjected to GFC techniques using LH-20 Sephadex.

High Molecular Weight Fraction Characterization and Low Molecular Weight Fraction TOC Characterization (Steps C1, C2, and D). Aliquots of the >500 dalton fraction were analyzed for TOC and TOX, in duplicate, with accompanying field blanks. Aliquots of the <500 dalton fraction were analyzed for TOC only. The analytical scheme originally called for MBAS analysis at Steps C1 and D; however, because MBAS were not detected in the Site 8 leachate during Step A, further MBAS analyses were eliminated. Furthermore, limited sample volumes precluded analyses for TOX and MBAS for Site 7 and 4 at Step C1.

The >500 dalton fractions were also scanned on a Hitachi 100-80 UV-VIS spectrophotometer (with recorder) from 800 nm to 900 nm. The scan was conducted with a distilled water blank in the reference cell using matching quartz cuvettes.

The >500 dalton fraction was lyophilized (freeze-dried) by placing approximately 75 mL of sample at a time in a 500 mL roundbottom flask fitted with a ground glass adapter. Vacuum tubing connected the flask and adapter to a trap which was placed in a dewar containing liquid nitrogen. A vacuum was applied to the system using a Welch Duo-Seal vacuum pump. The complete contents of the fraction were lyophilized in this fashion and the resulting solid was quantitatively recovered.

A small quantity of the solid lyophilisate was pressed into a potassium bromide (KBr) pellet, and subjected to IR using a Perkin-Elmer Fourier Transform

IR connected to a Perkin-Elmer 700 Series computer. Scans were from 4000 cm^{-1} to 600 cm^{-1} and referenced against air.

The solid was also subjected to C-13 NMR spectrometry, using a GE 300 series model. The samples were dissolved in deuterated water (D_2O), spiked with dioxane (as an internal standard) and run at a 45 degree pulse to allow for relaxation.

Extraction of Base/Neutral Fractions (Step E) and Acid Fraction (Step G). After TOC analysis, the entire <500 dalton fraction was extracted with methylene chloride at pH 7, then pH >12, and then at pH <1 (adjusted with sodium hydroxide).

Silica Gel Chromatography and GC/MS of Base Neutrals (Steps E1, F1, F2, and F3). The base and neutral extracts were then combined, and reduced to <2 mL over a hot water bath using a Snyder column, solvent exchanged into hexane, and reduced in volume in a concentrator tube to <1 mL. The reduced extracts were then subjected to silica gel chromatography, using a column of 10 mm I.D. by 240 mm with a 200 mL reservoir. Three fractions were collected (aliphatic, aromatic, and polar) corresponding to elution by: (1) 50 mL of pentane, (2) 200 mL of 1:1 pentane:-methylene chloride, and (3) 50 mL of methylene chloride. Each fraction's volume was reduced to approximately 2 mL, transferred to a crimp top vial, reduced further under a slow stream of nitrogen to a 500 uL final volume, and analyzed by GC/MS according to the procedure presented in Step C.

Characterization of the Acidic Semivolatiles Fraction (Steps G1 and G2). The acid extracts were reduced to 500 uL by the same methods, and screened by GC/MS for compound identification and quantification. After initial screening and compound identification, the extracts were derivatized with diazomethane or diazoethane to convert carboxylic acids to esters and alcohols to ethers. Diazomethane (CH_2N_2) (or diazoethane, for Site 4 and 7 samples) for the reaction was produced according to the method of Fales, Jaouni, and Babashak.[4] This method is of considerably lower hazard than most conventional methods, while producing CH_2N_2 in good yield. Once derivatized, the sample extracts were reanalyzed by GC/MS.

Aqueous GC/MS and LC/MS/MS (Step H). After acid extraction, the aqueous remainders were separated into aliquots for TOC, HPLC, LC/MS/MS, and aqueous GC/MS techniques. Aqueous GC/MS analysis was conducted using the methods described in Step C, with a J&W Scientific Carbowax Fused Silica capillary column instead of the DB5 fused silica column. HPLC was done using a Waters Association Model 244 LC with a R401 Refractive phase; therefore, runs were done with 100 percent methanol; 75 percent methanol and 25 percent H_2O; 25 percent methanol and 75 percent H_2O; and 100 percent H_2O. All runs utilized a C-18 reverse phase column.

