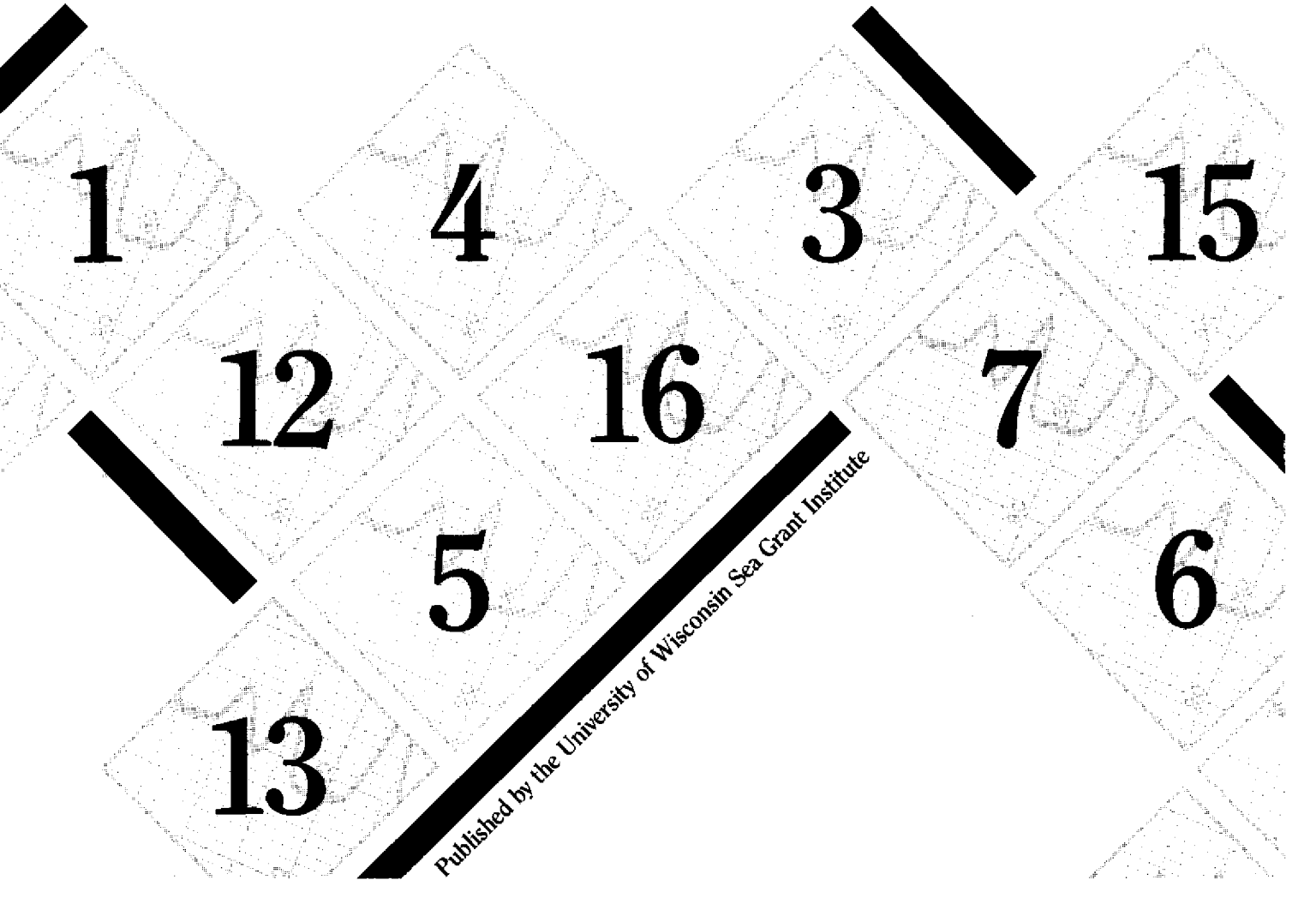
Methods for Analysis of Organic Compounds in the Great Lakes

II
Volume



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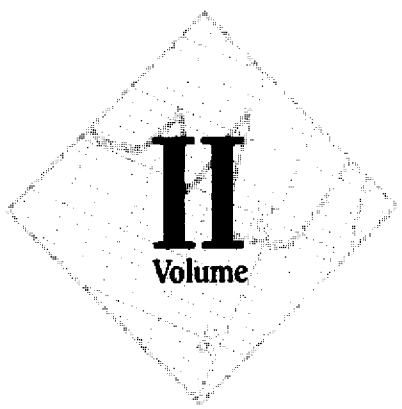

The Proceedings of an Invitational Workshop
October 10-11, 1985
Wisconsin Alumni Center
University of Wisconsin
Madison, Wisconsin

Chaired by

William C. Sonzogni and Douglas J. Dube
Wisconsin State Laboratory of Hygiene

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Preface

Since its creation in 1968, the University of Wisconsin Sea Grant College Program has actively addressed the toxic contaminants problem on many fronts. Multidisciplinary research in such program areas as aquaculture, resource policy, water quality, seafood technology, living resources and the Green Bay ecosystem have all dealt with the toxics problem to one degree or another. UW Sea Grant has also dealt with the problem through its public education and outreach activities.

Over the years, Wisconsin scientists have investigated the processes affecting the sources, pathways, fate and health effects of a variety of environmental contaminants. The levels of contamination are usually extremely low, however -- sometimes less than one part in a trillion -- which makes the ability to detect and quantify low-level contamination of the Great Lakes environment essential.

Given the fundamental importance of trace analytical chemistry, UW Sea Grant has twice cosponsored invitational workshops that have enabled trace contaminant chemists and scientists in related fields to share their knowledge and discuss common problems. The second workshop, held October 1985 in Madison, Wis., was cosponsored by the National Oceanic and Atmospheric Administration's National Marine Pollution Program. Like the first workshop in 1980, it attracted scientists from throughout the Great Lakes region.

We hope that, like the first, this methodology workshop summary will be a useful reference for state, federal and university laboratories working on Great Lakes contaminants (as well as other laboratories engaged in toxic research) and that it will further expand the information and technology transfer that occurred among workshop participants.

I would like to extend my thanks to all those who attended the workshop and so freely shared their special knowledge and expertise, and to the science journals and authors that generously allowed us to reprint the related research articles recommended by workshop participants.

Robert A. Ragotzkie
Director, Sea Grant Institute
University of Wisconsin-Madison

Workgroup Summaries

Methods for Analysis of Organic Compounds in the Great Lakes

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Introduction

by William C. Sonzogni
University of Wisconsin-Madison

In April 1980, U.S. and Canadian scientists from throughout the Great Lakes region gathered in Milwaukee for a workshop on methods for analyzing organic compounds in the Great Lakes. Cosponsored by the University of Wisconsin Sea Grant Institute and the University of Wisconsin-Milwaukee Great Lakes Research Facility, the workshop provided a timely and valuable forum for discussing a variety of analytical topics common to the Great Lakes.

Since then, the methods for detecting and quantifying xenobiotics have become more sophisticated and, in many cases, more complex. Furthermore, the data produced from these analyses are becoming increasingly vulnerable to misinterpretation. With these developments in mind, a second workshop on the analysis of Great Lakes toxic substances was held in October 1985. These proceedings summarize the discussions held during that workshop.

Concern over toxic compounds in the Great Lakes has, if anything, proliferated since the first workshop. Indeed, worldwide attention has focused on the Great Lakes as a harbinger of toxic contamination problems elsewhere. As a result, chemists working on Great Lakes contamination problems have not only been extremely busy but at the forefront in the development of innovative and sensitive contaminant measurement techniques. Furthermore, much of the data on toxic substances -- probably more than for any other water resource worldwide -- have been generated with regard to the Great Lakes. Thus, chemists and related scientists working on Great Lakes contaminant problems form a special group, and, judging from the attendance at both workshops, share a common need to discuss their work with their colleagues.

The purpose of the workshop was straightforward: to share recent information and experiences, and to discuss current issues regarding the analysis of organic pollutants in the Great Lakes. Those attending the workshop included representatives of industry, universities, and state and federal laboratories. Most of these scientists were from the Great Lakes Basin, but several from outside the basin also participated because of the leading role the region has played in contaminant research and monitoring.

The workshop was organized around four main issues:

1. Concentration techniques to measure low-level organics in water;
2. Pattern recognition of complex mixtures of organics;
3. Mass spectrometry techniques for dioxins, furans and other compounds;
4. New quality control issues in organic chemical analysis.

Each of these issues was chosen because of the current interest in these topics by both research laboratories and those production laboratories responsible for regulatory monitoring. These issues served to focus and organize the workshop and these proceedings, but it should be noted that many other topics were discussed that are not recorded in these proceedings. As at most workshops, some of the private discussions that took place were probably the most valuable -- discovering a time-saving modification used by

another laboratory, the source of a previously unavailable standard, or simply commiserating over the poor precision obtained from a method required by a regulatory agency.

One common theme of the workshop was the important role computers are playing in Great Lakes chemical analysis. Computers are used not only to manipulate and store the data that are generated, but also to control instruments and to acquire data directly from instruments. Robotics and other forms of automation also are beginning to have an effect on chemical analyses; automated samplers in chromatography, for example, are now commonplace.

While the use of computers and automation in the laboratory is not unique to the Great Lakes area, it was obvious from the workshop that Great Lakes chemists have been quick to implement the new technology. The result has been and will continue to be more rapidly produced, precise data at lower costs.

Regarding computers, a promising new area is the use of heretofore cumbersome mathematical techniques to recognize chemical mixture patterns. The patterns of complex chemical mixtures like polychlorinated biphenyls (PCBs) or toxaphene can now be reproducibly identified with relatively inexpensive personal computers. In fact, many of the routine decisions facing analytical organic chemists will soon be relegated to such "artificial intelligence."

The application of mathematical techniques to study chemical problems has developed into a new field called "chemometrics." As chemometrics expert and workshop participant D. Stallings (U.S. Fish & Wildlife Service, Columbia National Fisheries Laboratory, Columbia, Mo.) pointed out, chemometrics is likely to have a major impact on the way Great Lakes chemists go about their business.

Though pattern recognition techniques represent a significant advance in the traditional technique of matching the PCB patterns in environmental samples to commercial formulations, the workshop consensus was that the current PCB mixtures in environmental samples generally do not match known Aroclors. The alternative is to measure specific congeners with high-resolution capillary column chromatography.

Since the 1980 workshop, when it was discussed as a promising new technique, gas chromatographs equipped with capillary columns are now available in most trace contaminant laboratories. Even though PCB congener analysis will take longer to perform and thus will be more costly, the additional information obtained will be cost-effective. Knowledge of the concentrations of the different PCB congeners in Great Lakes environmental samples and the toxicity of these congeners may cause a shift in public health policy regarding environmental exposure to PCBs.

Measurement of very low levels of contaminants in Great Lakes water was of general interest to workshop participants, but few laboratories have attempted such analyses. However, more low-level data are needed to better understand the sources and fates of such contaminants (and the processes that control them), so more laboratories will be attempting such analyses. The good news from the workshop is that such analyses can be done; the bad news is that it will require very careful and time-consuming techniques.

Unlike before, this time there was no argument over the need for extensive quality control in the analyses of organics from the Great Lakes. The importance of determining the limit of detection (LOD) and limit of quantitation (LOQ) was especially emphasized. Most laboratories do not make these determinations routinely at present, but a demand

for these values is likely in the future. The LOD and LOQ will have to be customized for different matrices.

One interesting point that came out of the mass spectrometry discussion was that low-resolution mass spectrometers (the kind most Great Lakes laboratories now have) can be used for the analysis of dioxins and furans. The need for safety in handling these compounds was emphasized, but it was agreed that, with the proper precautions, these compounds could be handled safely in most laboratories. For example, it was noted that the use of a concentrated standard of highly toxic 2,3,7,8-tetrachlorodioxin might be avoided by obtaining prediluted standards.

Finally, it was generally agreed that mass spectrometers can and will play a greater role in the quantitation of organic contaminants. Until recently, the mass spectrometer played a greater role in qualitative identifications, as opposed to quantitative measurements. Several new computer techniques were discussed that could be used to measure contaminants, such as the U.S. Environmental Protection Agency's "priority pollutants" series. Also, low-cost mass spectrometers have recently appeared commercially and show promise of being useful for the analysis of many organics. The availability of such instruments should particularly be a boon to many small laboratories unable to afford conventional mass spectrometers. Further, these instruments might be a relatively inexpensive means of expanding the capacity of laboratories that already have conventional mass spectrometers.

What will the next five years bring to the Great Lakes contaminant field? One thing is certain: No matter what contaminants are in the limelight, automation and computers will allow faster, cheaper and more precise analyses. New types of instrumentation and equipment -- perhaps microbore columns, mass selective detectors and thermal desorbers -- will permit the analysis of compounds heretofore ignored, or will allow more analyses to be performed per day.

Whatever the technological advances, the analytical chemist will still be the most important resource, and the skill, patience and innovativeness of the Great Lakes organic analytical chemist will continue to be a major factor in the management of the Great Lakes ecosystem.

Workgroup Summary: Low Level Organics in Water

by James Hurley
Water Chemistry Program
University of Wisconsin-Madison

The measurement of low levels of organic compounds in water presents the researcher with a number of possible pathways to effectively obtain and analyze samples. Numerous sampling methods, extraction procedures and sample preparation techniques are available. The purpose of this discussion section was an exchange of information among researchers from different backgrounds and disciplines on the topic of measurement of low levels of organic compounds.

The introductory presentation at the morning session was given by D. Swackhamer (Indiana University, Bloomington). Her presentation outlined the considerations for effective sampling, extraction and clean-up prior to measurement by gas chromatography (GC) or other methods when analyzing low-level organic compounds. A number of different approaches to sampling strategy, isolation, concentration, clean-up and fractionation were outlined for initiating directed discussion in the afternoon subgroup meeting. At the afternoon subgroup meeting, about 20 researchers from government agencies (United States and Canada), industry and universities discussed their experiences with numerous approaches and techniques of isolation and analysis. The following sections represent the major topics discussed and include a short discussion of the conclusions or suggestions for each topic.

Sampling Strategy

The strategy for sampling low levels of organic contaminants in water reflects the goals of the specific research or monitoring program. It was not the objective of the group to develop one particular strategy, but rather to discuss some of the problems involved in developing a sound sampling strategy.

Composite sampling (a method of grouping samples) is recommended when a limited number of samples are taken at a sampling site (e.g., deep rivers and lakes). Samples are grouped on the basis of zones (e.g., the epilimnion or hypolimnion of a lake) or of time (e.g., monitoring effluents from industry).

For river studies, it was suggested that it is quite important to couple the results of analyses with a detailed flow study in an attempt to assess variability and to aid in flux calculations. The question of spatial and temporal variability, along with the number of samples needed to determine variability, must be considered when developing a strategy.

Other topics included mechanics (sampling equipment performance, repairs, etc.), the size of samples needed and the best methods of sample compositing. Perhaps the most important factor in determining sampling strategy is the capacity for the analytical laboratory to process the samples collected. Intricately designed strategies for obtaining the proper sample type, considering such factors as variability, may require modification due to the time and personnel required by the analytical laboratory to adequately analyze samples.

Fractionation and Extraction of Dissolved and Particulate Matter

The method of differentiating between dissolved and particulate matter in waters was an important topic of discussion. The operational distinction between the two phases depends on the collection efficiency of a filter or, in some cases, on the efficiency of centrifugation.

The most common filter type used is glass fiber. The Canadian Center for Inland Waters has used Gelman 0.3 µm glass fiber filters, and their sampling time and collection procedure were discussed. The use of membrane filters was discouraged for low levels of organics due to their interaction with hydrophobic compounds. Teflon filters are also used, but are cost-prohibitive in most cases.

The need to identify the particle cut-off size when using glass fiber filters was discussed. The manufacturers calibrate these tortuous-path filters with air particulates rather than particles in water. By contrast, membrane filters have calibrated pores. Therefore, the manufacturers' rated efficiency for glass fiber filters may not be directly applicable to particles encountered in water samples. Similar concern arises over the comparison of filter types when estimating suspended particulate concentrations. For instance, organic matter collected on precombusted glass fiber filters (which are difficult to weigh accurately) may not be directly comparable to the total suspended particulate concentration estimated by collection on polycarbonate membrane filters (Danielsson, 1982).

Continuous flow centrifugation has become a popular means of obtaining particulate matter because of the large amount of material collected over a relatively short time. M. Mullin (U.S. EPA Large Lakes Research Station, Grosse Ile, Mich.) is conducting a study of low levels of organic contaminants in particulate matter and comparing the collection efficiency of continuous flow centrifugation and glass fiber filtration. The results of this study will soon be available.

D. Armstrong (University of Wisconsin-Madison) reported that his group had investigated collection of Lake Michigan particulate matter by centrifugation. The samples were collected for analysis of metals rather than low-level organics. Preliminary results of this study indicate that the collection efficiency is quite good for particles larger than 1 µm; however, collection efficiency decreases for particles in the 1 to 0.4 µm size range. Efficiency is a function of a number of factors, including flow rate, particle size, density, shape and water temperature. Bates et al. (1983) have compared these collection parameters for hydrocarbon analyses. A major problem discussed is the lack of methods for the efficient collection of the "colloidal" fraction in water samples.

M. Fox (Canada Centre for Inland Waters, Burlington, Cnt.) discussed the use of the 200-L APLE solvent extractor as a means of onsite batch extraction of the dissolved phase. The sample is first pressure-filtered from a stainless steel beverage container through a Millipore filtering unit (glass fiber filter) and into the APLE extractor. The filter containing particulates is then rolled up in a test tube and stored in methylene chloride. Another method, suggested by D. Kuehl (U.S. EPA Environmental Research Laboratory, Duluth, Minn.), involves a continuous flow liquid-liquid extraction technique.

"Carboy" liquid-liquid extraction is perhaps the most extensively used extraction procedure. Alternatives to liquid-liquid extraction, such as XAD resins or polyurethane foam plug adsorbents, are commonly used. SEP-PAK cartridges (manufactured by Waters Associates, Milford, Mass.) are also used, but concern was expressed that the packing may not be uniform enough for this application. The sorbent surface area is also quite small with a SEP-PAK. With either technique, it is important to ensure sample integrity by

maintaining proper pressures and flow (to avoid channelling or disrupting filters, algal cells, etc.).

A common laboratory technique for the extraction of organic compounds from particulate matter, filters, resins, fish and sediments is through Soxhlet extraction. Similarly, a steam distillation technique has been proven to be a successful isolation technique, at least for certain compounds.

Sample Clean-Up Prior to Gas Chromatography

A number of clean-up steps were discussed. Florisil has been used extensively in column clean-up procedures. Some researchers have encountered problems with this adsorbent in that trace levels of certain organics (e.g., PCBs) have been shown to irreversibly adsorb on it. Reproducibility of packing character also presents a problem with Florisil. Silica gel and alumina are also widely used. However, J. Baker (University of Minnesota, Minneapolis) reported high losses of hexachlorobenzene using alumina. Researchers at the U.S. EPA Environmental Research Laboratory-Duluth commonly use a celite-sulfuric acid mixture with hexane elution. UM-Minneapolis researchers add sulfuric acid directly to the hexane as a clean-up technique. Both of these methods effectively remove biogenic interferences and, specifically, methyl esters. Scientists at the U.S. EPA Large Lakes Research Laboratory-Grosse Ile are also currently working on a gel permeation method for clean-up of water samples.

Sulfur in bottom sediments must be removed from samples due to interferences in GC. Though mercury has been used for sulfur removal, a number of participants expressed concern over the mercury wastes remaining. Copper has been used successfully by many laboratories. The copper must be cleaned prior to use by leaching with concentrated hydrochloric acid. A suggested technique is to place copper at the base of the Florisil or silica gel column, thus combining the clean-up steps.

Concentration Techniques

Two common methods of concentrating extracts are rotary evaporation under reduced pressure and Kuderna-Danish methods. Both are considered effective means for sample concentration, though there was some concern over volatilization with rotary evaporation. Kuderna-Danish methods may be more efficient in a production laboratory. The low cost of the necessary equipment (steam bath vs. rotary evaporator) and space requirements make Kuderna-Danish a better choice for certain laboratories.

Once a compound is preconcentrated by one of the above methods, nitrogen concentration is useful to achieve extremely small volumes. D. Swackhamer discussed a method that involves concentration of a sample down to volumes of approximately 100 µl by using narrow-bore vials. Care must be taken to assure proper low flows of nitrogen gas and temperature of the sample at or near room temperature. The method has been shown to prevent the loss of trace analyte in the sample and is effective because of the small surface area exposed to the nitrogen gas in the narrow vials.

Glassware Cleaning

The results of a study conducted at Indiana University were discussed by D. Swackhamer. Conventional glassware cleaning techniques -- such as solvent washing, acid washing

followed by solvent washing, and ashing -- were compared. Lowest background (blank) contamination was observed with the ashing technique. This technique involves wrapping glassware in aluminum foil and heating in a muffle oven for four hours at 450°C. The procedure is recommended not only for laboratory glassware, but for sample containers also. However, oven space and size may be a limiting factor in some labs, so this may not be a practical method for all glassware.

The use of a chromic acid wash solution for low-level organics was strongly discouraged, due to the possibility of residual chromic acid on the glassware. This may lead to the oxidation of sample organic matter or the creation of reactive surface sites on the glass walls.

Solvents

All laboratories use large amounts of organic solvents in sample work-up, and, in some cases, solvents represent the greatest fraction of the supplies and equipment budget. It is extremely important that the laboratory is supplied with solvents that are both analytically pure and cost-effective.

Previously, the solvent manufacturer of choice was limited to one. However, solvents of other manufacturers have now been found to be of similar analytical purity and, in some cases, less expensive. Records of solvent lot numbers should be kept to ensure quality control and protect against further contamination.

Volatile Organics

Though the topic of volatile organic compounds was not a primary discussion area, the problems with sampling for and analyzing these compounds were discussed. In general, the group recognized the need for better methods for sediment analyses, as U.S. EPA does not have an approved method.

D. Degenhardt (University of Wisconsin-Madison) discussed the attempts made at his laboratory to quantify volatiles in sediments. Both a purge and trap method and a headspace analysis were attempted. Both techniques, even though adequate for water samples, do not appear to be satisfactory for quantitation of volatiles in sediments.

Workgroup Summary: Pattern Recognition of Complex Mixtures

by Howard Drossman
Department of Chemistry
University of Wisconsin-Madison

Pattern recognition techniques can be used to solve many Great Lakes organic analysis problems. The sparsity of current data developed with pattern recognition techniques is largely due to the relatively short period of time that the techniques have been used for environmental analysis. The situation is sure to change according to D. Stallings (U.S. FWS Columbia National Fisheries Laboratory, Columbia, Mo.), who presented an excellent review and explanation of the current methods and applications of pattern recognition and other mathematical techniques, all part of a new field called chemometrics.

Chemometrics is the development of novel mathematical models to describe chemical phenomena. From a historical perspective, chemometrics has existed since 1915, when it was first suggested by Emil Fischer. Chemometric techniques have developed slowly since that time. With the availability of inexpensive personal computers and the progression of new algorithms, the use of chemometrics to solve environmental problems should be increasing rapidly.