Aqueous extraction residues were analyzed by Thermospray(R) ionization LC/MS by Oneida Research Services (ORS) to identify those species present. When those species were tentatively identified, standards were prepared and analyzed in order to quantitate the amounts present in each sample. Both positive and negative ion detection were utilized. Twenty-five uL injections of the water samples (neat) were made, and a mobile phase blank sample prepared at ORS was run. Instrumental settings were as follows:

Waters 600 Multisolvent Delivery System: 0.1M NH_4OAc in H_2O (mobile phase); 2.0

mL/min flow (MS); 1.5 mL/min flow (MS/MS); 4.6 x 250 mm ODS Phenomex LC column (5u); ambient LC oven temperature; 25 uL injection volume; U6K injector

Vestec Model 45 Thermospray(R) Interface: T_2 (probe tip) at 269° C; T_3 (vapor) at 257° C; T_5 (block) at 296° C

Finnigan 4600 TSQ: positive ions - Thermospray(R) ionization; negative ions-filament on; m/z 90-650, every 1.5 seconds (MS mode); m/z 10-280, every 0.5 seconds (MS mode); collision gas - Ar at 1.5 mtorr; collision energy - -4.0 to-29.5 V (manually operated)

RESULTS AND DISCUSSION

The results of the initial physical characteristics measurements and nonspecific analyses performed in the field and at the SAIC TECL are presented below in Table 1.

TABLE 1. INITIAL LEACHATE CHARACTERIZATION RESULTS

Analyte	Units	Site 4*	Site 7*	Site 8*
pH, field:	--	7.2	8.2	4.3
laboratory:	--	9.1*	8.1*	4.3*
Conductivity, field:	umhos/cm	>20,000	13,500	19,400
laboratory:	umhos/cm	10,500*	82,500*	19,500*
Eh, field:	mV	-329	-249	148
Nitrogen, total reduced:	mg/l	8.50*	8.15*	635*
MBAS	mg/l	2.1*	<0.1	<0.01
TOC	mg/l	1,400*	1,320*	16,000*
TOX**	mg Cl/L	5.4*	28.6*	166*

* Mean of duplicate tests.
** Total organic chloride.

The leachate from Site 4 was near neutral and became, like Site 7, moderately alkaline, possible due to reactions occurring in the leachate following sampling. Site 8 leachate was moderately acidic. The elevated conductivities of all the samples indicate significant amounts of electrolytes (salts) in the leachates. No apparent reason exists for the discrepancies between field and laboratory conductivity measurements. The field Eh (redox) measurements show that the leachates from Site 4 and 7 were slightly reducing, and the Site 8 leachate was very slightly oxidizing. The reduced forms of nitrogen were low in the Site 4 and Site 7 leachate, but the substantial level in Site 8 leachate (635 mg/L) suggested significant amounts of organic amines. Only Site 4 had any methylene blue active substances (usually anionic surfactants or detergents), and the level was quite low. The TOX analyses showed relatively small amounts of chlorinated organics in Site 4 and 7 leachates, and a substantial amount in the Site 8 leachate.

The TOC of the leachates was determined at different steps in the analytical scheme to track the progress toward identification of all of the organic constituents, and to maintain a material balance estimate through- out the sample fractionation process. Table 2 summarizes the results.

TABLE 2. TOTAL ORGANIC CARBON CONTENTS OF THE LEACHATE FRACTIONS

TOC Result	Site 4	Site 7	Site 8
Initial (Baseline) TOC (mg/l):	1,400	1,320	16,000
Percent Distribution of TOC by Molecular Weight,			
>500 Daltons Fraction:	79%	70%	39%
<500 Daltons Fraction:	27%	19%	32%
Left on GFC Column (by difference):	0%	11%	29%
Percent of Initial TOC Attributed to Specific Compounds:	14%	5.9%	2.8%
Percent of Initial TOC Not Attributed to Specific Compounds:	86%	94.1%	97.2%

Organic Constituents of the Leachates

The specific compounds detected by this study are presented in Table 3. Of the 58 compounds identified in the 1985 study and the 68 compounds identified in the current study, 37 were found at the same site in both studies.

Table 3 contains the concentrations for each organic compound identified in the three leachates in ug/L, and in terms of the theoretical organic carbon content of each compound (ug C/L), calculated from the concentration, molecular weight, and carbon weight of the compound, which facilitates mass balance comparisons between sites and between the 1985 and current studies.

Results of Spectral Analyses of the >500 Dalton Fraction

The proton NMR, C-13 NMR, and Fourier Transform infrared (FTIR) analyses results for the lyophilized GFC fraction of >500 daltons (Step C2) are summarized in the following paragraphs.

The Site 4 fraction contained a high inorganic salt concentration, which caused the sample to only partially dissolve. Methyl group portion peaks were present at a shift of 0.3 delta unit, and a peak at a shift of 3.2 delta units was probably protons on a carbon deshielded by heteroatom-containing functional groups. The peaks show a predominantly aliphatic character for the fraction, and suggest an oxygenated hydrocarbon such as an ether or ester. No organic carbon-13 was detected.

A high inorganic salt concentration was also apparent in the Site 7 high molecular weight fraction, limiting solution of the sample. Methyl peaks were observed at about 0.3 delta unit; a deshielded proton peak was seen at 2.3 delta units; and an aromatic proton multiplet was present at about 7.7 delta units. The spectrum suggests oxygenated aliphatic and aromatic hydrocarbons. No organic carbon-13 was detected.

The proton NMR of the Site 8 fraction showed proton resonance only at 7.1 delta units, indicating that the constituents were mainly aromatic hydrocarbons. A substantial absorption for HDO (mono-protonated deuterated water) suggested the

TABLE 3. CONCENTRATION OF ORGANIC COMPOUNDS IDENTIFIED IN THE THREE LEACHATE SAMPLES

Compound	Site 4		Site 7		Site 8	
	ug/L	ug C/L	ug/L	ug C/L	ug/L	ug C/L
VOLATILE ORGANIC COMPOUNDS--STEP B						
Methylene chloride	22800	3230	388	54.9	4.53	0.64
Acetone	3400	2110	5440	3370	66.7	41.3
Dichloroethylene, 1,2-	59.4	14.7	23.3	5.77	2.42	0.60
Vinyl acetate	<1	<1	<1	<1	114	63.8
Trichloroethylene	39.4	7.19	75.0	13.7	1.64	0.30
Benzene	174	160	204	188	2.06	1.90
Methyl n-butyl ketone	<2	<2	<2	<2	8.15	5.86
Methyl isobutyl ketone	1750	1260	341	245	1.29	0.93
Toluene	10300	9370	593	541	141	129
Ethylbenzene	408	369	22.9	20.7	1.84	1.66
Diethyl ether	<2	<2	<2	<2	26.9	17.4
Isopropyl alcohol	<2	<2	<2	<2	8.38	5.02
n-Butyl alcohol	<2	<2	<2	<2	41.7	27.0
Pentane	<1	<1	<1	<1	8.78	7.30
Pentanol, 2,2-dimethyl-1-	<2	<2	<2	<2	4.81	3.48
Xylene, o-	<1	<1	<1	<1	4.73	4.24
Unknown(Base m/e=57)	<2	<2	<2	<2	90.4	60.2
Dichloroethane, 1,1-	1070	260	37.2	9.02	<1	<1
Methyl ethyl ketone	3880	2580	195	130	<2	<2
Chlorobenzene	29.7	19.0	1450	925	<1	<1
Xylenes, m- & p-	1350	1210	23.1	20.7	<1	<1
Unknown(Base m/e=36)	254	169	<2	<2	<2	<2
Unknown(Base m/e=68)	74.1	49.4	<2	<2	<2	<2
Methanethiol	2720	678	<2	<2	<2	<2
Methyl sulfide	973	376	<2	<2	<2	<2
Unknown(Base m/e=43)	198	132	<2	<2	<2	<2
Dimethylpropionitrile, 2,2-	18.0	13.0	<2	<2	<2	<2
Dimethyldisulfide	64.4	16.4	<2	<2	<2	<2
Butanone, 3,3-dimethyl-2-,	109	78.4	<2	<2	<2	<2

< = the concentration of the compound is less than its detection limit. (continued)
the value is the detection limit of the compound.

* = the compound was quantified at a concentration below its detection limit (5 ug/L).

ug/L = micrograms compound per liter

ug C/L = micrograms carbon per liter

TABLE 3. (Cont.)