Pattern Recognition

One of the most important environmental applications of chemometrics has been the development of pattern recognition techniques for use in the analysis of complex mixtures. Several algorithms are available, but the one that was discussed in this workgroup is SIMCA (Soft Independent Method of Class Analogy). SIMCA appears to have many potential applications in Great Lakes organic analysis, such as the recognition of dioxins and furans. The SIMCA approach is unique, because it is designed to search for similarities rather than applying the method of differences. Interestingly, SIMCA has many applications outside of chemistry as well (it was originally developed to beat the odds at horse racing). SIMCA is largely based on equation (1):

$$X_{k,i} = X_i + \sum_{a=1}^{A} (\theta_{k,a} \times \beta_{a,i} + e_{a,i}) \qquad (1)$$

where:

- i,k = variables referring to variable and object
- X = mean values of variables
- β = scores for each variable
- θ = loading of the variable
- e = residuals (errors)
- A = number of variables

The equation is best solved in matrix form with the variables represented by a $\underline{k} \times \underline{i}$ matrix and the loading variables given by a $1 \times \underline{k}$ vector. These solutions are obtained by matrix manipulation that does not require matrix transposition. The solutions may then be

graphed in n-space using a computer-generated plot. Though the equations and solutions are somewhat complex, the user only needs to understand the significance of the input variables and how to interpret the output. A simplified way of looking at the method was presented by Stallings and is reproduced in Figure 1:

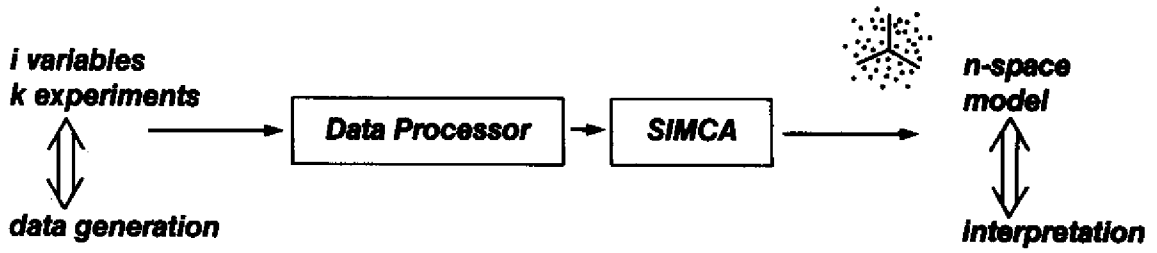

FIGURE 1

It is important that the user understand the significance of the output in physical terms, because -- as in any computer-generated information -- the output can be no better than the input, though it may display more information.

To date, the SIMCA approach has been used for identifying PCB patterns in fish, bird eggs, river water and lake water. The utility of the pattern recognition technique lies in the many ways that information can be extracted from the same data set. Up to this time, most PCB results from environmental mixtures have been reported as an Aroclor or a mixture of Aroclors. The SIMCA approach can be used to more precisely match PCBs in environmental samples to established patterns as well as determine when PCBs in an environmental sample do not match an established pattern.

The SIMCA algorithm can produce an n-space representation of different possible PCB mixtures, with each "pure" Aroclor mixture represented by one apex of an n-sided polygon. For a mixture represented by a basic set of four Aroclor mixtures, the surface would be represented by a tetrahedron. Any point lying on a line of the tetrahedron would best be described as a combination of two Aroclor mixtures. A point lying on a surface would best be described as a combination of three mixtures. The advantage of this algorithm is that it would represent a poor fit to any Aroclor combination with a point lying outside of and a distance from the tetrahedron. Such a case may indicate that the sample should be fitted to a different basis set of Aroclors or perhaps to an entirely different basis, such as a Cl_1 to Cl_{10} homolog representation.

Pattern recognition techniques may have their greatest environmental applications in the correlation of chemical data with physical or biological data. Examples of such applications that have already (or could in the future) take advantage of pattern recognition techniques include:

* correlation of Aroclor distribution in birds, eggs and fish with geographic area or distance from a source;

* correlation of chlorinated dioxin input with geographical distribution in Lake Superior and Lake Michigan;

* toxaphene distribution in the Great Lakes;

* correlation of bioassay results with chemical concentration data;

* correlating mass spectrometry data with functional groups;

* sequencing of DNA and RNA;

* the selection of variables in analytical tests to optimize sensitivity, and

* correlation of toxicity data with exposure data in fish analysis work.

Overall, it was obvious to workshop participants that the use of pattern recognition in solving problems will be limited only by the ability to think of creative uses.

Computer Needs

An important question concerning the use of pattern recognition techniques discussed at the workshop was, "What type of computer equipment is needed?" At the very least, a user would need a data-generating device, a quantitator, a data reduction program, a data transfer program and a computer powerful enough to manipulate the data. The data generator can be as simple as a series of test tubes or as complicated as a Fourier Transform instrument. The selection depends on the samples to be analyzed, methods of quantitation and the economic status of the laboratory. The quantitator can be any instrument or integrator in series with the instrument that can attach a numerical measurement to the observed property of the system under study.

Currently, the pattern recognition techniques do not include data reduction algorithms, because these must often be programmed to fit the desired application. Many of these programs are currently available commercially. The American Chemical Society publishes descriptions of some programs that might be useful to analytical chemists. The data transfer program must be capable of transferring the reduced data to the pattern recognition program in ASCII characters (ASCII is the computer acronym for American Standard Code for Information Interchange).

The size of the computer needed for pattern recognition work will vary with the job. For example, to correlate PCBs by homologous series, a larger computer would be needed to handle sediment or bird tissue samples than to do transformer oil samples (due to the greater complexity of the former). For transformer oil samples, a system consisting of a GC with an integrator coupled to a personal computer with 64K of Random Access Memory (RAM) might be sufficient. The more complex samples would require a larger computer. Note that a computer with 64K RAM can manipulate a 50 x 50 matrix. A typical MS/DOS computer system with expanded RAM can process a 100 x 100 matrix.

Another question asked was the set-up time and training needed to successfully utilize a pattern recognition system in a typical laboratory. The consensus was that set-up time would probably take two to three months, while another three months of training would be required for an operator to learn the system. The complexity and diversity of the samples to be analyzed would, of course, affect the time it would take to get a system operational.

Reporting PCB Data

One major issue of debate was the effect of pattern recognition techniques on data uses. In particular, will the reporting of PCBs as Aroclor mixtures, as in conventional techniques, become obsolete?

The tradition of reporting PCB mixtures found in the environment according to the Aroclor mixture they most closely matched was established for a number of reasons. These include (1) the ease of tracking PCBs to a point source; (2) familiarity with the components of Aroclor mixtures; and (3) reluctance to switch to a new standard, like the reluctance to switch from English units of measure to metric units.

On the other hand, compelling reasons now exist for reporting PCBs by an alternate system, such as by congeners or homologous series. These reasons include (1) the need for toxicity-congener correlations that are often masked when results are reported as Aroclor mixtures; (2) degradation properties can be better assessed by correlations with the congeners; (3) data can always be reconstructed with pattern recognition techniques to give the original Aroclor mixture; (4) information is distorted (i.e., inaccuracies occur) when a mixture is "forced" to fit an Aroclor mixture; and (5) analytical methodologies have improved to the extent that congener analysis is now more practical.

The general opinion at the workshop was that, in the near future, PCB mixtures will rarely, if ever, be reported as Aroclor mixtures. Instead, PCBs will be identified according to specific congeners or by homologous series.

Summary

In summary, the use that the data will be expected to serve will, in the end, dictate how the data are prepared. In this regard, relating PCB mixtures to Aroclors is likely to have limited uses in the future. On the other hand, advanced computer techniques like pattern recognition will greatly expand the number of ways data can be reported. Therefore, the question for the future is likely to switch from whether PCBs in environmental samples should be reported as Aroclors, to how should the data be reported to be most useful in a given situation.

Pattern recognition is one of several new techniques that will allow the chemist a variety of options to optimize the use of the results. Chemists working on Great Lakes organics have started to take advantage of these new techniques, and they are expected to give even greater attention to these techniques in the years ahead.

Workgroup Summary: Mass Spectrometry Techniques

by Philip J. Emmling
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Combined gas chromatography/mass spectrometry (GC/MS) techniques have received wide acceptance and expanded use for the analysis of organic compounds in the Great Lakes. In the five years since the previous methodology workshop and report (Delfino, 1980), the basic electron-impact, low-resolution mass spectrometer has remained relatively unchanged. In contrast, sample preparation, chromatography, data handling systems and commercially available, stable isotope compounds have made significant refinements to organic analyses by GC/MS. The mass spectrometry (MS) workgroup proceedings reflect the interest in these developing research topics. Participants in this workgroup represented Canadian and U.S. agencies, universities and industry.

Dioxin and Furan Analysis

An introductory presentation was given by D. Kuehl (U.S. EPA Environmental Research Laboratory-Duluth). His group has been investigating the bioaccumulation and depuration of polychlorinated dioxin and furan compounds by fish. By including internal standards (consisting of stable, isotope-labeled compounds) in the sample extraction and clean-up steps, GC/MS detection limits approach 1 part per trillion for 2,3,7,8-TCDD in fish. Extracts were quantified with capillary GC and low- or high-resolution, electron impact or ion trap MS. The preliminary conclusions reached by this research include:

1. The chemical 2,3,7,8-TCDD is bioaccumulated from municipal incinerator fly ash by freshwater fish.

2. The level of 2,3,7,8-TCDD in fish is not directly proportional to the level of 2,3,7,8-TCDD in fly ash.

3. 2,3,7,8-TCDD is the only TCDD isomer in fish.

4. The level of 2,3,7,8-TCDD in fish appears to follow a dose-response curve.

5. Freshwater fish (i.e., carp) readily accumulated 2,3,7,8-TCDD when exposed to contaminated natural sediments in a laboratory bioassay. However, the level of TCDD in the fish did not appear to reach steady state within 55 days.

6. Depuration of 2,3,7,8-TCDD from carp (1 kg) collected from water in a TCDD-contaminated watershed reaches half-life in 300 to 325 days.

7. Depuration of 2,3,7,8-TCDD from laboratory bioassay carp compares to carp collected from the environment -- 30 to 35 percent after 200 days.

8. The rate of PCDD/PCDF depuration from fish decreases with an increasing degree of chlorination.

9. Preferential uptake of 2,3,7,8-TCDD-substituted isomers previously observed for TCDDs appears to follow throughout the series of PCDD/PCDF.

10. 2,3,7,8-TCDD is preferentially deposited in fatty tissue covering the base of the brain of a carp. The concentration in this brain fat deposit is 5 to 15 times greater than in brain tissue and 5 to 30 times greater than in the fillet.

Future research plans of Kuehl's group include investigating dioxins and furans in the environment, studying why 2,3,7,8-TCDD is uniquely found in fish, determining bioaccumulation factors for several PCDD/PCDF ratios in several species of fish, and investigating the relationship between the level of PCDD/PCDF ratios in fish and sediments. Similar analytical procedures for the analysis of PCDDs and PCDFs have been presented by Smith et al. (1984).

Following Kuehl's presentation, an informal discussion of MS techniques was held, led by Kuehl and E. Marti (Wisconsin State Laboratory of Hygiene, Madison).

The discussions began with R. Norstrom (Canadian Wildlife Service, Cttawa, Cnt.) commenting on Kuehl's presentation. In Canada, chlorinated dioxin analysis includes gel permeation and carbon column enrichment followed by column chromatography clean-up. Capillary column chromatography and quantification with stable isotopes using low-resolution, electron-impact quadrupole MS completes the analysis. Biological samples were found to contain a limited number of PCDD and PCDF isomers and thus were analytically less complex than municipal incineration or sediment samples.

The group concluded that a high resolution mass spectrometer was not necessary to do dioxin and furan analyses; rather, a low resolution instrument could be used with high resolution chromatography, provided careful extraction and clean-up were performed.

Emerging Techniques

Computer-assisted MS has taken the technique from oscillograph data recording into an era of sophisticated analog to digital conversion and data storage. Computer software has been developed to aid in the identification and quantification of organic compounds. While the quantification of pesticides, PAHs and similar single-component compounds can be relatively straightforward, complex mixtures like toxaphene and Aroclors have challenged analysts.

A. Alford-Stevens (U.S. EPA-Cincinnati) presented software designed to identify peaks as Cl_1 to Cl_{10} PCBs and produce a final quantitation report for Cl_1 to Cl_{10} PCBs. The software uses a single response factor for each level of chlorination. The approach was generally accepted, but some participants noted that the use of a single response factor oversimplified PCB determinations. High resolution GC has brought PCB pattern recognition from packed column technology (Webb and McCall, 1973) to a more advanced level (Schwartz et al., 1984; Dunn et al., 1984). Relative retention times have been shown to increase directly with increasing PCB chlorine content, but there may be large differences in the magnitude of relative GC response factors within each isomer group (Mullin et al., 1984). With the availability of all 209 isomers and their response factors, this computer software program could possibly be changed to consider individual response factors. The software would then be compatible with the proposed U.S. EPA analytical methods 1624 and 1625 (discussed later).

The MS workgroup discussed the availability, cost and benefits of stable isotope standards. While the cost of some chemicals remains high, more compounds are becoming available. Stable, labeled isotopes and isotope dilution techniques can improve the precision and accuracy of calculated percent recoveries for the determination of priority pollutants in industrial effluents (Colby et al., 1980). Percent recoveries were uniformly unaffected by effluent sample matrix as long as sufficient material was recovered for measurement.

This was not found to be the case with the more conventional GC/MS methods 624 and 625 (Federal Register, 1979). Colby et al. (1980) concluded that (1) large numbers of compounds could be identified and quantified in environmental samples with isotope dilution methodology in a routine manner by using full spectrum data and readily available instrumentation, and (2) quality assurance/quality control (QA/QC) functions could be used with labeled analogs.

The Wisconsin State Laboratory of Hygiene currently uses the Fused Silica Capillary Column GC/MS Quality Control Protocol (Acurex Corp., 1984) for the determination of semivolatile priority pollutants, which represents the practical state-of-the-art in QA/QC for this technique. Relative retention times and response factors are determined with stable isotope internal standards representative of the compound classes of interest. Agencies are moving toward the next level of GC/MS standardization -- methods 1624 (volatile compounds) and 1625 (semivolatile compounds) (U.S. EPA, 1985) -- which will require stable isotope-labeled analogs for each compound analyzed. Response factors for compound classes will be replaced by this technique when all the compounds of interest are commercially available.

Liquid Chromatography/Mass Spectrometry

High-performance liquid chromatography (HPLC) and MS techniques have each become useful tools in analytical organic chemistry. Combining HPLC and MS hardware has not been easy, since the two techniques would seem to be mutually incompatible. Attempts to combine these tools may be either off-line (LC followed by MS with no interconnecting interface) or on-line (LC/MS). Arpino and Guiochon (1979) concluded that off-line LC/MS would be limited to a few difficult cases not directly amenable to on-line LC/MS. D.E. Games (1983) summarized the basic problems of on-line LC/MS as (1) the mass spectrometer is not capable of handling the high gas-flow volumes generated by conventional LC, (2) useful mass spectra are required from compounds that, in many cases, are thermally labile and/or have low volatility, and (3) chromatography performance must be maintained. Arpino and Guiochon (1979) more rigorously define the requirements of an ideal LC/MS interface.

The MS techniques group was led by L. Burkhard (University of Wisconsin-Superior) in a discussion of commercially available LC/MS systems. Three on-line interfaces that are currently being refined are moving belt, direct liquid injection and thermospray.

The moving belt interface receives an LC effluent directly onto a stainless steel or polymide (Kapton) moving band. The solvent is evaporated with an infrared heater and removed by vacuum pumps. A heater desorbs solute molecules, which enter the MS source block. Both EI and CI spectra can be generated from these systems. The moving belt interface has been simplified and reviewed by Stout and daCunha (1985).

Direct liquid injection (DLI) transfers the LC aliquot through a capillary tube directly to the MS source. A partially permeable restriction is placed in the interface to adjust the

solute/solvent flow. The relatively high source pressures require a chemical ionization (CI) source. An electron beam produces the CI reactions, leading to primary and secondary ions for analysis. Polar solvents are favored because they yield primary ions, which can easily protonate solute molecules. The use of microbore packed and capillary open tubular columns can improve the DLI interface performance and cost (Tsuda et al., 1985).

Thermospray is the latest of the LC/MS interface techniques and includes another ionization method. The LC aliquot enters a temperature-controlled, heated tube so that the solvent emerges as a very fine spray. The solvent droplets evaporate, and charge exchange occurs between salt buffer ions (0.1 M ammonium acetate) and the organic solutes. Both positive and negative ions are formed and recorded by the MS source and electronics. Thermospray technology has been improving (Blakley and Vestal, 1983) and can provide routine capability for solving environmental analytical problems (Covey et al., 1985).

Overall, the workgroup had little hands-on HPLC/MS experience. LC/MS methodology is evolving rapidly, but application by production laboratories concerned with priority pollutant analyses is still in the future.

Supercritical Fluid Chromatography/Mass Spectrometry

Supercritical fluid chromatography/mass spectrometry (SFC/MS) received limited discussion within the group. Solute and solvent phenomena of supercritical fluids important to SFC/MS have been discussed by Smith and Udseth (1983). The technology of SFC/MS systems has not been implemented by many laboratories in the United States. Supercritical fluids have viscosities and solute diffusivities intermediate between liquids and gases. SFC offers higher mobile-phase linear velocities and higher separation efficiencies per unit of time than HPLC (Fjelsted and Lee, 1984). The greater densities of supercritical fluids compared to gases (GC) lead to solute solubility and mobile-phase selectivity parameters.

Capillary chromatography MS has become the method of choice for the analysis of relatively low molecular weight volatile and semivolatile compounds. Capillary column SFC with small diameter columns (100 μm i.d.) has been demonstrated to provide high-resolution separations of complex mixtures of nonvolatile or thermally labile compounds with the use of MS detection (Smith et al, 1982). Smith et al. (1984a) have used 50 μm i.d. capillary columns to separate standard mixtures and a complex coal tar extract, using SFC/CIMS by adding a sufficiently fast pressure programming capability. Electron impact spectra have been produced by Smith et al. (1984b) with a modified SFC/MS interface.

SFC/MS was considered to be a replacement for LC/MS by F. Cnuska (Canadian National Water Research Institute, Burlington, Cnt.). The interfacing problems of LC/MS presented in the previous discussion do not appear as serious for SFC/MS.

The potential advantages of SFC/MS relative to GC/MS or LC/MS methods were listed by Smith et al. (1982) as (1) higher molecular weight, polymeric, heterofunctional and thermally labile compounds can be separated as well as the more volatile species; (2) capillary SFC columns can provide greatly enhanced chromatographic efficiency relative to HPLC due to solute diffusivities, which are about 100 times greater in the supercritical fluid than in the corresponding liquid phase and viscosities similar to the gas phase; (3) mixed mobile phases, gradient, temperature and pressure programming are feasible to control the solvating power of the mobile phase; and (4) SFC with capillary

columns can provide low mobile-phase flow rates which, coupled with high mobile-phase volatility, allows optimum interfacing of SFC and MS.

Laboratory Safety

These discussions were summarized briefly for the entire workshop by E. Marti (Wisconsin State Laboratory of Hygiene, Madison). In addition to these topics, the concern for laboratory safety and educating laboratory personnel about extremely toxic chemicals was discussed. Despite the limited time available for the safety issue, several useful guidelines were proposed:

* Laboratory areas require safety planning before highly toxic substances are used in a work area.

* Concentrated standards should be stored in well-secured areas.

* High-risk analytical operations, such as weighing out chemicals for stock solutions or spiking samples, should be performed by a limited number of researchers.

* These high-risk workers should be given medical checkups at regular intervals.

* Seminars need to be conducted to educate all personnel in the laboratory both to train persons working with toxic chemicals and to calm the fears of support employees.

These guidelines demonstrate the concern of the workshop for laboratory safety, a subject that deserves more rigorous examination in the future.

Workgroup Summary: New Quality Control Issues

by Judy L. Crane
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Nineteen individuals, representing municipal, state, provincial and federal agencies as well as private companies, met to discuss quality assurance (QA) and quality control (QC) issues. Included in the workgroup's agenda were discussions on QA programs, terminology problems, strategies on how to report low level data, computers, fish tissue check samples and sediment samples.

Need for an Effective QA Program

D. King (Ontario Ministry of Environment, Rexdale, Ont.) presented a seminar on how Great Lakes laboratories can develop effective QA programs. In brief, King made the following points: (1) perform quality control steps before using any equipment; (2) do performance monitoring after starting analyses (e.g., round-robin tests); (3) document activities to control problems and to set protocol for the staff; (4) plan to provide the quality control information that the user needs; and (5) make sure the quality management team can install QA and QC procedures.

The workgroup meeting echoed many of King's ideas. The group agreed that effective QA programs are essential to any laboratory. Quality control is especially important when dealing with low levels of organics. QA managers, scientists, and statisticians should state their data quality objectives at the beginning of a project. One of the best examples of QA preplanning is the U.S. EPA's requirement that QA plans be prepared before programs are started.

Terminology Problems

The group agreed that the terminology used to statistically describe analytical data is often confusing to the chemist as well as to the data user. A special committee of the American Chemical Society attempted to resolve some of the confusion in environmental analysis, publishing a set of guidelines for data acquisition and data quality evaluation (Keith et al., 1983). While this reference is very useful, workshop participants generally felt that the examples used to explain terminology were often confusing.

More specific to the Great Lakes, the International Joint Commission (IJC) has developed some special terminology to be used with its reference sample program that has caused some misunderstandings. In particular, the "t" and "w" codes, which are used in conjunction with low level data, have caused some confusion. The "t" code is the limit of quantitation (i.e., results below "t" are tentative), and the "w" code is the reporting increment (analogous to the limit of detection). While at first hard to understand, the use of these "t" and "w" terms seemed reasonable to the workgroup participants.

Keith et al. (1983) defined the limit of detection (LOD) as the lowest concentration level that can be determined to be statistically different from a blank (i.e., LOD = 3σ, where σ is determined from replicates near a blank). LOD definitions vary between Canada and the U.S. EPA. Definitions also vary among chemical disciplines (Foley and Dorsey, 1984). King observed that an analyst's idea of the LOD differs from a statistician's definition; furthermore, he warned that the analytical detection limit should not be confused with the instrumental detection limit. Several other comments were made about the LOD. The group agreed that as one approaches the LOD, accuracy goes down.

Keith et al. (1983) define the limit of quantitation (LOQ) as the level above which quantitative results may be obtained with a specified degree of confidence (i.e., LOQ = 10σ). This definition was not questioned by the workgroup. W. Sonzogni commented that, for enforcement-type work, one is often dealing with numerical standards below the LOQ. It was also brought out that reported LOQs and LODs are often estimated, as opposed to being determined statistically.

Another terminology problem cited was the differentiation between standard deviation and relative standard deviation. Everyone agreed that relative standard deviation usually increases as the concentration decreases. Some questioned whether the absolute standard deviation is likely to be larger when dealing with low levels of analyte. Most thought it would, but were not sure of the significance of the increase. If the absolute standard deviation did not increase, one would not have to determine σ at the low end of the analytical range. Another point was that the σ for a specific analyte will vary depending on the sample matrix. Thus, LODs or LOQs will be sample matrix-dependent.

How to Report Low Level Data

The workgroup agreed that chemists should supply as much information as possible to users; it is up to the user to interpret that data. D. King emphasized that all values above the LOD should be reported, even if they fall below the LOQ. He did not advise reporting values less than 3σ (i.e., values below the LOD should be reported as such along with the LOD). The IJC's "t" and "w" codes seemed reasonable to use for qualifying data. Chemists were typified as being fairly conservative about reporting their data. The group felt that chemists should not rely on gut feelings about the reliability of their data (e.g., setting arbitrary limits) or worry that a value will be misused if reported. On the other hand, no single value should be used for enforcement purposes, especially if it falls below the LOQ.

The workgroup also agreed the analyst should report data with a σ that is determined on a spike that is close to five times the LOD. This stipulation for σ is important, because most organic results are near the LOD. Some group members did not think the analyst needs to report σ for every sample. Instead, σ could be calculated for a batch of samples, or once for a client. The group agreed that, for low-level organic analysis, it is important to determine and report σ for key analytes in different matrices. Most labs do not currently do this because it would take considerable time and effort to develop such data; however, such work may be required in the future. The result will be an increase in the cost of analyses, though overall the data quality will be improved.

Another topic of discussion was whether samples should be corrected for blanks. The consensus was to give the users the option of using the blank data to make corrections. Typically, reagent blanks are corrected for, though matrix blanks are only used to correct for matrix spikes. Some problems with blanks were identified. Volatile blanks have a short holding time (i.e., 2 weeks), and trip blanks are easily contaminated.

It was generally agreed that the percent recovery of a matrix spike, while an important measurement, does not necessarily foretell sample accuracy. According to W. Sonzogni, most environmental results are not corrected for recoveries; this has been the convention in the environmental field. The Wisconsin State Laboratory of Hygiene reports percent recovery for a batch of samples. The use of surrogate spikes (i.e., isotopic compounds) calls for percent recovery corrections. If the surrogates decrease by 50 percent, some analysts indicate this as low recovery. Though R. Hesselberg (U.S. FWS Great Lakes Fishery Laboratory, Ann Arbor, Mich.) thought surrogate spikes were useful for water samples, he saw problems with applying them to sediments.

The U.S. EPA has suggested that internal spikes be added in the field. A few groups at the meeting spike samples in the field to check for adsorption of contaminants onto the sample containers. However, specially trained field people are needed to do this, and one also needs to know the volume of sample being spiked.

The procedure used to determine recoveries when dealing with sediments can affect the results. For example, subtle differences in extraction techniques and the amount of time the spike is allowed to stay in the sample can affect results. Some participants thought sediment data should be reported on a dry weight basis instead of a wet weight basis. However, most results are reported on a wet weight basis.

Computers

Computers are becoming increasingly important for instrument control, data manipulation and data reporting. For organics analysis, the trend will be towards the use of computers in big production labs; this will enable expensive equipment to be used more efficiently.

The trend toward computerization will also promote greater reporting and use of quality control data. The ability to report data in various forms will allow the user to see the quality control data more conveniently. Greater access by the data user can only increase the demand for QC information. Computer systems currently used by the workgroup participants included those manufactured by Nelson Analytical, Perkin-Elmer, Burroughs (Spectrophysics) and Digital Equipment Corporation.

For the Great Lakes organic chemist, the use of chromatographic computer systems will be especially important. New chromatography software can provide control over the collection, data reduction and data reporting process. Unlike the integrators that are used in most laboratories, computerization will allow the storage of all raw data collected by the instrument. Data reduction (or data analyses) routines can then be used to optimize data quality, among other things.

The group warned against a tendency toward overconfidence in computer results. The chemist should not use the computer as a "black box." He/she should check all results and make sure the results are reasonable. Particular attention should be given to the treatment of outliers and significant figures. The consensus of the group was that computers have become a valuable tool in the laboratory, but they cannot replace the chemist. In fact, the chemist will be busier than ever interpreting computer output.

Fish Tissue Check Samples

The use of fish tissue check samples was also discussed. Check samples should fall in the range of contaminants the analyst expects to measure. The U.S. EPA uses freeze-dried

fish for their check samples. However, according to D. King, there are problems associated with using freeze-dried fish because they do not simulate the frozen fish tissue normally encountered in Great Lakes work.

Frozen fish tissue can be used as a check sample because it is stable over a long period of time. R. Hesselberg reported he had a 4 kg control sample of fish tissue that lasted for four years. He measured the stability of DDT and PCB compounds in Lake Michigan lake trout. From four years of data, he concluded the samples were quite stable; only a slight decrease in concentration was noted. Thus, it appears that labs doing trace organic analyses of fish tissue can prepare long-lasting reference samples for their own use.

Sediment Sampling

The nonhomogeneity of sediments makes them difficult to sample meaningfully. The fraction of sediment being sampled needs to be differentiated. For monitoring purposes, the analyst may want to use different sediment units (e.g., mg/L^2) so that sediment type will be independent of depth.

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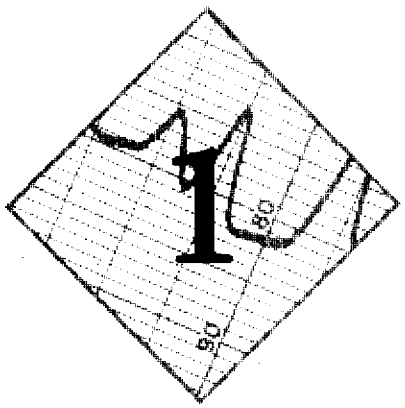

A Practical Sampling and Extraction System for the Quantitative Analysis of Sub-ηg/L Organochlorine Contaminants in Filtered Water and Suspended Solids

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A Practical Sampling and Extraction System for the Quantitative Analysis of Sub-ng/L Organochlorine Contaminants in Filtered Water and Suspended Solids

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ABSTRACT

An apparatus and procedure is described for the collection, filtration and subsequent extraction of 20 L water and suspended solid samples using readily available, inexpensive and sturdy equipment. Water collection, filtration and extraction of both phases can be accomplished in less than one hour. Recoveries of selected representative organochlorine contaminants spiked into "organic free" water and Lake Ontario water at environmentally realistic levels are presented.

INTRODUCTION

Numerous government agencies and private contracting laboratories in Canada and the United States are deeply involved in the complex task of analyzing water samples in the Laurentian Great Lakes watershed for a large and growing list of man-made organic contaminants. The purposes of these analyses are numerous and include loading estimates and studies on the fate and effects of specific contaminants.

Although analytical techniques have become extremely sensitive and sophisticated in recent years, there still exists a requirement for a practical large-volume water sampling and extraction system. Such a system is needed, when contaminants are present at low or sub-ng/L concentrations, in order to make meaningful loading estimates or quantitative assessment of the fate and effects of the contaminants on the aquatic ecosystem.

At the present time, a number of different sampling and extraction techniques are in use by the various laboratories, which makes the intercomparison of data sets from different laboratories difficult and sometimes impossible. The techniques range from the simple but inadequate extraction in a 4 L solvent bottle, to the combined use of a continuous flow centrifuge for suspended solids with the 200 L APLE (McCrea and Fischer, 1984) solvent extraction system. The latter technique is extremely sensitive but very time-consuming and bulky. Other systems with attractive features are sometimes complex and not widely available at the present time.

EQUIPMENT AND MATERIALS

* 20 L (5 U.S. gallons) stainless steel pressure containers, used to collect and store samples (Spartanburg Challenger VI, Spartanburg Steel Products, Inc.).

* 20 L stainless steel pressure container used as extraction reservoir (Millipore Cat. No. XX6700P20, Millipore Corp.).

* 142 mm stainless steel pressure filter holder (Millipore Cat. No. YY3014236, Millipore Corp.).

* 142 mm diameter, binder-free glass fiber filters, 0.3 μm nominal pore size (Gelman Cat. No. 61635, Gelman Sciences Inc.).

* Prepurified grade nitrogen, two-stage pressure reducing valve and 1/4" OD polyethylene tubing to pressurize the containers (any convenient supplier).

* Stainless steel quick-disconnect hose connections for the pressure containers (available with the pressure containers).

* Variable-speed laboratory bench stirrer, speed controller and impeller (Fisher Dyna-Mix, Fisher Scientific).

* Corrugated teflon flexible hose for transfer of water and extract (Penntube CT flex No. 400, Dixon Industries Corp.).

* Syphon tube: 1/4" OD teflon, and flexible "tygon"-type tubing (readily available from numerous sources).

* Solvents: Dichloromethane (DCM), 2,2,5-trimethylpentane and n-hexane, all distilled in glass, pesticide grade (Caledon Laboratories, Ltd.)

* 1 L glass bottles, narrow neck with teflon-lined screw caps; 50 ml glass culture tubes with teflon-lined screw caps for water and suspended solids extracts (any supplier).

* Anhydrous Na_2SO_4 (J.T. Baker Chemical Co., Ltd.)

* Various laboratory glassware.

PREPARATION OF EQUIPMENT

Two kinds of 20 L pressure containers are used. The sampling and storage containers are inexpensive stainless steel beverage pressure tanks and are modified by removal of all the internal pressure shut-off seals and replacing the dip tube seals with teflon replacements. The internal seals of the stainless steel pressure hose connectors are likewise removed. This avoids rubber/water contact and possible contamination of the sample.

The sample volume is most easily adjusted and measured by inserting a snug-fitting short piece of teflon tube in the storage tank inlet tube. A syphon attached to this will automatically reduce the water level to the

height of the bottom of the inlet tube. This adjusted volume was found to be 18.057 L ± 0.48 percent on a sample of five storage tanks.

The extraction container is a 20 L Millipore stainless steel pressure tank, chosen because it is equipped with two extra NPT threaded ports on the top surface. A 15 cm long by 1.25 cm diameter metal rod may thus be threaded at one end and installed in one of the ports as a support stand for the stirrer motor. The other spare port is plugged.

Transfer hoses for both water and DCM extract are teflon and have appropriate stainless steel connectors to mate with the corresponding connectors on the filter holder and pressure tanks.

Nitrogen for pressurizing the containers is most conveniently supplied through 1/4" OD polyethylene tubing with a toggle shut-off valve installed near the pressure container connector.

The stirrer is set up to clamp onto the support rod described above with a laboratory clamp such that it can easily be removed and replaced. The stirrer shaft should reach near the water-DCM interface, and the impeller should have large blades with a steep pitch so that a medium stirring speed will mix the tank contents thoroughly without producing a persistent emulsion in samples with high levels of natural organic compounds. A 2 L polyethylene graduate provides a convenient receptacle for the stirrer while not in use in the extractor.

METHOD

Fill the storage tank to overflowing and then adjust the volume precisely with a syphon as previously described. The amount syphoned off is approximately 1 L and may be used to determine the concentration of suspended solids.

Filter into the extraction container at 15 psi through a 0.3 µm glass fiber filter (binder free) which has been previously heated to 350–400°C.

Suspended Solids

Fold the damp filter in half, roll into a tube and place in a 50 ml screw-capped culture tube. Add 20 ml DCM. Cap and store at least 24 hours. Break up filter with a spatula and pour DCM extract into a fritted glass filter tube containing 2–5 cm anhydrous Na_2SO_4. Aspirate under water pump vacuum into a 250 ml RB flask. Add a further 20 ml DCM to the culture tube, cap and shake. Add the DCM to the Na_2SO_4 and aspirate as before. Repeat this procedure twice more and add a final 20 ml DCM directly to the Na_2SO_4. Add 5 ml 2,2,5-trimethylpentane to the 100 ml combined and dried extract as a keeper. The extract is ready for further concentration, cleanup and analysis.

Filtrate

Add 600 ml of DCM and stir for 10 minutes at a speed sufficient to ensure thorough mixing of the contents. Remove the stirrer and allow to settle 2–5 minutes, depending on emulsion forming potential of sample. Transfer DCM extract to a 1 L screw-capped glass bottle, using 1–2 psi nitrogen pressure.

Decant excess water from glass bottle back into the extraction container. Repeat this procedure twice more, using 100 ml DCM each time. After the final transfer, the excess water may be conveniently left in the 1 L bottle until the extract is further concentrated, cleaned up and analyzed. This part of the procedure may vary to suit the analysis requirements.

Sample Concentration and Cleanup

In this study, the following procedure was used:

The combined DCM extract was dried by passing through a 5 cm bed of anhydrous Na_2SO_4, and 5 ml of 2,2,5-trimethylpentane was added as a keeper. Both filtrate and suspended solid extracts were evaporated to approximately 2 ml on a rotovapor at 25°C. The concentrated extracts were allowed to flow by gravity through pasteur pipet minicolumns containing 2.5 cm 44 percent H_2SO_4 on silica gel topped with 0.5 cm anhydrous Na_2SO_4.

The extracts were eluted from the columns with 2x2 ml of n-hexane. The combined eluant was collected in a 10 ml Kuderna Danish tube and concentrated to a final volume of 1 ml under a stream of dry nitrogen on an N-Evap concentrator at 25°C. The 1 ml extract was analyzed for the spiked organochlorine contaminants by electron-capture gas chromatography with capillary columns.

RESULTS AND DISCUSSION

Selected organochlorine contaminants dissolved in acetone were spiked, in duplicate, into "organic free" water and Lake Ontario water to test recovery efficiency.

The "organic free" water was prepared by passing laboratory distilled water through an XAD-4 resin column. Lake Ontario water was collected at 1 m depth from the 1-mile marker buoy off Hamilton, Ont., and found to have 1.9 mg/L suspended solids.

100 µl of the spike solution was added to 17.3 L "organic free" water, stirred 5 minutes, allowed to stand 30 minutes and extracted as previously described. Recoveries, shown in Table 1, ranged from 81 to 132 percent, with a mean value of 102 percent.

17.3 L Lake Ontario water was spiked with 100 µl of the contaminant solution. The resulting solution was stirred for 16 hours to allow the contaminants to equilibrate between the liquid and solid phases. Filtration and extraction were performed as previously described. Lake Ontario water (17.3 L) was also extracted, unspiked, to determine blank values.

Recoveries of contaminants from Lake Ontario water and suspended solids are shown in Table 2. The compounds are listed in order of gas chromatographic elution, which is also approximately the order of decreasing solubility in water. This trend is clearly reflected in recoveries from the suspended solids, where 3 to 54 percent of the higher substituted PCBs and octachlorostyrene were observed, while the chlorobenzenes and chlorotoluenes were found almost exclusively in solution.

TABLE 1
Recovery of Spiked Organochlorine Contaminants from "Organic Free" Water

Compound	Amount Spiked (ng/L)	Percentage Recovery		
		Spike 1	Spike 2	Mean
1,2,4-trichlorobenzene	0.83	88	95	92
1,2,3-trichlorobenzene	0.42	110	121	116
2,4,5-trichlorotoluene	0.94	104	87	96
2,3,6-trichlorotoluene	0.93	93	97	95
1,2,4,5-tetrachlorobenzene	0.59	113	106	110
1,2,3,4-tetrachlorobenzene	0.24	120	107	114
Pentachlorobenzene	0.12	100	98	99
Pentachlorotoluene	0.18	112	107	110
Hexachlorobenzene	0.14	99	89	94
2,2',5-trichlorobiphenyl	0.79	83	88	86
2,2',5,5'-tetrachlorobiphenyl	0.52	109	112	111
2,2',3,3'-tetrachlorobiphenyl	0.34	128	134	130
Octachlorostyrene	0.14	102	91	97
2,2',4,4',5,5'-pentachlorobiphenyl	0.42	81	94	88
2,2',4,4',5,5'-hexachlorobiphenyl	0.33	93	132	113
Mirex	0.33	87	101	94
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	0.23	85	96	91
Overall mean recovery				102

Total recoveries were good, with a mean total recovery of 86 percent. A noticeably lower total recovery for the penta- to octachlorobiphenyls and mirex suggests some lack of recovery from the suspended solids, since these compounds were efficiently recovered from "organic free" water with no suspended solids.

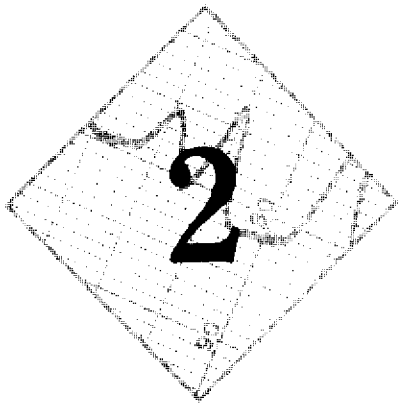
REFERENCE

McCrea, R.C., and Fischer, J.D. 1984. Evaluation of an aqueous phase liquid-liquid extractor (APLE) for the determination of organochlorine contaminants. IWD Environment Canada Report, 18 pp.

TABLE 2
Recovery of Spiked Organochlorine Contaminants from Lake Ontario Water and Suspended Solids

Compound	Amount Spiked (ng/L)	Percentage Recovery				
		Filtrate		Suspended Solids		Mean Total
		Spike 1	Spike 2	Spike 1	Spike 2	
1,2,4-trichlorobenzene	0.83	104	101	ND	ND	103
1,2,3-trichlorobenzene	0.42	117	119	ND	ND	118
2,4,5-trichlorotoluene	0.94	78	92	ND	ND	85
2,3,6-trichlorotoluene	0.93	85	77	ND	ND	81
1,2,4,5-tetrachlorobenzene	0.59	99	94	ND	ND	97
1,2,3,4-tetrachlorobenzene	0.24	91	122	ND	ND	107
Pentachlorobenzene	0.12	68	87	ND	ND	78
Pentachlorotoluene	0.18	105	95	ND	ND	100
Hexachlorobenzene	0.14	88	62	<1	ND	75
2,2',5-trichlorobiphenyl	0.79	65	67	<1	3	68
2,2',5,5'-tetrachlorobiphenyl	0.52	93	81	13	16	102
2,2',3,3'-tetrachlorobiphenyl	0.34	93	80	20	16	105
Octachlorostyrene	0.14	59	70	54	35	109
2,2',4,4',5,5'-pentachlorobiphenyl	0.42	54	35	19	11	60
2,2',4,4',5,5'-hexachlorobiphenyl	0.33	43	34	19	21	59
Mirex	0.33	28	35	23	25	56
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	0.23	36	38	19	23	58
Overall mean recovery						86

ND = Not Detected



Application of Soft Independent Method of Class Analogy (SIMCA) in Isomer-Specific Analysis of Polychlorinated Biphenyls

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Application of Soft Independent Method of Class Analogy (SIMCA) in Isomer Specific Analysis of Polychlorinated Biphenyls

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A principal components multivariate statistical approach (SIMCA) was evaluated and applied to interpretation of isomer specific analysis of polychlorinated biphenyls (PCBs) using both a microcomputer and a main frame computer. Capillary column gas chromatography was employed for separation and detection of 69 individual PCB isomers. Computer programs were written in ANSII MUMPS to provide a laboratory data base for data manipulation. This data base greatly assisted the analysts in calculating isomer concentrations and data management. Applications of SIMCA for quality control, classification, and estimation of the composition of multi-Aroclor mixtures are described for characterization and study of complex environmental residues.

Polychlorinated biphenyls (PCBs) are a class of synthetic chlorinated compounds with a total of 10 possible positions for chlorine attachment and 209 possible isomers, although the number of constituents observed in technical formulations is much smaller (1-3). PCBs have been produced by several industries worldwide in the form of technical formulations (4). Most PCBs produced in the U.S. originated as one of several products designated as Aroclors and were previously manufactured by the Monsanto Chemical Company (5). The major Aroclors produced were designated Aroclors 1242, 1248, 1254, and 1260, where the last two digits designate the percentage of chlorine by weight in the material. Each Aroclor is characterized by a somewhat different distribution of constituents having a characteristic chromatographic profile of about 70 to 100 chromatographic peaks (1,3).

After PCBs were identified in 1966 as pollutants in fish and wildlife (6), they were soon recognized as global pollutants

widely distributed in the biosphere and in tissues of most forms of life, including man (7-13). Concern about the occurrence of PCBs in the environment centers on their persistence, bioaccumulaton in organisms and toxicity (14, 15).

Of additional concern is the thermal conversion of PCBs to polychlorinated dibenzofurans, especially when PCB--filled electrical transformers are involved (16). Further, PCB isomers with no chlorine atoms substituted in the ortho, ortho'-biphenyl positions are particularly potent as inducers of aryl hydrocarbon hydroxylase activity. The potency of certain dibenzofurans and non-ortho, ortho' chlorine substituted PCBs in inducing activity of these enzymes is similar to that of the highly toxic 2,3,7,8-tetrachloro-dibenzo-p-dioxin (15, 17, 18).

Residues of PCBs exist as complex mixtures in almost every segment of the environment, and characterization of these residues poses a difficult challenge to the analyst. Routinely, PCBs have been quantitated by comparing selected peak areas observed in samples with those in one of several Aroclor mixtures (19, 20). Packed column gas chromatography has usually been used in these analyses, even though this technique provides poor resolution of individual isomers and congener groups (21). The problems associated with characterizing metabolically altered or weathered PCBs is a formidable task that requires an analytical approach with enhanced resolution.

Both the degree and position of chlorine substitution on the biphenyl rings influence the physical-chemical and toxicological behavior of the individual PCB constituents (15). When PCBs are released into the environment, the original isomer distribution pattern of any PCB formulation may be altered as a result of specific interactions with the environment (14, 22, 23).

Further complications are encountered in describing the residue profiles when more than one Aroclor mixture is encountered in an ecosystem. Thus, it is important to consider not only the total PCB concentration in a sample, but also to characterize the distribution of individual PCB isomers present in a sample.

Because information defining the distribution of the various PCB isomers among the compartments of lotic aquatic ecosystems (streams) is limited, the Columbia National Fisheries Research Laboratory began a detailed analysis of the distribution of PCBs in a segment of the mainstream and of a pool of the Upper Mississippi River. The objectives of this study were to model and understand the hydrological and biological distribution of PCBs, and to aid in assessing the environmental dynamics of PCBs in the Upper Mississippi River. Ecologically, Lake Onalaska, the river pool being studied, provides a major staging and feeding area for migration of the canvasback (Aythya valisineria) in the Mississippi River Flyway. About 1200 samples were analyzed by capillary gas chromatography for 69 PCB isomers. Sample types included water, suspended sediments, sediments, fish, benthos, and plants.

The amount and complexity of data resulting from these analyses prompted us to search for an improved method for characterizing and comparing information gathered from multicomponent analyses of large numbers of samples. Multivariate statistics were applied in the process of characterization of large numbers of complex residues. Such methods have been referred to as Chemometrics (24).

Soft Independent Method of Class Analogy (SIMCA), a pattern recognition technique based on principal components (25) was selected to evaluate and apply to the problems of establishing similarities among sample residue profiles. The development of a laboratory data management system to assist in the calculation and organization of results greatly enhanced the feasibility of this approach (26).

Materials and Methods

Gas Chromatographic Analysis. We used temperature programmed glass capillary gas chromatography to separate PCB residues. Use of an electron capture detector required an efficaceous sample cleanup for isomer quantitation (27). These combined techniques offered enhanced separations and enabled us to identify and quantitate individual PCB constituents (1, 27). Schwartz (27) separated more than 100 constituents from a 1:1:1:1 mixture of Aroclors 1242, 1248, 1254, and 1260.

A Varian Model 3700 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with a ^{63}Ni electron capture detector was used for sample analysis. The column was a glass capillary coated with a hydrocarbon stationary phase having the formula $C_{87}H_{176}$ and structure (24,24-diethyl-19,29-dioctadecylheptatetracontane) (Quadrex Corp., New Haven, CT). The column was a wall coated open tube (WCOT) (0.25 mm x 100 m) having a film thickness of approximately 0.3 microns and 3670 theoretical plates per meter. A 30 m section of the column was conditioned at 260 °C overnight and the temperature program rate, linear gas velocity, and detector makeup gas were determined by peak resolution of PCB isomers.