Compound	Site 4		Site 7		Site 8	
	ug/L	ug C/L	ug/L	ug C/L	ug/L	ug C/L
VOLATILE ORGANIC COMPOUNDS--STEP B (continued)						
Vinyl chloride	<1	<1	87.2	33.5	<1	<1
Chloroform	<1	<1	839	84.3	<1	<1
Tetrahydrofuran	<2	<2	197	131	<2	<2
Dichlorobenzene	<1	<1	1210	593	<1	<1
Trifluoroethane, 1,2-dichloro-1,1,2-	<1	<1	150	21.0	<1	<1
TOTAL		22102.09		6386.59		370.63
BASE/NEUTRAL EXTRACTABLES--STEP E						
n-Alkanes	<5	<5	<5	<5	52.6	44.4
Methylphenol, 2,6-di-t-butyl-4-	<10	<10	<10	<10	18.7	15.3
Unknown(Base m/e=69)	<10	<10	<10	<10	30.3	20.2
Unknown(Base m/e=180)	<10	<10	<10	<10	32.1	21.4
Phenol	190	145	<10	<10	13800	10600
Pentanediol, 2,2,4-trimethyl-1,3-	<10	<10	<10	<10	1740	1140
Methylphenol, 2-	21.5	16.7	<10	<10	18.5	14.4
Diethylphthalate	<10	<10	<10	<10	152	98.2
Bis(2-ethylhexyl)phthalate	28.1	20.7	<10	<10	86.7	63.9
Methylphenol, 4-	14.5	11.3	<10	<10	<10	<10
Methylphenol, 4-chloro-3-	1200	708	194	114	<10	<10
Toluene, 2,4-dinitro-	128	54.9	158	67.8	<10	<10
Pentachlorophenol	<10	<10	94.7	25.6	<10	<10
Phenol, 2-chloro	<10	<10	350	196	<10	<10
Chloroaniline, 4-	<10	<10	232	131	<10	<10
TOTAL		956.6		534.4		12017.8

(continued)

< = The concentration of the compound is less than its detection limit. The value is the detection limit of the compound.

* = The compound was quantified at a concentration below its detection limit (5 ug/L).

ug/L = micrograms compound per liter

ug C/L = micrograms carbon per liter

TABLE 3. (Cont.)

Compound	Site 4		Site 7		Site 8	
	ug/L	ug C/L	ug/L	ug C/L	ug/L	ug C/L
ACID EXTRACTABLES--STEP G						
Phenol	30.2	23.1	64.3	49.2	8720	6670
Benzoic acid	<10	<10	<10	<10	51500	35400
Pentanoic acid	<10	<10	<10	<10	1800	1060
Hexanoic acid	<10	<10	99.4	61.6	1480	919
Phthalic acid	<10	<10	<10	<10	223	129
Benzoic acid, 2-hydroxy-	<10	<10	<10	<10	92.7	56.4
Pentanediol, 2,2,4-trimethyl-1,3-	<10	<10	91.1	59.8	238	156
Phenol, 4-nitro-	193	100	253	131	<10	<10
Phenylpropanoic acid	<10	<10	107	76.8	<10	<10
Propenoic acid, 3-phenyl-2-	<10	<10	36.8	26.8	<10	<10
Propanediol, 2,2-dimethyl-1,3-	35.1	20.2	<10	<10	<10	<10
Unknown(Base m/e=97)	39.2	26.1	<10	<10	<10	<10
Benzoic acid, 4-methyl	14.7	10.4	<10	<10	<10	<10
Benzenesulfonamide, 4-methyl-	21.2	10.4	<10	<10	<10	<10
TOTAL		190.2		405.2		44390.4
DERIVATIZATION PRODUCTS FROM THE ACIDIC EXTRACTION--STEP G2						
Benzoic acid, methyl ester	<10	<5	<10	<5	87.5	61.7
Phenylacetic acid, methyl ester	<10	<5	<10	<5	49.2	35.4
Benzoic acid, 2-hydroxy-, methyl ester	<10	<5	<10	<5	742	468
Phthalic acid	<10	<5	<10	<5	211	122
Phthalic acid, butyl methyl ester	<10	<5	<10	<5	16.0	10.6
Tetradecanoic acid, 3-hydroxy-, methyl ester	<10	<5	<10	<5	12.6	8.8
Phthalic acid, dimethyl ester	<10	<5	<10	<5	30.9	19.1
Hexanoic acid	<10	<5	<10	<5	78.8	48.8
Hexanoic acid, methyl ester	<10	<5	<10	<5	5.70	3.7 *
Phenylacetic acid	<10	<5	<10	<5	80.0	56.4
Benzoic acid, 3-methyl-	<10	<5	<10	<5	12.8	9.0

(continued)

< = The concentration of the compound is less than its detection limit. The value is the detection limit of the compound.

* = The compound was quantified at a concentration below its detection limit (5 ug/L).

ug/L = micrograms compound per liter

ug C/L = micrograms carbon per liter

TABLE 3. (Cont.)