Chromatographic conditions were similar for all sample analyses. The temperature program was as follows: initial temperature 120 °C programmed at 2 °C/min to a final temperature of 255 °C; injector 220 °C; and detector temperature 320 °C. The carrier gas, hydrogen, was maintained at a column head pressure of 20 psi. The electron capture detector makeup gas was nitrogen with a flow rate into the detector of 17 mL/min.

The same chromatographic parameters were used in determining the molar response of the individual PCB isomers except, that the area responses were determined with a flame ionization detector. The flow rates of the hydrogen and air combustion gases were 30 and 300 mL/min, respectively. Response factors needed to

calculate individual congener concentrations were calculated by using the ratios of flame ionization to electron capture response for each standard peak (28, 29).

To integrate the digitized electrical signals from the gas chromatograph, we used a PDP 11/34 computer (Digital Equipment Corp., Maynard, MA) and the computer program PEAK-11, supplied from that corporation. An operational amplifier with variable gain was interfaced to the the gas chromatographic amplifier output to amplify the signal from the electron capture detector. Data were acquired at the rate of 20 points per sec and the integration and gas chromatographic operations were controlled by a Varian Autosampler (Model 8000), which also delivered a calibrated amount of sample to the gas chromatographic injection port. Extensive quality control and verification of analyses resulted in reproducible results (26).

Laboratory Data Base System. Data representing integrated peak areas were transfered to a second PDP-11/34 computer by magnetic tape, where programs written in Digital Standard MUMPs (DSM-11) created and maintained the chromatography data base. MUMPs was selected for development of this data base because this language is structured to efficiently deal with sparse arrays. The computer was configured with 96k words of memory, 20M words of disk storage, and a 9 track magnetic tape.

The data base programs accomplished the following tasks: (1) generation of retention values for each PCB isomer peak (relative to p,p'-DDE) in every chromatogram; (2) comparison of an Aroclor standard, or any mixture of Aroclors, to a master file of isomers containing structural identification, number of ortho, ortho'-chlorine substituents, and flame ionization response factors; (3) generation of linear and logarithmic (to accomodate non-linear detector response) regression equations for each of the constituents (69, 92, or 105 isomers) on the basis of Aroclor concentration and area of each component; (4) assistance in resolving ambiguities in the process of matching standard peaks to sample constituents; (5) generation of numerous reports on sample processing, including status of sample data, i.e., samples logged in, raw chromatograpic data matched to standards, concentration of constituents calculated from regression curve, data archived to magnetic tape, and (6) preparation and magnetic tape storage of final reports on concentrations of up to 105 isomers in samples, with notations on whether the measured concentration fell within, above, or below the calibration range for that constituent. A flow chart for the data analysis scheme is outlined in Figure 1.

In addition to these functions, other data base programs provided output formating and retrieval of concentration data from completed analytical reports and transfer of these data onto magnetic tape for subsequent examination by SIMCA programs. Additional features of the pattern recognition data management

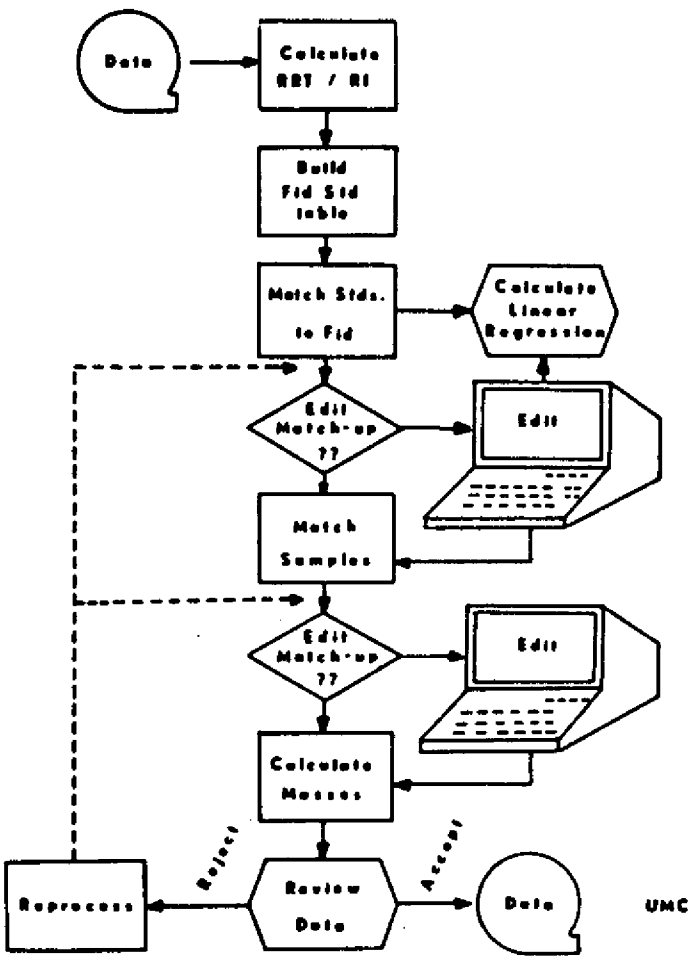


Figure 1. Flow Diagram of PCB Data Base.

subprograms provided peak frequency and ranking summaries within each selected sample set. Programs were written for graphic plots of concentrations of PCB isomer components and related statistics.

A summary of these functions follows:

1. Build the matrix of samples to output
2. Output the summary matrix of sample identity
3. Edit the summary matrix
4. Build the set of sample concentrations
5. Output the set of sample concentrations
6. Build the non-zero concentration matrices
7. Output the non-zero concentration matrices
8. Plot concentration histogram of a sample
9. Plot means and standard deviations
10. Generate principal components plots of data blocks

Results and Discussion

When large numbers of chromatograms are analyzed visually from chart paper or tabular data, it is difficult to detect minor (or perhaps major) significant differences that may be present in samples from different locations or differences that could be attributed to changing physiochemical processes. A typical gas chromatogram of a PCB mixture (Figure 2) represents a 1:1:1:1 mixture of Aroclors 1242, 1248, 1254, and 1260. It contains more than 100 component peaks, of which 69 were selected for use in these analyses.

An abreviated report from the analysis of the mixture of Aroclor standards just described is shown in Table I. The report indicates the individual isomer structure and degree of chlorination, ortho-, ortho-' substitution, isomer concentration, total concentration.

To illustrate the problems associated with evaluating such data, we conducted several studies with Aroclor standards and mixtures of these standards in an effort to determine what information could be readily obtained with the SIMCA method of pattern recognition (30-32). The following discussion illustrates some of the features of this approach and describes how the SIMCA method works when applied to Aroclor mixtures.

General Discussion of SIMCA. Consider a series of gas chromatograms obtained on a large number of samples (N). These samples can all contain the same number of constituents (P)--in the present study, 69 PCB isomers. Such data can be tabulated in matrix form as in Figure 3. Each row of the matrix is a chromatogram with the elements of the matrix, x_{ki}, representing concentration of peak i in sample k.

We first consider the hypothetical case when there is no variation in composition of the samples, either in relation to

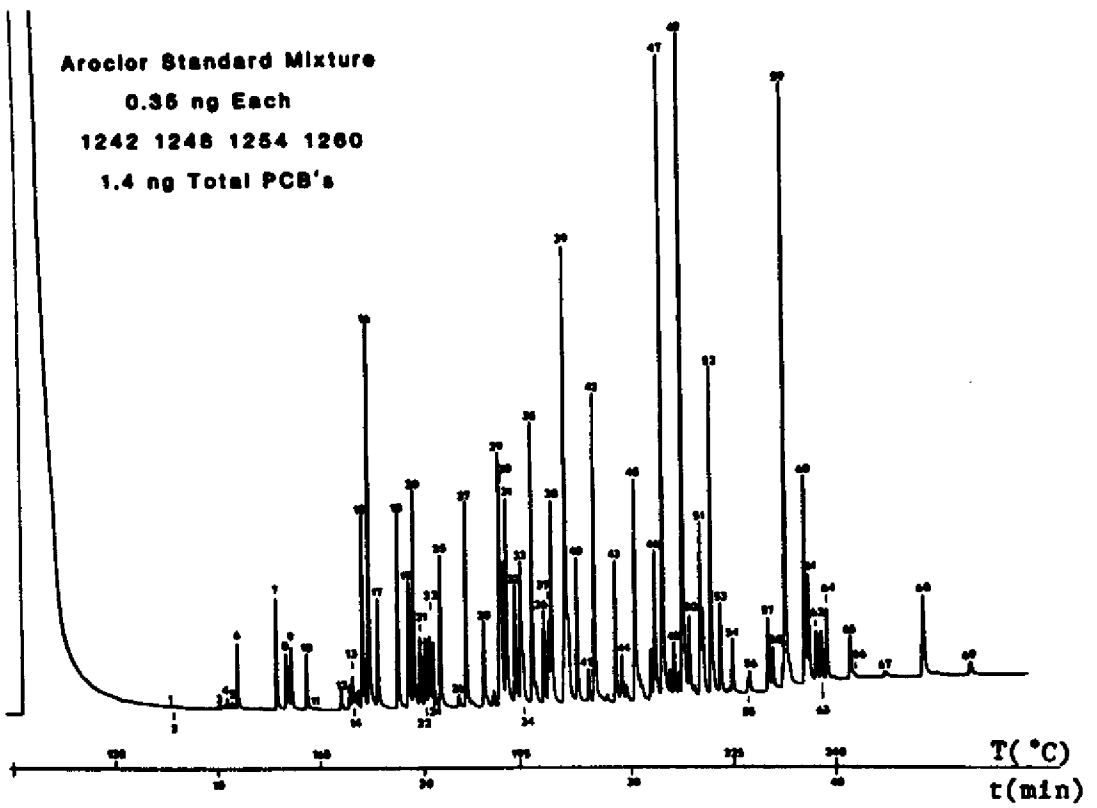


Figure 2. Capillary Gas Chromatogram of 1:1:1:1 Aroclor 1242:1248:1254:1260 Mixture.

the relative concentrations of constituents or in the absolute concentration of isomers. If our N samples are projected into P space, due to our prior requirement of identity, they would appear as a tight cluster, the only variation being due to measurement error.

Table I. Isomer Structure Assignment, Response Factors, and Concentration in 1:1:1:1 Mixture of Aroclor 1242;1248;1254 and 1260.

Peak	RRT[1]	Chlorines Total	Chlorines Ortho	Structure	F_i[2]	Conc.$_i$ ug/g
1	.2681	2	2	2,2'	.0098	2.53E-02
2	.2706	2	2	2,6	3.00E-5	9.00E-05
3	.3599	2	1	2,5	.0012	3.31E-03
4	.3695	2	1	2,4	.0046	1.40E-02
5	.3776	3	-	3CL	.0029	9.18E-03
6	.3903	2	1	2,4'	.0206	6.86E-02
7	.4615	3	2	2,2',5	.0468	1.53E-01
8	.4810	3	2	2,2',4	.0150	4.91E-02
9	.4873	3	2	2,2',3	.0152	5.05E-02
10	.5173	3	2	2,4',6	.0096	3.10E-02
11	.5352	2	0	4,4'	.0039	1.03E-02
12	.5803	4	3	2,2',5,6'	.0062	2.01E-02
13	.6031	3	1	2,3',5	.0076	1.93E-02
14	.6107	4	3	2,2',4,6'	.0040	1.39E-02
15	.6252	3	1	2,4',5	.0377	1.21E-01
16	.6390	3	1	2,4,4'	.0313	8.57E-02
17	.6525	3	1	2,3',4	.0106	3.42E-02
18	.6933	4	2	2,2',5,5'	.0623	2.00E-01
19	.7145	4	2	2,2',4,5'	.0226	7.33E-02
20	.7201	4	2	2,2',3,5'	.0574	1.83E-01
21	.7387	4	2	2,2',4,4'	.0080	2.72E-02
22	.7441	4	2	2,2',3,4'	.0085	2.75E-02
23	.7502	4	2	2,2',3,3'	.0125	4.12E-02
24	.7596	4	2	2,2',3,4	.0095	3.08E-02
25	.7758	4	2	2,3,4',6	.0159	5.10E-02
26	.8069	3	0	3,4,4'	.0034	1.16E-02
27	.8237	5	3	2,2',3,5,6	.0429	1.37E-01
28	.8547	4	3	2,2',4,5,6'	.0107	3,48E-02
29	.8897	4	1	2,3',4',5	.0351	1.10E-01
30	.8990	5	3	2,2',3,4,6'	.0100	3.14E-02
31	.9043	5	-	5CL	.0252	7.80E-02
32	.9174	4	1	2,3,3',4'	.0112	3.66E-02
33	.9320	4	1	2,4,4',5	.0236	8.01E-02
34	.9353	6	4	2,2',3,3',6,6'	.0002	6.28E-04
35	.9596	5	2	2,2',4,5,5'	.0560	1.77E-01

Table I. Continued

Peak	RRT[1]	Chlorines Total	Chlorines Ortho	Structure	F_i[2]	Conc.$_i$ ug/g
36	.9832	5	2	2,2',4,4',5	.0176	5.78E-02
38	.9913	5	2	2,2',3,3',5	.0122	3.98E-02
39	1.0205	5	2	2,2',3,3',4	.0571	1.83E-01
40	1.0499	6	3	2,2',3,5,5',6	.0192	6.10E-02
41	1.0726	6	3	2,2',3,4',5,6	.0021	6.59E-03
42	1.0815	6	3	2,2',3,4',5,6'	.0485	1.54E-01
43	1.1181	6	3	2,2',3,4,5',6	.0144	4.62E-02
44	1.1385	5	1	2,3',4,5,5'	.0044	1.40E-02
45	1.1613	5	1	2,3,3',4,4'	.0274	8.50E-02
46	1.1938	6	-	6CL	.0062	2.00E-02
47	1.2218	6	2	2,2',4,4',5,5'	.0425	1.45E-01
48	1.2382	6	2	2,2',3,3',5,5'	.0004	1.84E-03
49	1.2561	6	2	2,2',3,4,4',5'	.0273	8.79E-02
50	1.2730	7	3	2,2',3,4',5,5',6	.0033	1.01E-02
51	1.2998	7	3	2,2',3,3',4,5,6	.0145	4.52E-02
52	1.3146	7	3	2,2',3,3',4,5,6'	.0080	2.48E-02
53	1.3228	7	3	2,2',3,4,4',5',6	.0070	2.18E-02
54	1.3358	7	3	2,2',3,3',4,5',6	.0024	7.49E-03
55	1.3604	7	3	2,2',3,3',4,4',6	.0048	1.51E-02
56	1.3936	8	3	2,2',3,4,4',5,5',6	7.0E-5	2.24E-04
57	1.4280	6	1	2,3,3',4,4',5	.0028	8.73E-03
58	1.4444	7	2	2,3,3',4',5,5',6	8.0E-5	2.51E-04
59	1.4693	7	2	2,2',3,4,4',5,5'	.0250	9.45E-02
60	1.5032	7	2	2,2',3,3',4,4',5	.0096	2.99E-02
61	1.5208	8	3	2,2',3,3',4,5,5',6	.0025	7.84E-03
62	1.5295	8	3	2,2',3,3',4',5,5',6	.0013	4.13E-03
63	1.5454	8	-	8CL	.0020	6.28E-03
64	1.5575	8	-	8CL	.0025	7.73E-03
65	1.5948	8	3	2,2',3,3',4,4',5,6	.0016	5.05E-03
66	1.6119	9	4	2,2',3,3',4,4',5,6,6'	5.0E-5	1.25E-04
67	1.6502	8	-	8CL	1.0E-5	3.13E-05
68	1.7155	8	2	2,2',3,3',4,4',5,5'	.0011	3.58E-03
69	1.7380	9	4	2,2',3,3',4,4',5,5',6	2.0E-5	6.71E-05
					Total	3.24E+00

[1]Retention Time Relative to p-,p'-DDE
[2]Ratio of Electron Capture to Flame Ionization Response

	Peak Number							
Sample number	1	2	3	.	.	i	.	P
1						.		
2						.		
3						.		
.						.		
k	.	.	.	.	.	x_{ki}		
.								
N								

Figure 3. Matrix Representation of Sample Analysis for P Peaks and N Samples (Chromatography Data Matrix).

Each sample can be modeled by Equation 1, where m_i

$$x_{ki} = m_i + e_{ki} \qquad (1)$$

is the mean concenration of each peak in the matrix and e_{ki} contains the error of measurement and error associated with the mathematical modeling of the data array (model error).

We now relax our requirement for identity and allow the samples to be similar. This is analogous to having a group of N samples of the same species, type of Aroclor, or mixture of Aroclors. Such data can be shown to be modeled by Equation 2,

$$x_{ki} = m_i + \sum_{a=1}^{A} t_{ka} b_{ai} + e_{ki} \qquad (2)$$

in which A-product terms have been added to account for the variation in the data (30). This is shown in 3-dimensions below in Figure 4.

This is a principal components model in which b_{ai} is the loading of peak i in term a, and t_{ka} is the score of object k in term a; b is a peak specific term and t is an object or sample specific term. The variation about the mean, m_i, can be random or systematic. If random variation is observed it can be due to measurement error, and this variation can be used in quality

assurance of the data. If the measured variation is systematic, it can be the result of class specific internal variation, which can be used in classification or correlation studies.

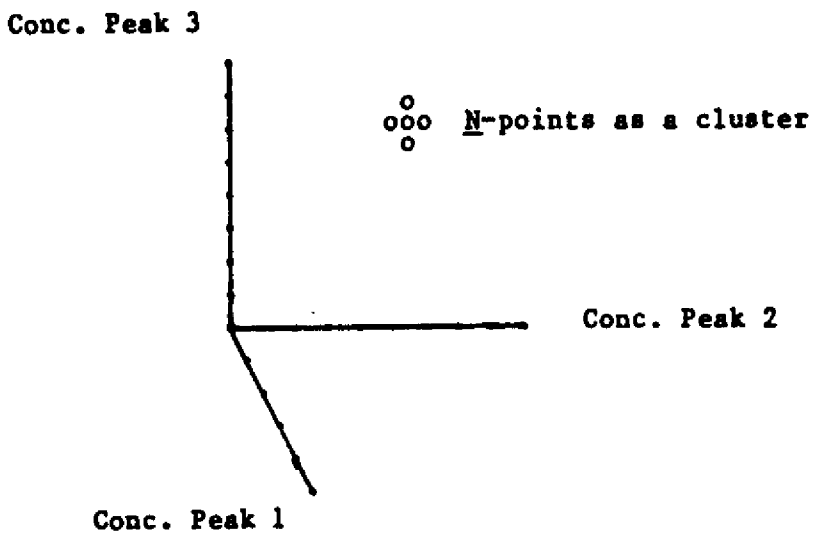


Figure 4. Clustering of a Class of Identical Samples.

Figure 5 illustrates the cases in which the data are represented by a point ($\underline{A}$=0), line ($\underline{A}$=1) or plane ($\underline{A}$=2). $\underline{A}$ is the number of product terms in Equation 2. Samples clustered in a point represent replicate analyses of a single sample in which there is no variation other than measurement error, and the product term in Equation (2) is 0. In these last two situations, the data vary about the mean, $\underline{m}_i$, and the position of each object on the line or plane given by the peak coordinates. An example of data that would form a line are those based on an analysis of a range of concentrations of a single Aroclor ($\underline{A}$=1). Data that could be represented in a plane result from the analysis of the fractional composition of two (or more) Aroclor mixtures ($\underline{A}$=2). In Fiqure 5, $\underline{q}$ designates the class number of the these hypothetical samples.

One can use principal components plots to visually inspect higher dimensional data. Their use is equivalent to projecting the higher dimensional data onto a two-dimensional plane. Such plots are helpful in interpreting chromatographic or other scientific data composed of many measurements (peaks or dimensions).

If it is known from the history of the samples illustrated in Figure 3 that the items represent $\underline{q}$-distinctly different groups or classes, a classification problem can be formulated. These classes may result from samples being derived from different locations or of different species, etc. In this study,

sample data from the analysis of five Aroclors were used (Aroclor 1242, 1248, 1254, 1260 and a 1:1:1:1 mixture of these four Aroclors). Together, these sample data compose five classes.

Using SIMCA, one can derive q-disjoint principal components models for distinct classes. This derivation is given in Equation 3 and a hypothetical set of data representing three classes (q=3) of data of increasing complexity is shown in Figure 5. The axes in Figure 5 may represent either the coordinates of the original data or those resulting from a transformation of the data such as will be discussed later in the section on data pretreatment. In the examples used in this paper, data have not been transformed. Disjoint principal components models are bounded by limits derived from the data used to calibrate the class model.

$$\underline{x}_{ki}^{q} = \underline{m}_{i}^{q} + \sum_{a=1}^{A} \underline{t}_{ka}^{q}\, \underline{b}_{ai}^{q} + \underline{e}_{ki}^{q} \qquad (3)$$

From the $\underline{e}_{ki}^{q}$-values in Equation 3, a standard deviation for each object can be calculated and from these a residual standard deviation for each class (q) can be obtained. Twice the residual standard deviation around the line or above and below the plane for a class defines a volume in P-space where classes have a high probability of occurrence. Classification of an unknown can be based on its projection into P-space and determination of its position. It may be inside one or none of the defined classes. This method has a number of other features that have previously been treated (32, 33) and we present only a limited discussion here. Principal components analysis and the closely related factor analysis, as applied to scientific data, have been well reviewed (25, 30).

Another feature of SIMCA that is of considerable utility lies in the assistance the technique provides in selecting relevant variables. Information contained in the residuals, $\underline{e}_{ki}$, can be used to select variables relevant to the classification objective. If the residuals for a variable are not well predicted by the model, the standard deviation is large. An expression defined as modeling power has been derived to quantitatively express this relationship. The modeling power (MPOW) is defined as:

$$\text{MPOW} = 1 - \underline{s}_i / s_{i,y}$$

where $\underline{s}_i$ is the standard deviation of a variable after being fitted to a model and $\underline{s}_{i,y}$ is the standard deviation before it is fitted to a class model. As MPOW approaches 1, the variable contributes strongly to class description.

Principal Components Plots. To obtain a visual representation of the data structure for the class or classes being examined, one can project the data onto a two-dimensional plane. A convenient plane for mapping is that described by the two first principal components. These plots are derived by plotting the t_{1i} and t_{2i} values for each sample and designating the location of the sample with the sample number. The axes are designated in these principal components plots as Theta 1 and Theta 2. Information about the similarity of samples is derived in most cases, by their close proximity in the principal components plot. Usually, separation of different classes are clear. By examining the clusters of samples in the graphical projections, the analyst gain insight into sample outliers from the principal components plots.

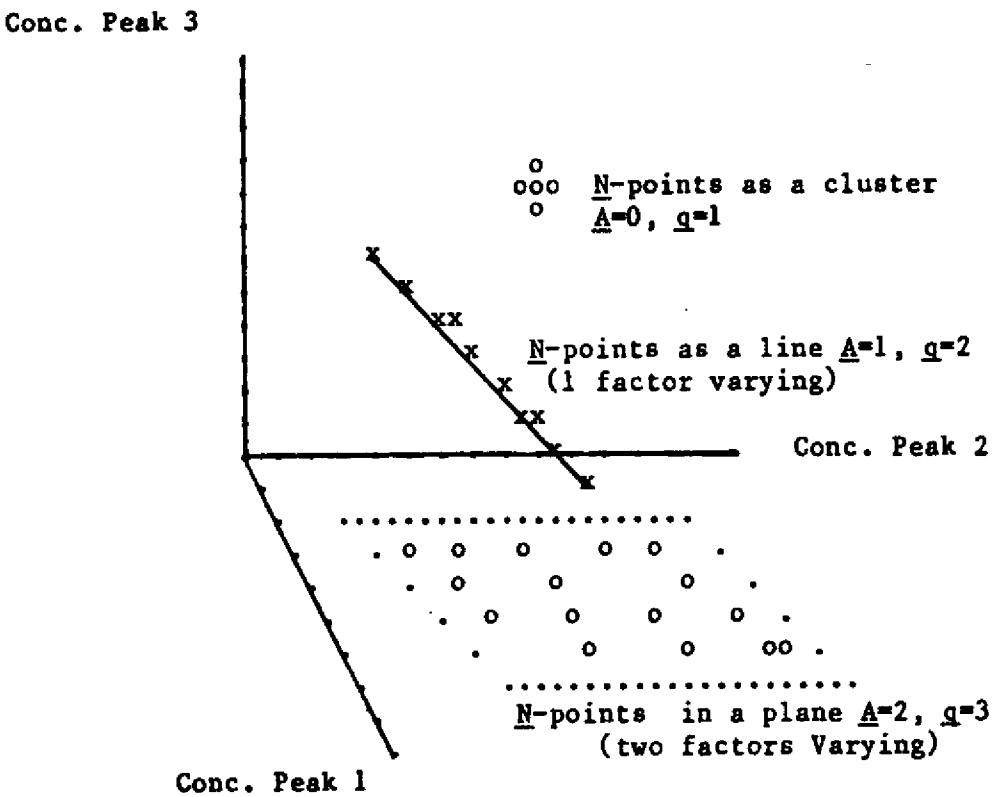


Figure 5. Clustering of Three Classes of Samples. Samples that are identical (A=0, q=1); or have one factor (A=1, q=2); or two factors varying (A=2, q=3). Class number = q and A = number or product terms in Equation 2.

Information about relation among variables in samples belonging to a data set can be obtained from an examination of plots of the sample loading terms (b_{1i} vs b_{2i}). In these plots the axes are designated Beta 1 and Beta 2. Variables responsible for certain samples being different from the main cluster of

samples are readily discerned. Three dimensional plots derived from the first, second, and third components can sometimes clarify additional relations among samples. Such a case is discussed later in discussions of modeling differences both in concentration and composition.

The similarity of samples can be evaluated by using geometrical constructs based on the standard deviation of the objects modeled by SIMCA. By enclosing classes in volume elements in descriptor space, the SIMCA method provides information about the existence of similarities among the members of the defined classes. Relations among samples, when visualized in this way, increase one's ability to formulate questions or hypotheses about the data being examined. The selection of variables on the basis of MPOW also provides clues as to how samples within a class are similar, and the derived class model describes how the objects are similar, with regard to the internal variation of these variables.

One must consider the number of product terms that should be included in a model. For chromatography data obtained from similar samples, it can be expected that the data will contain a high degree of correlation. In our experiments, two- or three-component models usually accounted for >90% of the variance in the data for a class of similar samples. Results from crossvalidation should be considered as the primary criteria in selecting the number of principal components to be extracted from a given data set (34).

In the principal components plots presented in this paper, the number plotted corresponds to the sample identification number given in the appendix. If more than one sample has the same locus in the score (Theta's) or loading plots (Beta's), the letter M is plotted. The values for the sample coordinates in the principal components plots can be listed by the SIMCA-3B program.

Data Pretreatment

A number of data pretreatments (scalings and transformations) are possible with SIMCA (and other methods). The appropriate scaling for a given data set is difficult to anticipate and depends on the nature of the problem being addressed as well as on the structure of the data being examined. This aspect of pattern recognition has not been been adequately investigated for gas chromatography data. In the applications discussed here, we have used two techniques--normalization and regularization.

Normalization. Each peak is weighted to its fractional (or percentage) composition. This weighting prevents samples with large total concentrations from heavily influencing an analysis. Also, this scaling technique focuses the classification on questions about variations in relative composition of samples independently of the absolute concentration measured. Although

instrumental limits of detection can strongly influence this data pretreatment, this feature, when used with caution, can be effective in characterization of the range of total concentrations that can be analyzed.

Regularization. Regularization, the autoscaling of Kowalski, (35) and scaling of Massart, (36) transforms the data so that the data set has a zero mean and a variance of one for each variable. This method equalizes the influence of peaks or measurements.

A risk with this approach is that if there are a large number of constant non-zero entries in the data matrix, they can act as binary variables and perhaps weight the analysis toward yielding trivial results. When cross validation is used, however, this risk is reduced.

Calculation of Composition of Mixed Aroclors

SIMCA can be applied to the problem of classification when attempting to correlate measurable effect variables with composition of the classified samples. In correlation analyses one may wish to determine how other sample variables, such as sediment composition, organic content, lipid concentration, etc., influence the composition of measured residues or concentrations of PCBs.

This determination cannot be made by using multiple regression techniques because there are too many independent variables, $\underline{x}_{kj}$, relative to dependent variables, $\underline{y}_{kj}$. The SIMCA-3B program, PLS-2, uses the partial least squares (PLS) method. This method has been proposed by H. Wold (37) and was discussed by S. Wold (25). In such a problem there are two blocks of data, $\underline{Y}$ and $\underline{X}$. It is assumed that Y is related to X by latent variables $\underline{u}$ and $\underline{t}$; $\underline{t}$ is derived from the $\underline{X}$ block and $\underline{u}$ is derived from the $\underline{Y}$ block.

These relations are mathematically stated in Equation 4 and 5.

$$\underline{Y} \leftarrow \underline{u} \leftarrow \underline{t} \leftarrow \underline{X} \qquad (4)$$

With the PLS method the X-block is modeled by the equation

$$\underline{x}_{ki} = \underline{m}_i + \sum_{a=1}^{A} \underline{t}_{ka} \; \underline{b}_{ka} + \underline{e}_{ki} \qquad (5)$$

and the Y-block by the equation

$$\underline{y}_{kj} = \underline{m}_j + \sum_{a=1}^{A} \underline{u}_{ka} \; \underline{c}_{aj} + \underline{f}_{kj} \qquad (6)$$

Both resemble principal components models, but are derived so as to simultaneously minimize e_{ki} and f_{kj} in the least squares sense while yielding t_{ka}'s and u_{ka}'s that optimize the correlation between the x's and y's. The u_{ka}'s and t_{ka}'s are analogous to the components in principal components analysis whereas the b and c terms correspond to loading values. The loadings reflect the importance or influence of the individual peaks in the PLS model.

A predictive relation between the latent variables is constructed as:

$$U_{ka} = d_a t_{ka} + e_k \qquad (7)$$

and can be used to estimate u (and the y's) from the X-block for PCB compositions similar to those modeled.

Applications of SIMCA to Chromatography Data

In the discussion that follows, the SIMCA method is illustrated by applying it to three problems: (1) quality assurance of chromatography data, (2) classification of unknowns, and (3) predicting the composition of unknown samples. This third problem is one of deconvolution of a mixture and calculation of the relative concentration of the constituents (25, 38).

The data modeled are from gas chromatograms obtained for Aroclors 1242, 1248, 1254 and 1260. The unknown samples are from the anaysis of used transformer oil obtained from a waste dump in New Jersey. The concentration of individual isomers in selected Aroclor and transformer oil samples are given in Appendix I. The data are organized in a matrix in which the first four data entries for each sample in row 1 of the data array (Table 2, Apendix I) designate the composition of the sample. For standards, these four variables represent the fractional parts of Aroclor 1242, 1248, 1254, or 1260, respectively, that were combined. Results from the analysis of transformer oil (samples 21-23) are of unknown fractional composition and variables 1 through 4 are null entries. In the examples that follow data from samples analyzed (Table 1, Appendix I) were used in part or in total to illustrate the PLS method.

As in many such problems, some form of pretreatment of the data is warranted. In all applications discussed here, the analytical data either have been untreated or have been normalized to relative concentration of each peak in the sample.

Quality Assurance. Principal components analysis can be used to detect large sample differences that may be due to instrument error, noise, etc. This is illustrated by using samples 17-20 in Appendix I (Figure 6). These samples are replicate assays of a 1:1:1:1 mixture of the standard Aroclors. Fitting these data for the four samples to a 2-component model and plotting the two first principal components (Theta 1 and Theta 2 [scores] in

Equation 3 for the samples shows that samples 19 and 20 are different from samples 17 and 18 (Figure 7).

The plot of the loadings for each chromatographic peak (Beta 1 vs. Beta 2; $\underline{b}_{1i}$ and $\underline{b}_{2i}$ Equation [3]) reveals information about the sources of the variance in the four samples (Figure 8). Information in Table II confirms these findings, as it is seen that the variability is largely the result of the failure to detect two peaks (peak 1 [variable 5], and peak 4 [variable 9]). Also, peak 63 (variable 67) in sample 20 exceeds its average concentration measured in all samples by 9%.

The data show that peak 1 is zero in sample #19 and #20, that peak 5 is zero in sample 19, and that there is about 12% variation in peak 63 in samples 19 and 20 (Table II). Only after a careful examination of the data plotted for each analysis are these deviations apparent in the chromatograms. These results illustrate the utility of principal components analysis (PCA) for checking the internal consistency of complex chromatograms.

Table II. Partial Peak Summary of Replicate Analysis of an Aroclor 1242:1248:1254:1260 Mixture

Sample	Concentration (ng) of Peak Number					
#	1 (5)[1]	4 (8)	5 (9)	6 (10)	63 (67)	ng Total
17	0.054	0.020	0.014	0.106	.0933	4.38
18	0.058	0.020	0.014	0.101	.0956	4.19
19	0[2]	0.016	0[2]	0.104	.0929	4.22
20	0[2]	0.020	0.020	0.101	.1030	4.10

[1](SIMCA Variable Number)

[2]Not Detected by PEAK-11 Integration Program

To illustrate the influence of changes in sample composition resulting from failure to detect constituents because of decreasing concentration or sensitivity, we offer a hypothetical set of data in histogram form (Figure 9). This histogram illustrates how the results of analyzing of a mixture of five compounds would change as a result of varying the sensitivity of the detection system or from sample dilution at a fixed sensivity.

The changes in fractional composition resulting from analyzing the sample under different sensitivities in this

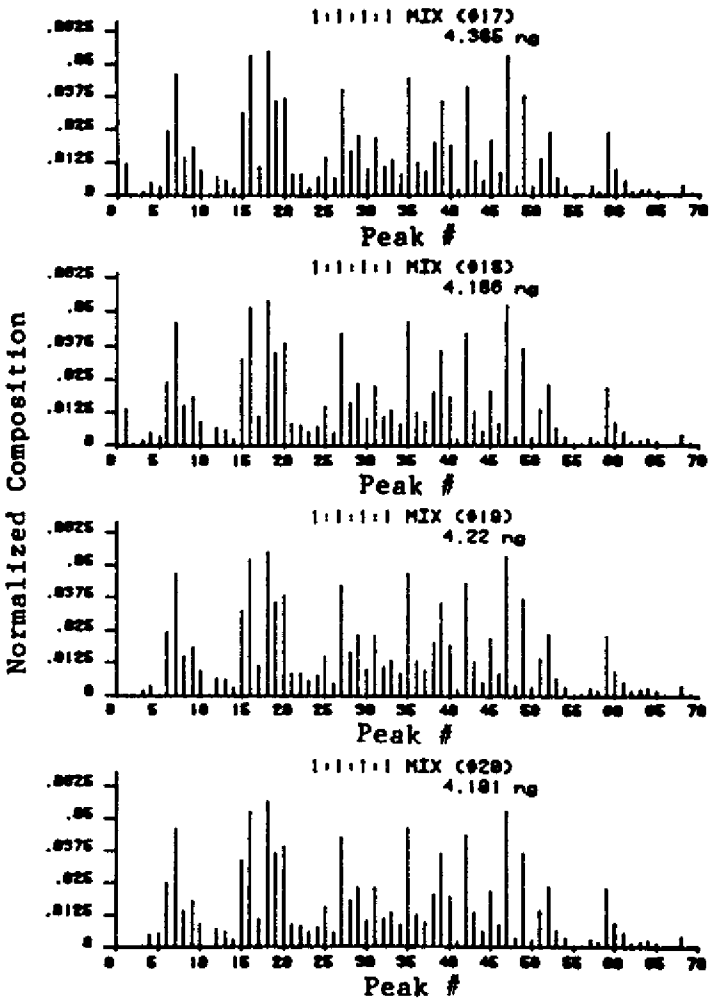


Figure 6. Fractional Composition Histograms from Four Replicate Analyses of Aroclor Mixture.

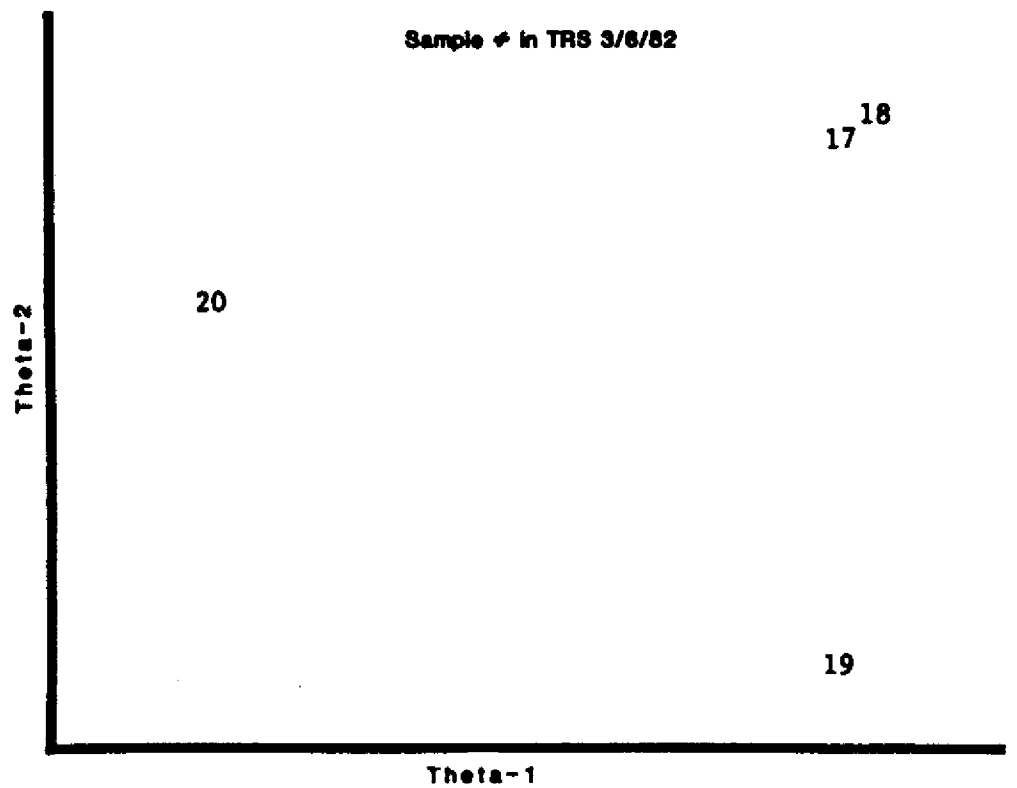


Figure 7. Principal Components Plot Derived from Fractional Composition Replicate Aroclor Analysis (Figure 2).

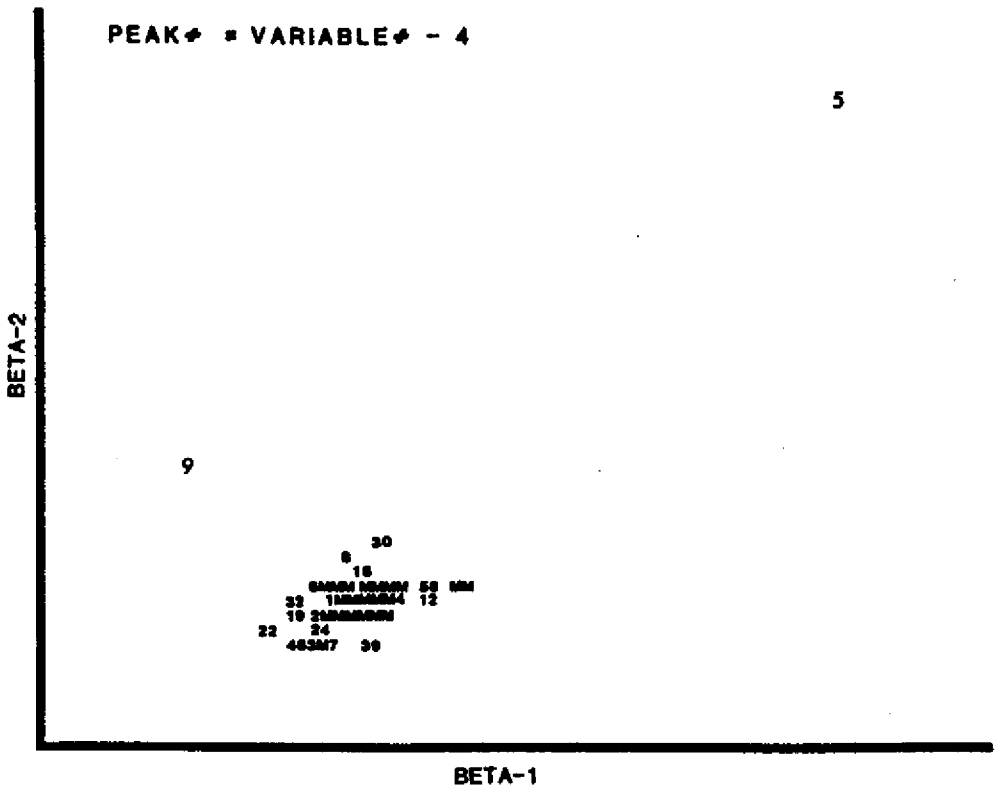


Figure 8. Plot of Variable Loadings (Betas) in Aroclor Mixtures.

example are summarized in Table III. The hypothetical total of the constituents is 30 arbitary units and correct results are obtained at sensitivity level I. Results determined at each of the lower sensitities are presented in brackets. Sensitivity decreases from level I to IV and the dashed lines represent the baseline at the different sensitivity levels. These results are commonly encountered when concentration data are expressed as proportions and this example serves to point out the importance of controlling this problem when the examining fractional composition data. It is shown later that SIMCA is able to accomodate for this type of change, if the change is a consistent function of concentration.

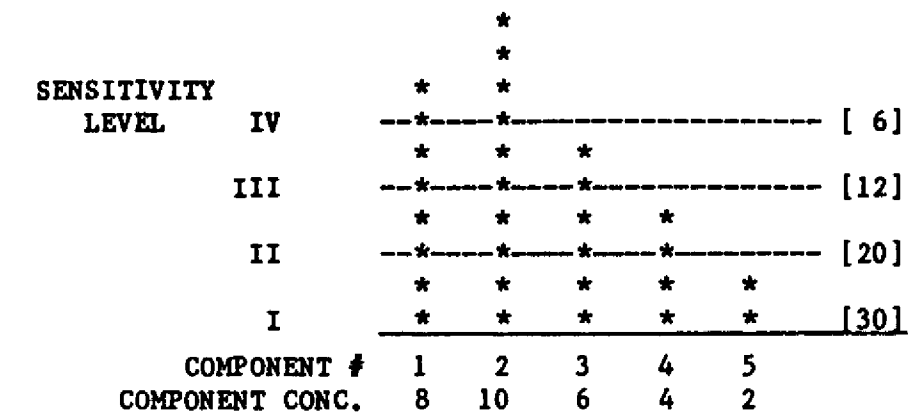


Figure 9. Example of changes in composition resulting from decreased sensitivity.

Table III. Changes in fraction composition of a hypothetical mixture due to decreasing concentration or decreasing sensitivity.

SENSITIVITY LEVEL	FRACTIONAL COMPOSITION OF COMPONENT # 1	2	3	4	5	TOTAL
I	.266	.333	.24	.133	.067	30
II	.333	.400	.20	.10	ND[1]	20
III	.333	.50	.166	ND	ND	12
IV	.333	.667	ND	ND	ND	6

[1]ND=Assumed to be not detected due to limited sensitivity

To further illustrate the utility of the SIMCA method we

examined an even more complex selection of Aroclor samples in which both composition and concentration of samples were modeled. Data for this illustration were from a group of 27 samples of four Aroclor types, where three Aroclors were analyzed over a range of concentrations (Table IV).

In this set of samples, the concentration data for the Aroclor 1254 concentration series were from another set of data in which there were 105 constituents instead of the usual 69. In this study, the concentration of the first 69 peaks of the total of 105 were selected. A sample of Aroclor 1254 having the same 69 constituents as Aroclor 1248 and 1260 was included to test the discrimination ability when concentration and composition were being modeled.

The data were modeled by a principal components model with three components. The statistical results method (25, 31) are presented in Table IV and V. In addition, the measured total PCB concentration is included in Table IV. One of the three sets of two-dimension plots (Theta 1 vs Theta 2) is presented in Figure 10. Individual samples of a given Aroclor were distributed regularly in these plots and samples were ordered according to concentration. The sums of squares decreased from 4,360 to 52.4 (Table V.) and approximately 88 percent of the standard deviation was explained by the three term component model.

Because of this structure in the plots, three dimensional representation of the data was investigated. The three principal components score terms ($\underline{t}_{k1}$, $\underline{t}_{k2}$, and $\underline{t}_{k3}$) from the statistical analysis of this data set were used as plotting coordinates for each sample (Table IV). The data were displayed and rotated about the axes in 3-D using a Dazzler TV graphics board (Cromemco, Inc., Mountain View, CA). Following this display of the data, the coordinates for each sample were used to generate a 3-D plot with a Texas Instruments Plotter driven by a MUMPS program in the laboratory data base.

Two graphics viewpoints were selected that allowed us to discern the three clustering of Aroclors as lines (Figure 11). The single Aroclor 1254 sample (point "3") that was composed of data from an alternate set of data is readily observed in these 3-D plots as not being similar to any of the other sample types.