Compound	Site 4		Site 7		Site 8	
	ug/L	ug C/L	ug/L	ug C/L	ug/L	ug C/L
DERIVATIZATION PRODUCTS FROM THE ACIDIC EXTRACTION--						
STEP G2 (continued)						
Benzoic acid, 3-hydroxy-, methyl ester	<10	<5	<10	<5	7.70	4.9
Nonanoic acid, 7-methyl-, methyl ester	<10	<5	<10	<5	9.00	6.4
Hexadecanoic acid	<10	<5	<10	<5	37.0	27.7
Anthraquinone	<10	<5	<10	<5	59.2	47.8
Benzoic acid	<10	<5	<10	<5	9260	6370
Phenol	121	92.6	54.3	41.5	4240	3240
Propanediol, 2,2-dimethyl-1,3-	39.1	22.5	<10	<5	<10	<5
Pentanediol, 2,2,4-trimethyl-1,3-	21.2	13.9	85.7	56.3	<10	<5
Chlorobenzoic acid, ethyl ester	7.80	4.6 *	10.4	6.1	<10	<5
Phenol, 4-nitro-	<10	<5	128	66.3	<10	<5
Hexanoic acid, 2-ethyl	<10	<5	141	94.0	<10	<5
Phenylpropanoic acid	<10	<5	30.1	21.6	<10	<5
Phenylpropenoic acid, ethyl ester	<10	<5	11.6	8.7	<10	<5
TOTAL		133.5898		294.4798		10537.33
AQUEOUS GC/MS ANALYSES--STEP H						
Benzoic acid	<0.05	<0.05	<0.05	<0.05	331000	228000
Phenol	<0.05	<0.05	<0.05	<0.05	38700	29600
Unknown(Base m/e=36)	<0.05	<0.05	<0.05	<0.05	1200	800
Propanol, 2-phenyl-2-	0.137	0.109	0.100	0.079	<0.05	<0.05
TOTAL		0.109		0.079		258400
LC/MS/MS--STEP H2						
Phthalic acid diamide	290,000	170,000	120,000	70,000	240,000	140,000
TOTAL		170,000		70,000		140,000

< = The concentration of the compound is less than its detection limit.
The value is the detection limit of the compound.

* = The compound was quantified at a concentration below its detection limit (5 ug/L).

ug/L = micrograms compound per liter

ug C/L = micrograms carbon per liter

presence of exchangeable protons such as found in alcohols, phenols, or amines. The carbon-13 spectrum confirms the aromatic character of the constituents, with peaks at shifts of 130.2 and 139.7. The FTIR spectrum showed alcohol or amine absorbance (possibly moisture in the sample), and fairly strong peaks characteristic of aromatic hydrocarbons.

Results of LC/MS/MS Analyses

In the aqueous sample from Site 8, three LC peaks were observed. A species of 166 daltons showed by MS a protonated form (m/z 167), confirmed by an ammonium adduct at m/z 184, a sodium adduct at m/z 189, and by negative ion detection. The second peak appeared to be a mixed spectrum of co-eluting species, the major species being 164 daltons. The third peak also gave ions at m/z 167, 184, and 189, suggesting that it was an isomer of the first peak.

Two LC peaks were observed for the Site 4 aqueous fraction. One peak resembled the 164 daltons species in the sample from Site 8. The second very sharp peak eluting at 6.0 min gave a protonated molecular ion at m/z 105 and an ammonium adduct at m/z 122. The sodium and potassium adducts were also observed at m/z 127 and 143, respectively. Negative ion detection confirmed the 164 daltons species and gave an ion (m/z 185) which could substantiate the species. The aqueous sample for Site 7 gave a LC/MS trace very similar to the sample for Site 4.

To identify the species found in the three water leachate samples, direct injection (no column) MS/MS daughter ion experiments, both positive and negative ion detection, were performed on selected ions that possessed appreciable parent ion currents. The 166 daltons species appeared to be phthalic acid. The ion seen at m/z 165 (164 daltons) also possessed some phthalate type structure and was proposed to be a diamide of phthalic acid. The structure of other ions observed was uncertain, partly because of extremely low daughter ion currents. Analytical standards were then run, and the phthalic acid and phthalamide daughter ion spectra matched those found for the samples. Semiquantitative concentrations were determined using external standards. A six ion selected ion monitoring (SIM) descriptor was used to obtain signals for the samples and the prepared analytical standards. Using this descriptor aided the overall minimum detection limit, as well as the accuracy of the measurement.