Classification To illustrate the use of SIMCA in classification problems, we applied the method to the data for 23 samples of Aroclors and their mixtures (samples 1-23 in Appendix I). In this example, the Aroclor content of the three samples of transformer oil was unknown. Samples 1-4, 5-8, 9-12 and 13-16, were Aroclors 1242, 1248, 1254, and 1260, respectively. Samples 17-20 were 1:1:1:1 mixtures of the Aroclors. Application of SIMCA to these data generated a principal components score plot (Figure 12) that shows the transformer oil is similar, but not

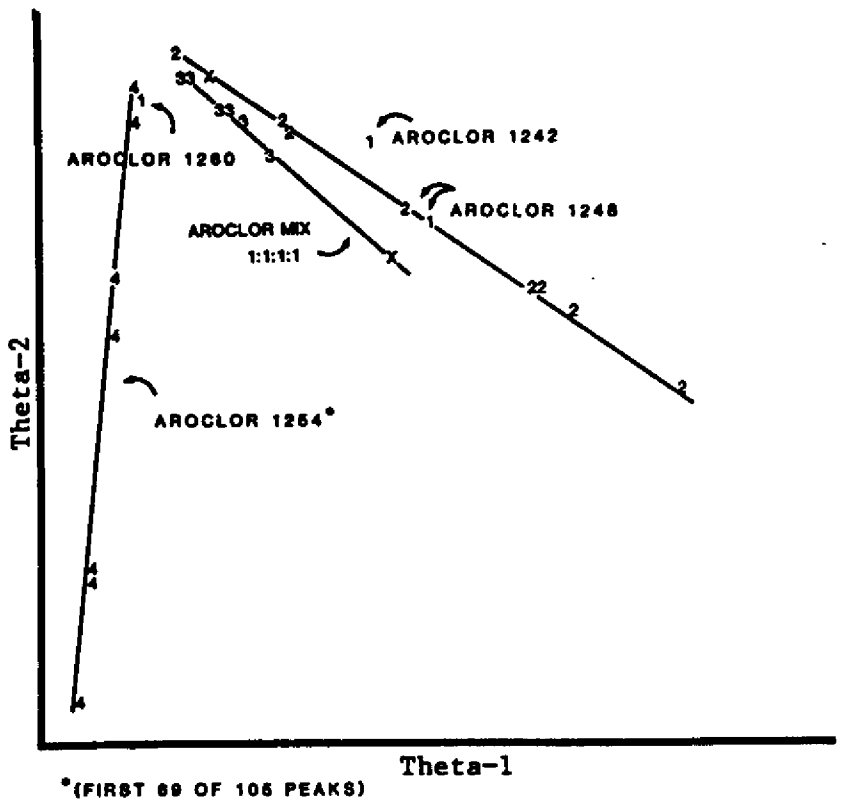
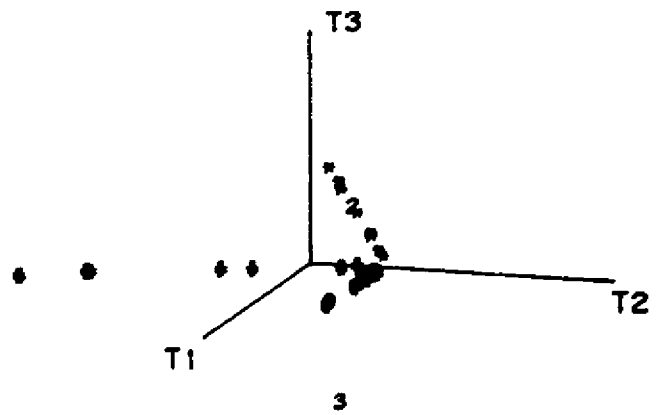


Figure 10. Principal Components Plot (Theta 1 vs. Theta 2) from Aroclor Classes (Table IX).



* Aroclor 1248 Series
@ 1:1:1:1 Aroclor Mix
\# Aroclor 1254 Series (69/105)
2 1248 Test
3 1254 (69) Test

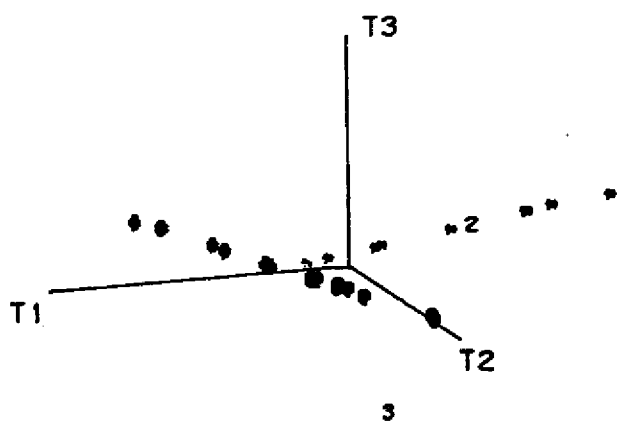


Figure 11. 3-D Views of Theta Values Derived from PC Analysis of Aroclors. Key: * = Aroclor 1248; @ = 1:1:1:1 Mixture; # = Aroclor 1254 (69/105 pks); 2 = Aroclor 1248 (test); and 3 = Aroclor 1254.

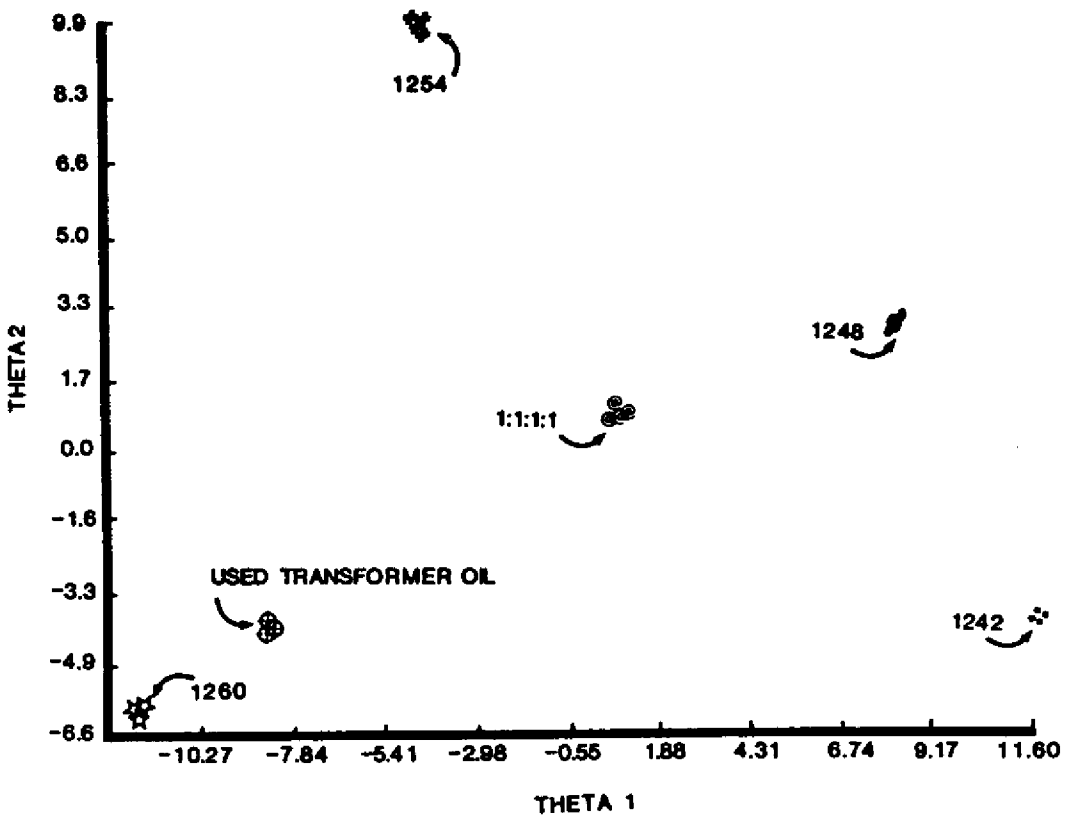


Figure 12. Principal Components Plot from Five Aroclors Classes and a Used Transformer Fluid (most similar to Aroclor 1260).

identical, to Aroclor 1260. A more detailed discussion of classification using these data is presented by Dunn et al. (39).

Table IV. Theta Values from a Three Component Principal Components Class Model (A=3) and Total Measured Total Concentration of PCBs.

Aroclor and SAMPLE #	THETA 1	THETA 2	THETA 3	CONC.
1248				
1	-0.813	-0.003	0.227	6
1254				
2	-0.180	0.11	-1.39	6
1248				
3	0.524	0.621	0.21	0.76
4	0.351	0.541	0.216	1.58
5	0.352	0.541	0.211	1.61
6	-0.0173	0.369	0.211	3.46
7	-0.0641	0.352	0.225	5.63
8	-0.645	0.077	0.22	6.56
10	-1.32	-0.246	0.231	9.85
11	-1.29	-0.236	0.231	9.74
9	-1.52	-0.334	0.245	10.9
12	-2.07	-0.592	0.240	13.7
1:1:1:1 mixture				
19	0.486	0.570	0.048	1.48
14	0.442	0.546	0.038	1.76
13	0.255	0.418	-0.075	3.22
18	0.264	0.430	-0.089	3.24
17	0.167	0.370	-0.14	3.89
15	0.0237	0.273	-0.253	5.11
20	-0.587	-0.099	-0.629	9.7
16	-0.603	-0.122	-0.67	9.99
1254[1]				
24	0.721	0.513	0.173	0.80
26	0.733	0.41	0.159	1.38
25	0.839	-0.139	0.126	3.82
23	0.866	-0.353	0.112	4.76
27	1.08	-1.64	0.000	10.7

[1]Data Set Obtained from first 69 of 105 Isomers Quantitated

Prediction of Composition of Unknown Samples PLS.

Because many samples are analzyed in which the analyst is interested in determining which Aroclor mixtures are present, we applied the PLS method to the data obtained from the analysis of

the used transformer fluid previously discussed. In order to estimate the Aroclor content of the previously classified the used transformer fluid previously discussed. In order to estimate the Aroclor content of the previously classified the used transformer fluid previously discussed. In order to estimate the Aroclor content of the previously classified transformer oil samples, we obtained additional data from the analysis of Aroclors of varying proportions. In Appendix I, the data are ordered in an array and the first four variables designate the fractional part of each Aroclor composing the sample in the order 1242, 1248, 1254, and 1260. This composition data represents the Y-block and the 69 peaks represent the X-block of data analyzed with the PLS-2 program.

Table V. Statistical Summary for A=3 Principal Components SIMCA Analysis of Aroclor Samples.

Step	Par.	A	NDF	SS	SD	1-SD/SDY	SS(TETA)	ITET
0		0	1862	4.36E+03	1.53E+00	0.000	0.	0
1	Alpha	0	1794	2.25E+03	1.12E+00	0.268	0.	
2	Beta-	1	1700	5.83E+02	5.86E-01	0.617	1.7E+03	14
3	Beta-	2	1608	1.36E+02	2.91E-01	0.810	4.5E+02	9
4	Beta-	3	1518	5.24E+01	1.86E-01	0.879	8.4E+01	17

The samples of unknown composition--21-23 and samples 1-20, 24-34 (Appendix I) were those of Aroclors of variable composition. Variables 5-73 are isomer concentrations (Variable 74, the total PCB concentration in ppm was not included in the analysis). Variables 5-73 represent the fractional composition or isomer proportional concentration values. Representative concentration histograms of the data set are presented in Figure 13. Four PLS components were extracted and then used to estimate the Aroclor content of the unknowns and of a standard sample (No. 24). The Aroclor standard is a mixture of three Aroclors in the ratio of 0.33:0.33:0:0.33. Chromatograms of the samples for which the PLS estimates were made (Table VI) were similar when compared to a chromatogram of a similar mixture of standards.

The partial least squares (PLS) method has been applied to structure activity problems by Wold *et al.* (38). Recently, Lindberg *et al.* (40) employed this approach to resolve mixtures of humic acid and ligninsulfonate on the basic of fluorescence spectra.

This example demonstrates that the PLS method gives a stable estimate of the Y-block, even though there are many more X-variables than samples, a condition that removes the possibility of applying multiple regression. Another advantage of the method

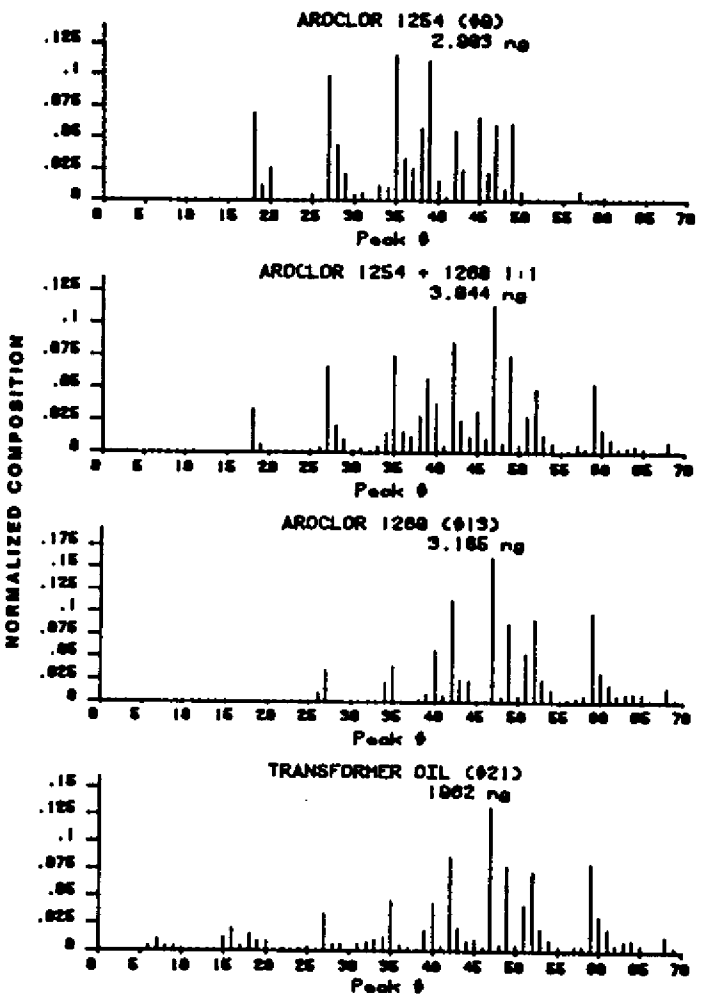


Figure 13. Fractional Composition Histograms of Used Transformer Fluid and Aroclors.

that makes it attractive for use in analytical problems is its computational efficiency and simplicity, which makes it possible to use microcomputers and minicomputers to carry out such calculations.

The SIMCA software is available in two forms, both developed by Wold (25, 31): 1) an interactive, Fortran version which runs on Control Data Corporation (CDC) machines, and 2) an interactive version, SIMCA-3B. Additional information on these programs is contained in Appendix I. Only the SIMCA-3B version contains the CPLS-2 program used for PLS analysis.

Table VI. Fractional Composition of Aroclors in Transformer Oils Estimated by partial least squares.

Sample number	Aroclor 1242	1248	1254	1260
21	.03	.03	.08	.84
22	.03	.03	.08	.84
23	.03	.03	.08	.84
25	.37 (.33)[1]	.36 (.33)	.05 (.00)	.24 (.33)

[1]Actual composition

Environmental Applications

To illustrate the environmental application of the SIMCA method we examined a set of isomer specific analyses of sediment samples. The data examined were derived from more than 200 sediment samples taken from a study site on the Upper Mississippi River (41). These analytical data were transferred via magnetic tape from the laboratory data base to the Cyber 175 computer where principal component analysis were conducted on the isomer concentration data (ug/g each isomer).

The first principal component values (Theta 1) for each sample were determined and these values were correlated with the total PCB concentration (Figure 14) recorded for each sample in a separate computer data base that contained other environmental data such as hydrology and sediment texture. The results indicated that certain samples deviated by factors of about two. Upon examining the sample records, the recorded dilution values

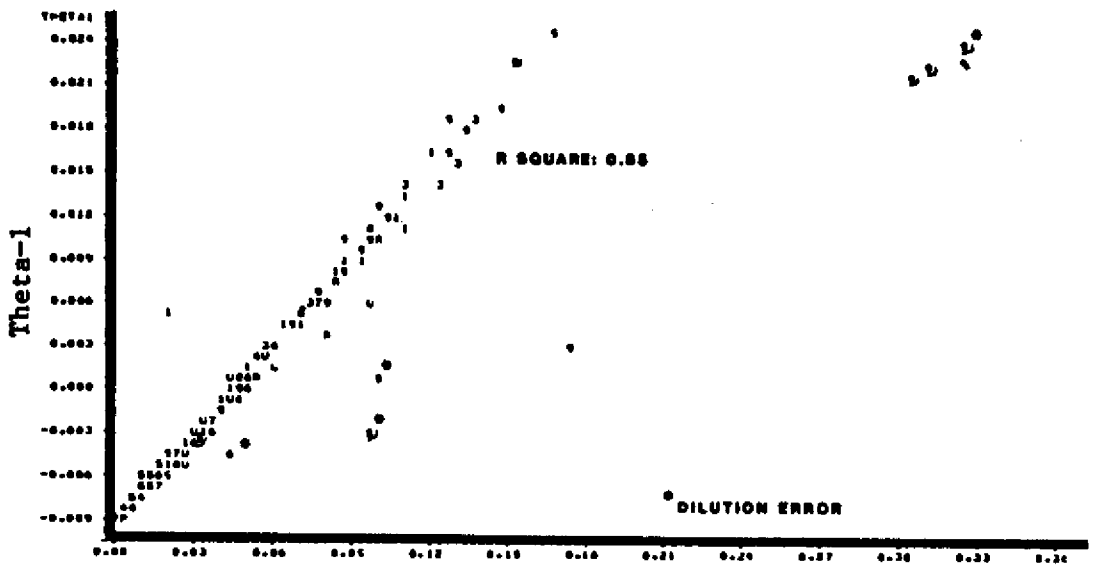


Figure 14. Correlation of Theta 1 vs. Total PCB Concentration Measured in 201 Lake Onalaska Sediment Samples. Plot symbol is site number.

for samples designated with an asterisk in Figure 14 were found to be in error by a factor of 2. When the values were corrected, these samples fell on the correlaton line. Little or no correlation of the second principal component value existed with total composition (Figure 15).

An examination of the sample distributions observed in principal components projections using isomer concentration data expressed as fractional composition, as well as the clustering of samples by similar values of their second principal component score term, revealed consistent differences existed in sample profiles. The next step in this data evaluation is to statistically analyze correlations of the PLS components from analyses with the external variables such as percent sand, clay and silt, and total organic matter in samples. These correlations may play an important role in identifying factors resulting in changes in PCB composition and enable one to more clearly understand the forces determining the distribution and fate of PCB in a complex ecosystem.

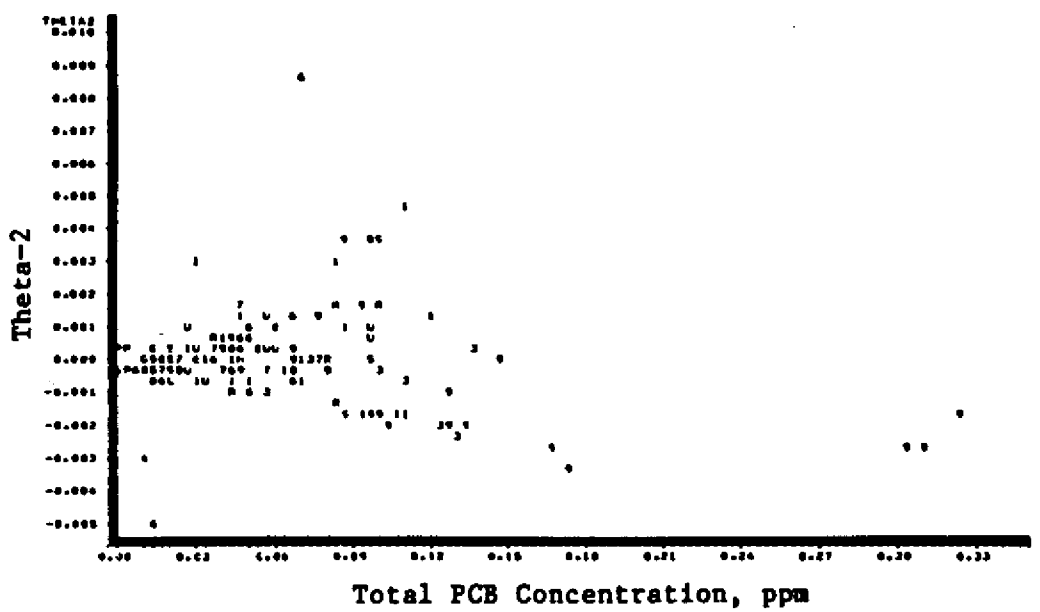


Figure 15. Plot of Theta 2 vs. Total PCB Concentration measured in 201 Lake Onalaska Sediment Samples.

Appendix I.

This appendix contains some of the data generated for the SIMCA and PLS analyses. The complete data set is available from the authors. Upon request the data will be provided on 8" single density single sided floppy disks in IBM 3740 format for CP/M based systems or on 5 1/4" double sided double density floppy disk for the IBM/PC or other MS/DOS based computers. The requestor, however, must supply a properly formated floppy disk.

Software Availability. The SIMCA software is available in two forms, both developed by Wold (25): 1) an interactive, Fortran version which runs on Control Data Corporation (CDC) machines. The second set of programs are an interactive microcomputer version, SIMCA-3B, are available from Principal Data Components, 2505 Shepard Blvd., Columbia, MO 65201. The SIMCA-3B pattern recognition programs includes the CPLS-2 program used for PLS analysis and are available for CP/M (Digital Research, Pacific Grove, CA) and MS-DOS (Microsoft Corporation, Bellueve, WA) for 8088 or 8086 based microcomputers.

The Fortran version used in this study was located at the Computer Center at the University of Illinois at Champaign/Urbana. The Fortran version is useful for analysis of very large data sets, i.e. 400 x 70 matrices. The SIMCA-3B version for microcomputer systems is interactive, menu driven, and is applicable to intermediate sized data sets and runs under CPM or MS-DOS. In this study, the SIMCA-3B program--CPLS-2, was used to obtain the results in the PLS examples discussed.

An earlier Fortran version of SIMCA is available for use in the ARTHUR package available from Chemical Information Systems, Box 2227, Falls Church ,VA. Recently, the operating system was changed on the CDC Cyber computer system at the University of Illinois. The new operating system does not allow the earlier SIMCA-2T version used to perform the environmental analyses to operate correctly. The authors expect that a new version of SIMCA will be installed that will function with the current operating system in use on the CDC Cyber computer.

Partial Summary of Data from the Gas Chromatographic Analysis ot Aroclor, Aroclor Mixtures, and Transformer Oil analyses.

Table 1. Identity of Samples Analyzed—Aroclor 1242, 1248, 1254, 1260, Their Mixtures and a Transformer Oil. Data are included in this appendix for sample numbers designated with an asterik.

SIMCA ID	Description
1*	Aroclor 1242 Replicate
2	Aroclor 1242 Replicate
3	Aroclor 1242 Replicate
4	Aroclor 1242 Replicate
5*	Aroclor 1248 Replicate
6	Aroclor 1248 Replicate
7	Aroclor 1248 Replicate
8	Aroclor 1248 Replicate
9*	Aroclor 1254 Replicate
10	Aroclor 1254 Replicate
11	Aroclor 1254 Replicate
12	Aroclor 1254 Replicate
13*	Aroclor 1260 Replicate
14	Aroclor 1260 Replicate
15	Aroclor 1260 Replicate
16	Aroclor 1260 Replicate
17*	Aroclor42:48:54:60 1:1:1:1
18*	Aroclor42:48:54:60 1:1:1:1
19*	Aroclor42:48:54:60 1:1:1:1
20*	Aroclor42:48:54:60 1:1:1:1
21*	Used Transformer Oil Replicate
22*	Used Transformer Oil Replicate
23*	Used Transformer Oil Replicate
24	Aroclor 42:48:54:60 1:1:0:1
25	Aroclor 42:48:54:60 1:0:1:1
26	Aroclor 42:48:54:60 0:1:1:1
27	Aroclor 42:48:54:60 1:1:0:0
28	Aroclor 42:48:54:60 1:0:1:0
29	Aroclor 42:48:54:60 1:0:0:1
30	Aroclor 42:48:54:60 0:1:1:0
31	Aroclor 42:48:54:60 0:0:1:1
32	Aroclor 42:48:54:60 0:1:0:1
33	Aroclor 42:48:54:60 1:1:1:1

Table II. Data Matrix Organization for Aroclors and Samples.