Extreme differences in molecular ion adducts (most notably the sodium adduct) can cause quantitative errors as a result of preferences in adduct formation due to sample matrix differences (i.e., presence or absence of sodium). When the results were compared with the TOC results for the aqueous fractions, it was apparent that the calculated results required correction for differences in the salt content of the standards and the samples. For Sites 4 and 7, the measurements were corrected based on a phthalic acid amide spike recovery result. For Site 8, the phthalamide spike hydrolyzed to phthalic acid during analysis. This led to the measurement of the sample pH (about 2), and to the need to correct the calculations for differences in ratios of molecular ion adducts, and to report the results for the acid and amide together as just phthalic acid amide. The acid was assumed to have been formed entirely by hydrolysis due to the low pH of the sample solution. The results of the LC/MS/MS analyses are included in Table 3.

Effectiveness of the Analysis Scheme

Certain leachate fractionation and analysis steps were performed in the present study that were not performed in the 1985 study, to allow more complete identification of the constituents of the leachates. Although some comparisons can be made between the results of the 1985 study and the present study, the almost 2-year lapse between sampling for the studies limits the comparability of the leachate sampled from any site.

GFC was performed on each leachate after the volatiles were purged, to remove high molecular weight compounds, such as natural or synthetic polymers, which might interfere with subsequent separations and analyses, especially of the base/neutral and acid extractables. The GFC may have contributed to the overall increase in compounds identified by decreasing the interference of high molecular weight compounds with fractionations and analyses, but the expected improvement in the number of base/neutral and acid extractables was not observed.

GFC of the Sites 7 and 8 leachates resulted in a significant loss of organic carbon on the Sephadex G-10-120 column. The LH-20 Sephadex GFC was much better: all eight fractions contained some organics, and the total recovery of organic carbon was 102 percent.

Silica gel column chromatography of the base/neutral extractables did not increase the number of extractables identified in the present study over the 1985 study.

The acid fraction was derivatized with diazomethane or diazoethane, because the esters which form are much more easily separated by GC than the corresponding acids, which tend to tail badly or not form distinguishable peaks upon GC. The acid fraction derivatization significantly increased the number of acidic compounds identified in the present study. While 11 acidic extractables were identified by standard analytical procedures, 10 additional acidic compounds or their esters were identified following the azoalkane treatment of the acid extractables fraction.

GC/MS was performed using a column compatible with water as an injection solvent, to detect compounds which were so polar and water soluble that they would not have been extracted or detected. Additionally, LC/MS/MS was also performed on the aqueous fraction, to identify compounds not identifiable by GC/MS. The analysis performed on the final aqueous fraction of the leachates yielded only one added compound for each step, but most of the organic carbon identified for the three sites was identified by these two steps. Of the 14 percent of organic carbon identified in the Site 4 leachate, 12 percentage points of that was attributable to the LC/MS/MS analysis step. Of the 5.9 percent of organic carbon identified in the Site 7 leachate, 5.3 percentage points was due to the phthalic acid diamide identified in the LC/MS/MS step. Of a total of 2.8 percent of organic carbon identified in the Site 8 leachate, 1.4 percentage points was attributable to the aqueous GC/MS step, and 0.9 percentage points was attributable to the LC/MS/MS step. The aqueous fraction GC/MS and the LC/MS/MS steps clearly improved the leachate characterization.

Leachate Constituents by Functional Group Class

Leachate constituents belonging to each of six general classes of organics

which attack landfill liners were identified in the leachates. The six classes, in an order approximating their tendency to damage polymers used for liners, from least damaging to most damaging, are as follows: (1) organic bases, (2) organic acids, (3) oxygenated/heteroatomic hydrocarbons, (4) aliphatic hydrocarbons, (5) halogenated hydrocarbons, and (6) aromatic hydrocarbons. Of the 68 compounds identified, 44 were found at only one of the sites, and only 11 compounds were found at all three sites. Phthalic acid diamide was the dominant compound, representing 88 and 90 percent of the identified organic carbon in the leachates from Sites 4 and 7, respectively. Both Sites 4 and 7 had relatively little organic acid compounds, while the Site 8 proportion of identified organic carbon in the form of organic acids was 69 percent. The highest proportion of the organic acids was benzoic acid, present at a mean of 19 percent of identified organic carbon.

All three of the sites had a substantial proportion of phthalic acid derivatives. Almost all of the phthalic acid was in the diamide form, plus some of the free acid and three different phthalate esters were found in the Site 8 leachate. The totals of phthalic acid derivatives for Sites 4, 7, and 8 were 88, 90, and 31 percent of the identified organic carbon, respectively. Phthalate esters are common industrial chemicals, but the presence of phthalic acid diamide rather than phthalate esters must be explained. Higher concentrations of phthalate esters may have been present initially in the leachates, but a substantial concentration of ammonia may have been present. The alkaline pH of the Site 4 and Site 7 leachate samples is consistent with this hypothesis. The ammonia would probably react with the phthalate esters to form phthalic acid diamide. This hypothesis is also consistent with the observation that very little of the esters are present in those leachate samples containing the highest proportion of the diamide. Phthalate esters were nearly absent in the Site 4 leachate; no phthalates were found at Site 7. The Site 8 leachate, with the smallest proportion of the diamide, also had phthalic acid and three phthalate esters.

CONCLUSIONS

A substantial proportion of the organic carbon content of all three hazardous waste leachates was derived from phthalic acid. The dominant compound by far was a weak organic base, phthalic acid diamide, which may have been formed from ammonia and phthalic acid or from ammonia and phthalate esters through reactions occurring in the landfill or in the leachate collection system. The second most dominant category of compounds was the organic acids. The large proportion of carboxylic acids and phenols (an average of 23 percent of the identified organic carbon with molecular weight <500 daltons) may also have been caused by reactions in the landfill or leachate. Either chemical oxidation or oxidative microbiological degradation of the landfill contents, or both, are strongly suggested by the abundance of acidic compounds, including short chain fatty acids.

The more careful analysis of the leachates led to a more thorough assessment of their chemical content than in the 1985 study. Ten more volatiles were identified than in the previous study, but since no change was made to the volatiles analysis step, the increase is likely due to actual changes in the leachate compositions since 1985. However, analysis of a fraction not usually characterized in standard environmental sample procedures, namely the aqueous residue from base/neutral and acid extractions, led to identification of 31 to 91

percent of the characterized organic carbon. (It was in this fraction that the large proportion of phthalate acid diamide was found for all three sites.)

The analytical methods that permitted the more complete analysis of the aqueous fraction included: (1) direct aqueous injection GC/MS and (2) LC/MS/MS using the Thermospray(R) technique. The major compounds identified and quantified by these techniques were too polar and hydrophilic to be extracted efficiently from water. These compounds would have gone undetected by analytical methods usually applied to hazardous wastes.

Another method used to enhance the analysis of the polar, hard to chromatograph, compounds was the diazoalkane derivatization of the acid extractable compounds to form methyl (Site 8) or ethyl (Sites 4 and 7) esters. This added step allowed the identification of 10 compounds that had not been detected in the fraction before derivatization. Again, the polar nature of the compounds was the problem; the GC separation of organic acids is often poor because of the tailing associated with compounds containing carboxylic acid (-COOH) groups.

Two methods for molecular weight fractionation by GFC were tested, using two different Sephadex gels. The first method, using G-10-120 Sephadex, caused irreversible material loss from two of the leachates. The second gel, LH-20 Sephadex, produced 102 percent of material recovery when pilot tested with the Site 7 leachate.

The major conclusions from results of the leachate analyses are as follows. The organic content of the leachates is 0.1 to 1.6 percent by weight. Only 19 to 32 percent of the organic carbon in the leachates has a low enough molecular weight (<500 daltons) to be considered solvent type compounds likely to cause damage to landfill liners. Of the low molecular weight fraction, about 9 to 52 percent could be identified by the analytical methods, including direct aqueous injection GC/MS and LC/MS/MS. From 2.8 to 24 percent of the initial, or baseline, total organic carbon (TOC) could be assigned to specific organic compounds, although the unidentified portion could be partially characterized with respect to functional groups.

The mean contributions of organic classes to the identified content of the leachates are as follows:

Organic bases -	70 percent
Organic acids -	23 percent
Oxygenated/heteroatomic hydrocarbons -	3.1 percent
Aromatic hydrocarbons -	2.3 percent
Halogenated hydrocarbons -	1.4 percent
Aliphatic hydrocarbons -	much less than 1 percent

The two dominant organic classes (organic acids and bases) found in the leachates tend to be the least damaging to polymeric landfill liners, according to literature and pamphlets distributed by various synthetic liner manufacturers.

RECOMMENDATIONS

A recommended synthetic leachate based on the results of the current study and based on the percent organic carbon content of the compounds could contain the following:

- Organic bases: Phthalic acid diamide - 70 percent
- Organic acids: Benzoic acid, 19 percent (aromatic carboxylic acid); Phenol, 3.5 percent (phenol); Hexanoic acid, 1 percent (fatty acid)
- Oxygenated/heteroatomic hydrocarbons: Acetone, 1.8 percent (polar ketone); Methyl isobutyl ketone, 0.8 percent (fatty ketone); Methanethiol, 0.2 percent (thiol); Tetrahydrofuran, 0.3 percent (ether)
- Aromatic hydrocarbons: Toluene, 2 percent
- Halogenated hydrocarbons: Methylene chloride, 0.6 percent (chlorinated aliphatic); Chlorobenzene, 0.8 percent (chlorinated aromatic).