Data Matrix - Variable #				
Sample # ID code				
1(1242)[1]	2(1248)	3(1254)	4(1260)	1
6(peak 2)[2]	7(peak 3)	8(peak 4)	9	10
11	.	.	.	.
16	.	.	.	.
21	.	.	.	.
26	.	.	.	.
31	.	.	.	.
35	.	.	.	.
41	.	.	.	.
46	.	.	.	.
51	.	.	.	.
56	.	.	.	.
61	.	.	.	.
66	.	.	.	.
71	72	73(peak 69)	74[3](Total conc.)	

[1]Weight fraction each Aroclor in sample variable 1-4
[2]Variables 5-73 are fractional concentration of each PCB isomer
[3]Variable 74 designates total PCB concentrationin sample

Table III.Representative Analyses of Aroclors, Their Mixtures and a Transformer Oil Sample. Refer to Table 2 for key to data organization.

Sample # ID code				
	1 1 42			
100	0	0	0	.3318
0	.03722	.1324	.1501	.6295
.8347	.3023	.3691	.1762	0
.07662	.07048	.02987	.5528	1.14
.2134	.3437	.3375	.3169	.09121
.08603	.02791	.07272	.1301	.1613
.04002	.0355	.1661	.08603	.2138
.1051	.09709	0	.03421	.02422
.01593	.02423	.02633	0	0
0	0	0	.01582	.01318
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	7.511	

Table III. Continued

	5 5 48			
0	100	0	0	.02185
0	0	0	.02243	.04143
.3688	.09205	.1105	.07285	0
.1108	.08407	.03458	.3342	.4488
.09049	.6112	.4933	.5239	.1222
.1164	.04218	.1092	.2177	.05379
.1572	.1294	.3208	.1477	.3446
.1657	.1605	0	.1479	.09148
.06115	.09849	.1533	0	0
0	0	0	.09543	.05943
0	0	0	0	0
0	0	0	0	0
.0009057	0	0	0	.0003501
0	0	1.463E-05	0	0
0	6.426E-05	0	6.257	
	9 9 54			
0	0	100	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	.2006	.03684	.07303	0
.001386	0	0	.01534	0
.2877	.1281	.06263	.01397	.01731
.003889	.03496	.03043	.3352	.09876
.07539	.1657	.3222	.04839	.007758
.1595	.07247	0	.1939	.06609
.1752	.02599	.1777	.01992	0
.005103	.002069	.001326	0	.002222
.02406	0	.007326	.0102	0
.001136	0	0	0	0
0	0	0	2.903	
	13 13 60			
0	0	0	100	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	.02807
.1134	0	0	0	0
0	.0004755	.06472	.1251	0
0	.006675	.02628	.1837	.02169
.3606	.07934	.07441	.0009664	0
.5124	.01567	.2802	.01874	.1711
.2944	.08182	.04352	0	.006068
.01198	.02128	.3146	.1039	.06354
.01951	.02727	.03142	.02323	.002931
0	.04784	.009139	3.185	

Table III. Continued on next page

Table III. Continued

17 17 M4

25	25	25	25	.05351
0	.007108	.02049	.01411	.1062
.2019	.06352	.0797	.04155	0
.03203	.02606	.01205	.1385	.2308
.04948	.2394	.156	.1621	.03699
.03584	.01305	.02929	.06368	.03058
.1765	.07449	.09968	.04474	.09625
.04801	.05987	.03558	.1951	.05449
.04109	.08646	.1563	.08263	.01062
.1798	.05681	.02459	.09232	.03886
.2308	.01501	.1661	.01465	.06061
.1034	.03089	.01593	0	.002988
.01464	.007318	.103	.04175	.02351
.007217	.009673	.01155	.007575	.0004781
0	.01765	.002694	4.385	

18 18 M4

25	25	25	25	.0582
0	.009318	.02033	.01366	.1012
.1923	.06195	.07673	.03757	0
.02852	.0246	.01163	.1362	.2158
.04677	.2254	.1456	.1597	.03718
.03311	.02247	.03049	.06241	.02041
.1754	.06777	.0975	.04221	.09346
.04581	.0564	.03511	.1943	.05288
.03923	.08372	.1494	.07889	.01055
.1755	.05492	.02173	.0869	.03402
.2197	.01502	.1514	.01265	.05624
.09693	.02829	.01499	0	.002718
.01202	.006532	.09292	.03756	.02181
.006168	.008577	.01005	.007134	0
0	.01566	.002565	4.186	

19 19 M4

25	25	25	25	0
0	.00758	.01591	0	.1035
.1979	.06332	.07692	.03998	0
.02768	.02566	.01181	.139	.2205
.04811	.2343	.1521	.1627	.0366
.03671	.02365	.03228	.06273	.01992
.1798	.07212	.09911	.04306	.0963
.04658	.05797	.03613	.198	.05478
.04075	.08714	.1496	.08243	.01029
.1816	.05516	.02203	.09154	.03546
.2255	.01544	.1564	.0134	.05881
.09807	.02874	.01439	0	.002419
.01253	.00762	.09556	.03932	.02241
.006722	.009386	.01079	.006948	0
0	.0158	.001706	4.22	

Table III. Continued

	20 20 M4			
25	25	25	25	0
0	0	.01953	.02258	.1012
.1888	.05618	.07373	.03659	0
.029	.02383	.01114	.1375	.2149
.04563	.2313	.1486	.159	.03436
.03259	.0219	.03021	.06214	.0217
.1742	.07348	.09438	.04086	.09305
.04368	.05558	.03355	.1881	.05164
.03871	.08279	.1486	.07911	.008697
.1776	.05508	.02429	.08819	.03461
.2167	.0147	.1513	.01244	.05699
.09493	.0272	.01298	0	.002228
.01184	.007262	.09327	.03842	.02261
.006812	.008346	.01053	.006725	0
0	.01538	.001846	4.101	
	21 21 TO			
0	0	0	0	0
0	0	0	0	9.761
21.21	9.022	7.668	4.709	0
0	0	0	22.01	37.65
9.456	29.72	18.87	16.88	0
5.23	0	3.79	6.906	0
67.07	11.2	13.59	0	13.89
16.32	19.48	25.79	90.13	10.21
7.393	2.339	36.12	83.84	9.548
169.9	41.68	17.97	19.92	7.416
259.6	10.67	150.3	11.58	82.64
141.5	39.53	20.73	9.535	0
9.323	8.306	156.3	62.34	39.39
10.12	18.16	20.98	10.67	0
0	27.11	6.732	1962	
	022 22 TO			
0	0	0	0	0
0.	0	0	0	7.651
18.84	6.861	6.402	4.596	0
0	0	0	20.97	36.33
9.354	28.37	16.44	16.38	0
4.054	0	0	7.090	0
68.15	11.62	13.79	0	13.65
15.17	17.98	24.69	89.59	8.900
6.871	2.331	36.60	82.98	9.401
167.6	42.73	18.34	18.26	69.10
258.6	10.65	150.1	11.48	81.87
140.7	39.08	20.85	9.035	0
8.846	8.124	156.7	61.77	39.50
10.08	17.99	20.90	10.63	0
0	26.88	6.672	1929.	

Table III. Continued on next page

Table III. Continued

	023 23 TO			
0	0	0	0	0
0	0	0	0	9.198
20.07	8.285	5.973	4.554	0
0	0	0	21.05	33.38
8.918	27.98	15.53	15.96	4.408
0	0	0	6.221	0
63.37	10.25	13.64	0	12.98
15.78	19.00	24.82	86.27	8.967
0	2.185	37.18	79.38	9.282
161.7	39.50	17.65	20.34	6.783
244.7	9.602	141.9	10.87	77.59
131.9	36.90	19.59	8.435	0
8.368	7.868	146.8	56.95	36.46
9.241	16.73	19.50	9.806	0
0	24.92	6.579	1835	

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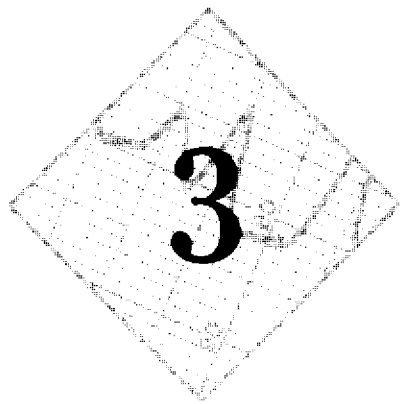

Collection of Suspended Particulate Matter for Hydrocarbon Analyses: Continuous Flow Centrifugation vs. Filtration

Timothy S. Bates, Susan E. Hamilton and Joel D. Cline

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Notes and Discussions

Collection of Suspended Particulate Matter for Hydrocarbon Analyses: Continuous Flow Centrifugation *vs.* Filtration[a]

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High-speed, continuous-flow centrifugation and glass-fiber filtration were used to collect suspended matter from river water and waste water effluent for hydrocarbon analyses. Both methods collected equivalent weights of total suspended matter and similar particle size distributions and were >90% efficient for particles >4·0 μm. Glass-fiber filters retained more lipid-extractable organic matter and saturated hydrocarbons than did continuous-flow centrifugation. Adsorption of dissolved or colloidal organic compounds onto glass-fiber filters resulted in an overestimate of the organics associated with particulate matter.

Introduction

Particulate matter plays an important role in the transport of organic matter. More than 85% of the hydrocarbons entering estuaries from waste water effluents and storm water runoff are thought to be associated with suspended particulate matter (Van Vleet & Quinn, 1977; MacKenzie & Hunter, 1979; Eganhouse *et al.*, 1981). The adsorption of these hydrophobic compounds to particulates is dependent upon both their solubility and the organic content of the suspended matter (Herbes, 1977; Lopez-Avila & Hites, 1980; Eganhouse & Kaplan, 1981). Association of hydrocarbons with particulates greatly affects the physical movement of these organic compounds and their availability for biological consumption. The organics transported on particulates are ultimately trapped in sediments either by direct deposition or by incorporation into fecal pellets (Wade & Quinn, 1979; Prahl & Carpenter, 1979). Analysis of the organic compounds in surface sediments is not necessarily an accurate record of the organics that were initially deposited. Prahl *et al.* (1980) have demonstrated a significant recycling of organic matter in surface sediments. The only means of quantifying the transport of particulate organics is actually to sample the suspended matter.

There are several methods by which suspended matter can be collected. Sediment traps have been shown to collect sinking particulate matter efficiently (Lorenzen *et al.*, 1981), but

are not practical for sampling particulates in waste water effluent, rivers, or confined estuaries where strong currents and ship traffic interfere. Particulate matter in these environments can be more easily collected by either filtration or centrifugation. Filtration is the traditional method by which suspended matter is collected (Sackett, 1978). Glass-fiber filters are used to recover samples intended for organic analyses since these filters can be readily cleaned by combustion. A third method of collecting suspended matter is by continuous-flow high-speed centrifugation. This paper summarizes studies conducted to determine the size fraction of material collected by continuous-flow centrifugation and the hydrocarbons associated with this material. These results are compared with river and waste water effluent particulates collected by glass-fiber filtration.

The work reported here is part of a larger study, under the NOAA/OMPA section 202 program, involving the sources and transport of hydrocarbons on the Duwamish/Green River, Washington (Hamilton *et al.*, 1983). This small river system (~35 $m^3 s^{-1}$ mean flow) enters Elliott Bay, an embayment of Puget Sound, and the port of Seattle. A secondary waste treatment plant, located 20·5 km upstream, discharges about 2 $m^3 s^{-1}$ into the river.

Methods

River water was pumped through an intake manifold at 50 l min^{-1} with a Peabody Barnes submersible pump. Approximately 400–500 ml min^{-1} of the flow were diverted through a 400-μm screen prefilter to a Sorvall Model SS-3 or RC-5 high-speed centrifuge. Sedimentation of the particulates was accomplished with the centrifuge operating at approximately 21 000 gravitational units (15 000 rev min^{-1}). Composite waste water effluent samples from the Renton secondary treatment plant were stirred continuously and gravity-fed through the centrifuge under the same conditions.

River and waste water samples were periodically filtered through Gelman type A/E, 47-mm glass-fiber filters (nominal pore size 1 μm), Reeve Angel 934 AH, 42·5-mm glass-fiber filters and Nuclepore 47-mm polycarbonate membrane filters (pore size 0·4 μm). The glass-fiber filters were loaded with approximately 5 mg and the membrane fibers with approximately 1 mg of material. The filters were weighed on a Cahn Model 4700 Electrobalance before and after sample filtration. Analytical methods used for hydrocarbon analyses are reported elsewhere (Hamilton *et al.*, 1983).

Size analyses of waste water particulates were performed with a Model ZBI Coulter Counter. Particle size distributions were determined with a Coulter C1000 Channelyzer. Apertures of 50 and 200 μm were used to size particles from 1 to 10 μm.

Results and discussion

Size analysis

Coulter Counter size analyses were performed on secondary treatment waste water samples. Particle counts of centrifuge influent and effluent and the glass-fiber filtrate were used to calculate the percentage particle retention. The results (Figure 1) show similar particle collection efficiencies for the centrifuge and glass-fiber filter. Both attain efficiencies of greater than 90% for particles greater than 4 μm. The median retention (the size at which 50% is retained) for the glass-fiber filters is less than 1 μm, which is in agreement with the filter data of Sheldon (1972). The median retention for the centrifuge is also less than 1 μm. On the basis of particle size analysis, the centrifuge and glass-fiber filters appear to collect material in the same size–frequency range.

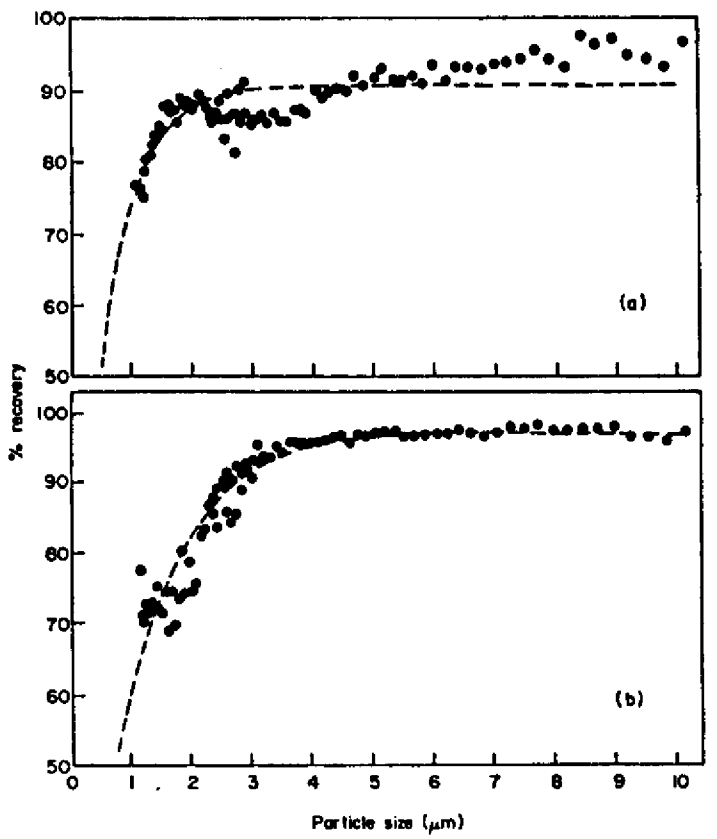

Figure 1. Percentage recovery of waste water suspended particulates collected by (a) filtration (Gelman A/E glass fiber) and (b) continuous centrifugation (21 000/*G*) as a function of particle size.

Weight comparisons

Total suspended matter (TSM) was determined using Gelman and Reeve Angel glass-fiber filters and the continuous-flow centrifuge. The waste water sample TSM values for the three methods averaged 6·0±1% mg l^{-1} (one standard deviation expressed as a percentage of the mean). The clean-up combustion (15 h at 450 °C) of the Gelman glass-fiber filters did not affect the weight of TSM collected by the filter. These results show that Reeve Angel filters (used in the waste treatment industry for determination of total suspended solids), Gelman glass-fiber filters and the centrifuge are directly comparable techniques for the measurement of TSM in waste water effluent.

The collection efficiency of the centrifuge was tested further by filtering the centrifuge influent and effluent through 0·4-μm Nuclepore membrane filters. The traditional cut-off point between dissolved and particulate matter is 0·4 μm (Riley, 1975). The centrifuge collected 87% of the total waste water particulate weight as determined by the 0·4-μm membrane filter.

A similar comparison was done on suspended matter from the Duwamish/Green River, Washington (Hamilton *et al.*, 1983). The collection efficiency of the centrifuge based on TSM values obtained with 0·4-μm Nuclepore filters ranged from 63 to 92% (mean 78± 11%), depending upon the tidal stage (Figure 2). At periods of low tide the river flow increased, suspending larger particles and thereby increasing the apparent collection

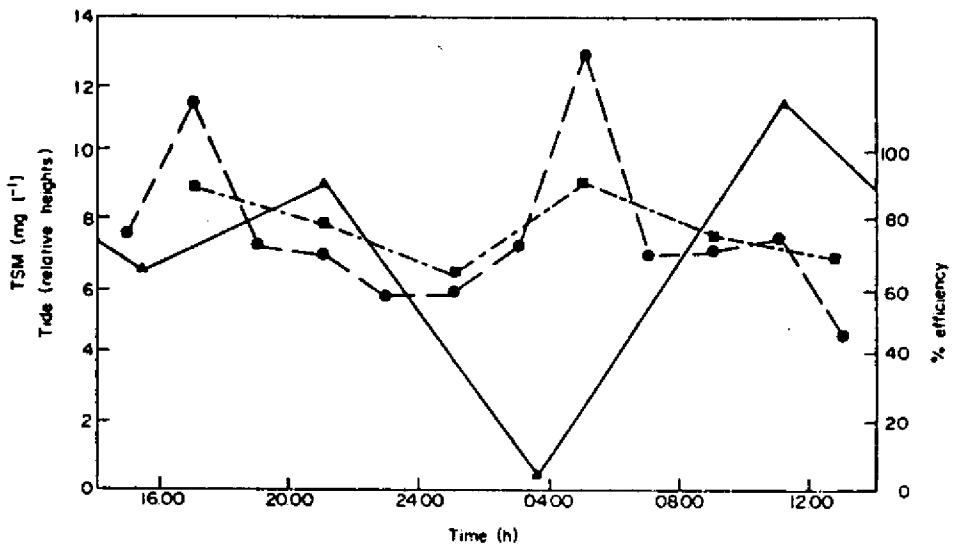

Figure 2. Centrifuge collection efficiency (■ — · — ■), total suspended matter concentrations (● - - ●) and relative tidal amplitudes (▲—— ▲) over a 24-hour period (29 30 October 1980) at river km 20 on the Duwamish/Green River. Relative tidal heights are derived from predictions at river km 7.

efficiency of the centrifuge. At high slack water, the river flow slowed or stopped, allowing larger particles to settle out, thereby causing the apparent centrifuge efficiency to decrease. The retention of riverine suspended matter on glass-fiber filters showed efficiencies similar to the centrifuge. Sampling with the centrifuge for a 24-h period integrated the fluctuations in TSM. Although both the glass-fiber filter and the centrifuge collected comparable weights of material, neither collected all material greater than 0·4 μm.

Hydrocarbon comparisons

Two centrifuges were used to collect suspended matter from the Duwamish/Green River. One centrifuge was capable of refrigerating the sample at ~2 °C to quantify temperature effects. The refrigerated centrifuge effluent temperature was maintained at the influent temperature (10 °C). The effluent temperature from the non-refrigerated centrifuge averaged 17 °C. Refrigerating the sample had no effect on the saturate hydrocarbon composition. It can therefore be assumed that the samples were not chemically or biochemically altered during the 24-h collection period.

There were several differences, however, between the organic material collected by glass-fiber filtration and that collected by centrifugation. The filter collected two to three times as much lipid-extractable material as did the centrifuge. The weights of total saturated hydrocarbons and unresolved complex mixture (UCM) averaged 1·4±11% times higher for the filtered samples than for the centrifuged samples in both river and waste water. Internal ratios (pristane/C_{17}, carbon preference index (CPI) etc.) were the same for both filtered and centrifuged samples (Table 1). Centrifuge effluent and glass-fiber filtrate were also analysed for hydrocarbons. The compositional differences between the 'dissolved' and particulate samples are a result of an enrichment in the planktonic and algal hydrocarbons, heptadecane and pentadecane, in the particulate phase (pristane/C_{17}, CPI 14-20, Table 1).

The discrepancies in the organics collected by filtration and centrifugation are probably not sampling artifacts. Samples collected by continuous-flow centrifugation integrate

TABLE 1. Hydrocarbon composition and parameter comparisons for centrifuged and filtered samples

	TAH ($\mu g\ g^{-1}$)	UCM ($\mu g\ g^{-1}$)	Pris/C_{17}	Pris/Phyt	Phyt/C_{18}	CPI 14–20	CPI 20–32
Waste water effluent							
Centrifuge particulate	200	6400	0·22	1·0	1·7	5·4	1·4
Filter particulate	260	9200	0·27	0·9	2·1	4·6	1·3
Centrifuge dissolved	45	1700	0·52	0·9	1·9	2·5	1·2
Filter dissolved	25	900	0·57	0·9	1·4	2·0	1·5
Centrifuge particulate	140	6200	0·24	1·0	0·88	2·2	1·5
Filter particulate	220	11 000	0·29	1·0	0·96	2·4	1·3
Centrifuge dissolved	83	610	0·67	1·4	0·58	1·0	1·1
River							
Centrifuge particulate	13	50	0·60	2·2	0·60	1·5	7·6
Filter particulate	18	60	0·59	1·5	0·58	1·3	7·0

The first four samples are from October 1980, the remainder are from February 1981. TAH indicates total aliphatic hydrocarbons, UCM indicates unresolved complex mixture, and CPI indicates carbon preference index for either C_{14-20} or C_{20-32}.

variations in river TSM and hydrocarbons over the sampling period, whereas filter samples represent instantaneous concentrations. The filter samples, however, were composites of filters taken every 2 h during the sampling period in an attempt to average temporal variability. The waste water samples were well mixed composites. The material collected by filtration and centrifugation should have been equivalent.

The most likely explanation for the hydrocarbon discrepancies is adsorption. Glass-fiber filters and the material collected on their surface adsorb organics. The adsorbed organics can be either dissolved or colloidal (Sharp, 1973). Feely (1975) calculated that 23% of what he measured as particulate organic carbon was actually adsorbed dissolved organic carbon. Shultz (1974) found that 20% of the fatty acids retained by glass-fiber filtration were dissolved. This hypothesis is partially supported by the analysis of the centrifuge effluent and glass-fiber filtrate from the October 1980 waste water sample. The 'dissolved' saturated hydrocarbons were 28% of the particulate hydrocarbons by centrifugation and 12% by filtration. Thus, the adsorption of dissolved or colloidal organics by the glass-fiber filter causes an overestimate of the hydrocarbons associated with particulate matter.