This recommended leachate formula contains compounds actually found in the leachates, and representative of the physical and chemical characteristics of the functional group classes found in the leachates, except for the aliphatic hydrocarbons class, its proportion in the leachates being negligible.

The TOC content of a synthetic leachate should be 0.6 percent by weight, and the balance should be water. The synthetic leachate should also contain enough of a salt (e.g., sodium chloride) to yield a solution conductivity of about 30,000 umhos/cm. The TOC of 0.6 percent represents the mean total percent content of organic compounds found in the leachates, both identified and unidentified. It is assumed that the compounds chosen to represent the organic content are representative of all organics in the leachate, both low and high molecular weight. (The spectral data on the high molecular weight fraction supports this assumption.)

The GFC separation into high and low molecular weight fractions with Sephadex, the analysis of the aqueous residual fraction by direct injection onto a water-compatible gas chromatography (GC) column, the derivatization of the acid fraction and reanalysis, and LC/MS/MS are all appropriate techniques for future similar studies. Up to 52 percent of the <500 dalton organic content of the leachates was identified, proving that the analytical modifications improved the assessment. A more statistically significant assessment of a typical leachate would require sampling a larger number of landfills. In this study, many of the constituents were found at only one of the three sites.

QUALITY ASSURANCE AND QUALITY CONTROL

A quality assurance program was followed throughout the study to ensure that analyses of the leachate samples yielded results which were precise, accurate, and representative of the leachates sampled. When possible, samples were collected directly from the leachate collection system without using beakers or bailers, and where the leachate was unaltered (e.g., not exposed to open air) to eliminate any potential for sample contamination by the sampling equipment or sample degradation, respectively. At the lab, the QA/QC procedures contained in the standard methods were followed, and extended to the nonstandard procedures when appropriate. Field instruments were routinely calibrated against external standards (e.g., standard buffers). Field/method blanks were shipped with each set of leachate samples sent to the laboratory. Duplicate analyses were performed in the laboratory by splitting a single leachate sample.

These QA/QC procedures helped control and assess accuracy and precision in the results. However, the complex series of analyses required for the study made it impossible to analyze samples within the holding times typically required by the USEPA. We felt that the possible bias which may have resulted from loss of volatiles or by chemical transformations would be small, and was less important than developing a new scheme for thorough analysis of leachates.

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ANALYTICAL REFERENCE STANDARDS AVAILABLE FOR ENVIRONMENTAL ANALYSIS

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ABSTRACT

The quality of all analytical measurements rests ultimately upon the quality of the analytical standards. In order to assist in the quality assurance of environmental measurements involving toxic substances, the Environmental Monitoring Systems Laboratory - Las Vegas, Nevada (EMSL-LV) maintains a Standards Repository which is located at Research Triangle Park, North Carolina. Since 1981, EMSL-LV has maintained and continued to expand an inventory of analytical reference standards in support of the requirements of Superfund, the Federal Insecticide, Fungicide and Rodenticide Act, and the Office of Solid Waste. Depending upon Program eligibility, these standards are distributed without charge to U.S. government laboratories, foreign laboratories (government and commercial), federal contract laboratories, regional, state, and local government laboratories, and universities. Therefore, the standards are available to organizations engaging in environmental research, monitoring and regulatory activities for toxic substances.

The Repository distributes approximately 2,800 chemical compounds which are EPA purity-assayed analytical reference standards; 800 of these compounds are available for worldwide distribution (presently to 90 countries) through our pesticides standards index. The remaining 2000 compounds are because of limited supply, available only to U.S. government, government contractor laboratories, and at times to foreign governments under special arrangements. All standards are for research and monitoring and are supplied so that laboratories can demonstrate traceability of their working standards to a single known source. The standards distributed by the EPA Repository are analyzed to confirm identity and to establish purity. Identity is verified by mass spectrometry, supplemented by other techniques (IR, NMR), when needed and purity is determined by independent analytical methods (DSC, GC/FID and HPLC). Only a small amount of a particular standard is supplied per request. The Repository also supplies technical information for environmental analysis.

United States Environmental Protection Agency Symposium on Solid Waste Testing and Quality Assurance Volume 1 – Proceedings
July 11 – 15, 1988

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