Collection of suspended matter by centrifugation offers other advantages over filtration. In a 24-h period on the Duwamish/Green River, the centrifuge can process 700 l of water. A 47-mm glass-fiber filter clogs with 1·5 l of water. It would require more than 450 filters (or fourteen 273-mm filters) to collect the amount of material recovered by the centrifuge. The larger centrifuge sample increases both analytical accuracy and precision. Both sample size and accuracy of weight measurements may have contributed to the total weight disparity in Table 1 where the dissolved-plus-particulate centrifuge sample is less than the dissolved-plus-particulate filter sample. The centrifuge sample is also free of interferences from the filter. The absence of the filter allows a more accurate sample weight and facilitates other analyses such as total carbon and nitrogen where the suspended matter is often removed from the filter before analysis. High-speed, continuous-flow centrifugation can also be accomplished at sea. The Sorvall SS-3 centrifuge has been fit to a gimballed carriage allowing suspended particulates to be collected under most sea conditions (Young & Cline, 1983).

Summary

The basis for particle retention by centrifugation is distinctly different from that of glass-fiber filtration. Sample collection by centrifugation is based upon particle density and flow rate (residence time in the centrifuge tube). Glass-fiber filters select particles on the basis of pore size which is continually changing as the filter is loaded. Material collected by glass-fiber filtration has been correctly termed 'retained organic matter' as opposed to particulate organic matter (Shultz, 1974). Centrifugation and glass-fiber filtration are both efficient (>90%) at collecting particles >4 μm and have median retentions at <1 μm. Although both centrifugation and glass-fiber filtration collect similarly sized particles, the glass-fiber filter has been shown to give an overestimate of the organics associated with those particles. The continuous-flow centrifuge permits a more accurate estimate of the concentration of organics associated with suspended particulate matter.

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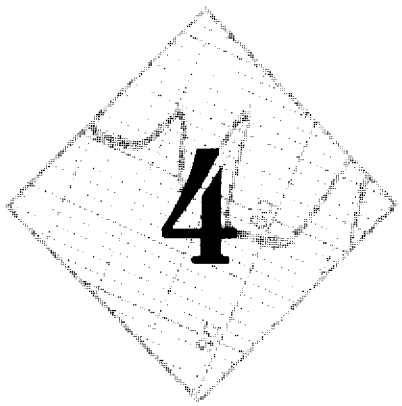

Credibility: The Consequence of Quality Assurance

Donald E. King

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CREDIBILITY:
THE CONSEQUENCE OF
QUALITY ASSURANCE

BY

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Summary

This paper reviews the relationships between quality control, quality assurance and credibility. Various factors important to the establishment of data credibility are enumerated with particular emphasis on those which can be readily addressed within the laboratory environment. Quality assurance is described as a management function which rests on the documentation and establishment of quality control protocols, and on the evaluation and summarization of their outcomes. Quality control is a technical, operational function which investigates and confirms the proper conduct of all those procedural components necessary to a successful conclusion. The outcome of quality control operations in the laboratory allow one to determine the repeatability, reproducibility and bias of the analytical system, in particular the analyst, as well as to establish the precision and accuracy of the analytical procedure, and the stability of the instrumentation used. Techniques for establishing bias and error are briefly outlined. The terminology involved is reviewed. Finally there is a brief discussion of the problems of reporting data, particularly at or below the detection limit. The difference between a detection criterion, and the detection limit is reviewed.

Credibility requires action by more than just the laboratory analyst. If the result does not reflect the real world because of inadequate program planning, field operations or inappropriate data use and interpretation, the promulgation of laboratory quality assurance documentation can work against credibility. However, the laboratory analyst is central to the entire operation, since he can see the results of inappropriate activity by his associates and is best able to advise them on remedial action.

Credibility: The Consequence of Quality Assurance

Introduction

Credibility is fast becoming the catch-word of the 80's for those involved in the study of environmental and health effects arising out of the treatment and disposal of waste material. In the face of contradictory evidence from analysts supporting opposing view-points, the public, and even data users within the same agency as that providing the analytical service, are left uncertain as to whom to believe. Expressions of confidence from the analyst based on even the best in-house control activity is increasingly being perceived as insufficient or at worst as a whitewash. One needs only to become involved in the evaluation of interlaboratory comparison data to realize, in spite of the emphasis placed on quality control during the 70's, that something significant is missing.

Credibility lies in the eye of the beholder. There is always a strong tendency to attach credibility to facts which support one's own particular beliefs. (All other facts are incredible!) Analysts, like anyone else, believe strongly in their own credibility because facts, to which they alone are privy, support the viewpoint that what they do produces reproducible data. When faced with a challenge from another analyst, they know the degree of credibility of their own operation, and automatically assume that the other fellow's results are very nice but wrong. Of course the feeling is mutual. No matter how much time one spends on establishing one's own confidence, there is no guarantee that anyone ese will be convinced, unless there are well documented facts to back it up.

Establishing Credibility

The subjective and volatile nature of credibility demands that it be established in an organized logical fashion, and that the facts supporting it be well documented. Each facet of the operation must be examined in minute detail, with particular regard given to those which are most subject to attack. In the case of environmental investigations several factors must be addressed. In broad terms these include:

a) the rationale for the investigation. (What are we trying to prove and, equally important, disprove?)

b) definition of resource needs relative to existing capability. (Will we be able to find what we are looking for?)

c) the design of the field sampling program. (Will it produce all data required, both in support of and against taking the action proposed by the topic under investigation?)

d) the field activity necessary to obtain the samples in the form required for analytical evaluation. (Will the samples be worth analyzing?)

e) the maintenance of sample integrity and identity. (Are samples properly and sufficiently identified? Will the reported data actually match the right samples?)

f) the proper interpretation of data. (Will our conclusions be supported by the data presented? Will all data be evaluated?)

The above factors tend to be beyond the scope and, to some extent, beyond the influence of the analytical scientist in most large agency laboratories. But there is growing concern that unless they are properly looked after, the analyst will be left holding the bag. It is difficult to establish data credibility when one is unable to establish the credibility of the sample or the sampling process, the chain of custody or suitability of transportation facilities.

While we realize that the larger issue of credibility of an analyst with the general public mainly falls outside the immediate control of a laboratory quality assurance program, it is apparent that any data user would be extremely foolish to ignore the consequences of lack of public acceptance of his data. As stated above, many of the factors influencing the analytical result are beyond the control of the analyst. To this extent therefore, the analyst cannot be held responsible if the sample which he examines does not truly represent the situation under study. Under such circumstances, however, where the analytical result does not then reflect the real world situation, the promulgation of QA documentation becomes a cruel joke on the organization concerned and can actually work to destroy credibility.

It would seem, therefore, that some power beyond that of the laboratory analyst is necessary to establish and maintain the public credibility of scientific organizations. We can all cite examples of organizations whose scientific pronouncements are seldom questioned. Examination of these situations generally reveals that the spokesperson for the group is a charismatic individual of demonstrated sagacity. Closer examination of his support group may reveal dedicated commitment to the aims of the organization or it may reveal sloppy methodology leading to highly dubious analytical results. In either case, however, the organization makes progress by demonstrating an ability to use the results in a positive fashion for the publicly perceived betterment of a situation.

In view of the fact that charismatic leaders are in short supply these days, what can be done to enhance the overall acceptance of our data? I am afraid that there is no simple answer to this question. There is, however, a philosophic approach which when assiduously pursued minimizes those occasions when published data assails the public credulity.

The first step is to apply the test of reason to each analytical result, i.e. could the sample, as described, contain this substance in this concentration? The second step is to question a reasonable result, i.e. is this the most likely number to apply to that substance in this sample? The third step is to assess the acceptability of the result to the client, i.e. is this the result which will be most gratifying to the submitting agency? The fourth step is to prepare an appropriate dialogue for use when the answer to step 3 is negative, perhaps including a written comment on the report to explain the result.

In summary the same painstaking care with which the result is determined should be applied to ensure that the result is appropriate for the intended use. The end product of laboratory analysis should not be numbers, but solutions to the client or data user's problems.

Credibility and the Laboratory

Within the laboratory there are of course more than enough areas to be addressed in order to establish credibility. Factors to be considered include:

a) the physical structure and condition of the laboratory facility. (Is the lab clean and tidy and properly laid out for the safe performance of analytical functions?)

b) the mechanical condition of equipment used for analysis. (Is it properly maintained and correctly, and safely, connected to all power, water, gas, etc. sources required?)

c) the source and quality of analytical reagents, distilled water or other solvents, and their storage. (Are precautions in place both to protect the user and to ensure reagent integrity?)

d) the availability of completely documented analytical methodologies appropriate to the sample types being examined. (Are they taken from standard reference texts?)

e) the existence of documented quality control and quality assurance protocols

f) the proficiency of the analyst, and his/her experience with the sample types and analytical procedures routinely encountered. (Is she/he trained to recognize and handle special cases?)

g) the source, reliability, preparation and maintenance of calibration materials, in-house controls and external reference material. (Can someone else backup your values?)

Most of these factors are defined and recorded at a given point in time. They should be reviewed periodically to ensure the necessary standard is being maintained. Procedures for inspection, verification and documentation of current status must therefore be developed. In fact it is this activity that involves the concept of a laboratory quality assurance program.

Quality Assurance

The rationale behind establishing a laboratory quality assurance program is to provide a basis for defining and documenting lab and data credibility in terms

acceptable to both the individual client and the public-at-large. It is completely immaterial that an analyst performs any of a number of controlled operations in order to ensure that he can confidently attach his name and reputation to an analytical report, if, in the face of a challenge from another analyst or the opposing side, he cannot provide adequate proof that these operations were carried out, that their outcomes were reviewed and evaluated, and that continuing control was demonstrated.

Many laboratory managers and analysts do not yet appreciate fully the difference between quality control and quality assurance. Because they are exposed at first hand to the data generated by their own control operations, they develop a "feel" for their data quality. They become "confident" because the numbers generated or the observations made in order to monitor the variation of a particular component in their analytical process, are repetitively contained within a narrow range. When pressed they can casually refer to their past experience with respect to the "precision" and "accuracy" of their data. However if pressed further they are often forced to admit that raw data required to substantiate their claim is not readily available. Even when it is in a more or less presentable format, the fact that another confident analyst has produced results which appear to be in direct conflict, makes it all too clear that part of the story must be missing.

Quality assurance therefore rests on one's ability to retrieve documents:

a) which establish the daily protocols implemented to monitor the various factors which have been known, or are most likely, to affect the total measurement system, and specify what action is to be taken if trouble occurs.

b) which review the quality assessment techniques employed to evaluate data produced in support of the above monitoring protocol, indicating their frequency of application.

c) which identify supervisory responsibilities and duties in order to maintain quality assurance.

d) which summarize and review the degree of success achieved in daily operation, i.e. the limits within which the system was actually maintained,

the number of occasions when it failed or almost failed, and the significance of such for the various data user groups being served.

e) which provide evidence summarizing the comparability of data, i.e. against what standards, or in association with which other analysts, and with what frequency and success has comparability been established.

f) which review the historical development and implementation of the current procedures, i.e. against what other methods were they compared, what range of sample types, and how many, were used to establish the suitability of the method, and how does current data compare to that provided under the previous methodologies.

It should be apparent that quality assurance is a middle-management function. Supervision, evaluation and review, summarization and documentation, are the key words. These do not represent tasks generally assigned to a junior technician, or a scientist working on the bench. It is only recently becoming apparent that quality assurance protocol for supervisors and managers has not been given the attention it requires. There is no question that familiarity with the usual procedures, and the general routine tends to preclude any urgency in documenting them or their outcome. Scientific staff in such positions must give consideration to documenting how their QC protocol works, the rationale behind it, and the expected impact on data and laboratory credibility in order to ensure that their staff are knowledgeable and "on track". In this regard, the quality assurance oficer, if such a position exists, provides guidance and assistance in this documentation effort, but the effort must originate with, and reflect, actual lab management and supervisory activity.

Quality Control

Quality control activity provides the data upon which quality assurance reports are based. There is no question that a technician can maintain an analytical process in control by carefully checking each of the many factors involved. This may or may not require a tabulation of the specific observations. However "control" implies preventive maintenance rather than emergency response. Prevention implies foresight and a predictive capability. Therefore true "control" requires firstly, on the spot tabulation or charting of the observations required by the established protocol, and secondly, documentation of action taken if required.

Control charting allows one to observe trends toward an out-of-control situation as well as pick out specific instances of loss of control. It has been noted that:

a) a system must be established to be in control, in order to be maintained in control.

b) a system is not in control if it is observed to produce unexpected data more than once every twenty to twenty-five runs.

c) control limits usually become tighter once a process is placed under a control protocol as a reflection of the fact that the original limits set were based on data produced by an uncontrolled operation.

d) if the process, under control, produces information which is better than that determined as essential for the data use intended, the establishment of acceptance limits looser than the control limits is permissible. The two modes of operation should not be confused however. (In some systems, day to day variation is more difficult to control than within-day variability would suggest. If one loosens the within-day control process it becomes even more difficult to detect the between-day changes which are potentially more serious since they are related to maintenance of accuracy rather than simply precision.)

Quality control should be present from start to finish. It not only provides evidence of product quality, but it also ensures that product quality will be maintained at a high level by providing a check on the components that go into it. As noted earlier a system is not "in control" unless it has been consistently within the control limits over an extended period of time. If an analytical run is set up every working day, failure to meet the control limits more than once a month suggests some component of the process is out-of-control.

Component control, then, is the preventive action necessary to maintain product control. It includes regular checks on analyst proficiency, method ruggedness, reagent quality, media preparation, solvent and distilled water purity, laboratory glassware, cleanliness and storage. It also monitors equipment operation in the form of maintenance schedules, etc. for replacement of parts before they wear

out, and in the form of charts for temperature, humidity stability, etc., where important. The preparation of analytical calibration standards should be controlled to ensure they agree with previous standards or in-house controls before use. Equipment response should be regularly checked against appropriate standard materials i.e. thermometers, balances, spectrophotometric wavelengths, GC retention times for freshly packed columns, etc.

All observations made for component control should be recorded in a permanent, organized fashion for ease of retrieval and review. Control charts may be necessary where the ruggedness of the system depends on tight control over that particular factor. This recording or charting activity does not of itself provide quality assurance, but does provide for easier supervision to ensure protocol is being maintained and appropriate action is being taken. Product control is generally assured if component control has been properly maintained. The truth of this must, however, be documented in order to determine the precision and accuracy of the analytical process is being maintained.

Method Documentation

Laboratory credibility is generated as a result of the existence of documented protocols which specify the tasks undertaken to ensure that an analytical result is properly measured and correctly interpreted. A carefully described analytical method is critical to the analytical outcome. Traditionally analysts have depended upon the texts of "standard methods" promulgated by various standards-setting organizations such as APHA, ASTM and US-EPA, but the explosion in technological progress since the 1950's which has seen the development of automated, microprocessor-controlled instrumentation in a variety of applications, has made it difficult for such texts to keep up-to-date. Increasingly the instrument manufacturers have provided new detection systems based on physical rather than chemical processes, and have incorporated into their systems the calibration and calculation functions. Under these conditions the nature of the methodology document, and the quality control functions which must be an integral part of the method, must change to include the specific instrumentation employed, in relation to the extent to which it has displaced the analyst. We must now control the instrument as well as the analyst. Methods must be described relative to the time and place and conditions in which they are employed. Traditional "standard methods" do not

adequately address this need, therefore the analyst must provide his own documented procedure, incorporating all those steps required for sample preparation, instrument and reagent and standards preparation, as well as the quality control checks performed along the way.

Quality Control Terminology

Control is exerted in order to prevent error, not to monitor it after it has occurred. There are two types of control; component control investigates the suitability of the ingredients and the correct operation of all necessary equipment (including the human), whereas product control establishes the quality of the data produced. "Standard methods" are accepted and defined as such partially on their "ruggedness", i.e. non-susceptibility to small changes in procedure. Therefore some factors need less control than others. A particularly critical factor, however, is and will always be the human operator.

Product control is, in fact, a measure of the performance of the chemical/physical, instrumental, operator team. In general terms we wish to observe and thus control the "precision and accuracy" of this team. In order to do this effectively we must break the total down into manageable pieces. This requires careful definition of terms. The following are recommended because they provide clarification of the distinction between terms often considered to be synonymous.

Precision - an inherent property of the method, which a very proficient operator may ultimately achieve. Basically it is the ability of the method to produce a very tight range of values for repeat analysis of standards.

Accuracy - an inherent property of the method, including the physical-chemical principles involved, which determines how close to "truth" the average of a series of precise measurements will be. Truth is generally defined by international agreement.

Repeatability - a property of the analyst dependent on proficiency and analytical conditions, the nature of the sample and the range of concentration of the analyte. It indicates ability to produce a tight range of values for repeat analyses of samples once the analytical system has been set-up and calibrated.

Reproducibility - a property of the analyst and the stability of the "instrumentation" employed. It indicates ability to achieve essentially the same calibration from one day to another (or from one analyst to another). It should be not more than 50% larger than the repeatability if proper calibration control is established.

Repeatability and reproducibility are estimated respectively by, the within-run standard deviation (S_w) obtained usually from duplicates analyzed within the same calibrated run, and the between-run standard deviation (S) obtained usually from one or more control samples analyzed on a day-to-day basis. The use of paired controls permits estimation of both S_w and S and thence identification of systematic error which is the source of non-reproducibility.[2]

Deviation - the natural random variability introduced in any measurement process due to indeterminate changes from the exact procedure.

Error - deviations which are so large that one must conclude that the exact procedure has not been followed correctly. The source of the error may not be apparent. Obviously a reference point is required, either a previous average value or a single other result. In the latter case error is suspected rather than confirmed. It may result from randomly contaminated glassware, etc.

The terms Indeterminate and Determinate Error are not needed if the above discrimination between Deviation and Error is recognized. This is also consistent with the use of the term Standard Deviation as a measure of range of Deviation.

Systematic Error - error introduced (most typically) in the calibration process. It will affect all data produced in a given run in the same way. An intercept error has a constant effect whereas a slope error shows a proportional effect dependent on concentration.

Bias - difference on average between results produced by different analysts and/or different methods. The difference may be constant if an intercept, blank or background estimate differs between the analysts etc., or it may be propor-

tional to concentration if the standards used for calibration do not agree. A bias of less than 10% is to be considered good. Bias at or near zero is of greater concern, and should be stated in absolute (i.e. mg/L) rather than relative (i.e. %) terms.

Inaccuracy - difference between an in-house set of standards and an external reference standard. (In order to be confident that a difference equivalent to one standard deviation will be detected requires at least 13 replicate analyses of both the in-house and the reference materials.)

In general, imprecise data cannot be used to determine the degree of inaccuracy in absolute terms. We can only say that the magnitude of any inaccuracy will not exceed some value based on the imprecision of the method and the number of replicate analyses performed, neglecting for the moment any consideration of bias.

Specificity - a characteristic of the method and its ability to isolate and detect a specific target element or compound. Many tests are "specific" only under the assumption that other interfering constituents will not usually be present in the routine samples for which the method has been developed. The test-method nomenclature should, but does not always, properly identify the target compounds or elements.

Recovery - a factor properly associated with the sample preparation process but generally confused with a correction for the effect of the sample matrix, etc. on the calibration curve. In some instances the recovery factor may be corrected for, but in others, particularly organics, it is often reported separately.

Calibration - the complex protocol of establishing, at a point in time, the relationship of response against concentration. It estabishes the traceability of in-house standards and controls against the values obtained on external reference materials, as well as the linearity and stability of the analytical system.

Standardization - the day to day process of confirming the measurement system is kept in calibration.

Any proper methodology documentation process will address these topics and incorporate specific quality control operations, where necesary, to ensure that data quality can be maintained and demonstrated to exist, in each of these areas of system proficiency. The protocols for gathering, evaluating, and summarizing the outcome of such quality control activity are not incorporated into the methodology. In the same manner as instrument operation, they form part of the training process to which the new analyst is exposed, and will be documented as standard operating principles.

Repeatability vs Reproducibility

For the individual analyst, it is suggested that true precision and accuracy cannot be independently established. Precision must be considered as an attribute of the perfectly operated process rather than of the analyst. Even a perfectly operated system will have an inherent bias or inaccuracy which depends upon the physical or chemical prinicples involved, and which may remain undetermined if an alternative independent system is not available.

Repeatability, on the other hand, is an analyst attribute. It changes in magnitude from one to another, and from time to time, dependent on experience and proficiency. It should be determined/estimated for the analyst, before he is allowed to work alone, based on his ability to obtain satisfactory replicate results from real samples. Usually duplicates are employed. The analysis is repeated on a series of samples, within the same calibrated run, in such a way that the duplicates are non-adjacent and, preferably, in a "blind" location in the run.

Repeatability establishes that a state of "simple statistical control" exists for the measurement process on a within-run basis.[1] However chemical analytical procedures depend on restandardization from run-to-run. Therefore a state of "complex, or multistage, statistical control" must be recognized for between-run data. While it is possible "to act for the moment as if" simple statistical control exists, the same is not valid for complex statistical control unless a calibration control protocol has been established to demonstrate it.

Thus, once within-run repeatability has been established, the analyst's ability to be reproducible from run to run must be established. Reproducibility is largely

dependent on the stability of the calibration process. The validity and variability of the daily analytical calibration step must be confirmed by use of paired, long-term, in-house or external control materials.[2] These two "controls" are checked immediately after the standardization of concentration versus response has been established. The difference of the two results monitors stability of the calibration slope, and its standard deviation can be used to estimate within-run repeatability because any bias in the individual values tends to be cancelled by subtraction. The sum of these results, on the other hand exaggerates any systematic error so that the standard deviation of the sums is larger than that for the differences. A great deal of information about the measurement process can be obtained by reviewing plots of sums and differences over time. Thus, if the difference is generally in control, then excess variability in the sum can be attributed to lack of control over the intercept or blank.

If the in-run repeatability of the analyst is established from duplicates, the standard deviation of the difference between paired calibration controls will be approximately $\sqrt{2}$ larger (because two datum are used). Tolerance limits for the sums can therefore be established from in-run repeatability. It can be shown that, for about 50 pairs of calibration controls, the standard deviation of the sums should not exceed 1.87 times that of the differences, to ensure that between-run reproducibility is (statistically) not significantly larger than in-run repeatability, and that a significant calibration variability will be detected if it exists.[3]

Establishing Bias

The above process is required to ensure that the analyst is in a sufficient state of day-to-day control to make it worth while to participate in interlab or intermethod comparison studies. At this point both reproducibility and bias are controlled. The level of error is as yet unknown.

External standards and reference materials are most useful for establishing the presence of bias. Their use, however, is only of value if the findings can be incorporated into the daily operation. The expense and/or relative difficulty of obtaining these materials usually requires the establishment of in-house controls the concentration of which is traceable to external reference materials by repeated analysis of both, within an analytical run which is under both calibration

and repeatability control. This permits translation of findings from today's run into both past and future runs.

When analyzing external materials, the objective should be to detect, and estimate, the level of bias present. Traditionally they have been used to demonstrate that the bias present is not detectable under the conditions of the experiment. Since these conditions are often unfavourable, (insufficient replicate analysis of both the internal and external materials, and inadequate control over reproducibility) it is fairly unlikely that bias would be detected. It can be estimated[4] that in order to detect a difference equal to one standard deviation with a "power" of 90% at a level of significance of 5% (i.e. $\alpha = .05$ and $\beta = .10$) requires at least 13 replicates of each material. If one wishes to ensure that any bias is negligible (i.e. no greater than one standard deviation) the correction factor must be determined very precisely and accurately. More than 50 replicates of each would be required to detect a difference of one-half a standard deviation.

Split-Sample Comparisons

It is possible to compare labs or methods based on analysis of several samples once each way. Over time (given a reproducibility-controlled system) data will be gathered covering a range of concentration. This is amenable to linear regression, and the slope and intercept estimated will indicate the relative bias. The residual sum of squares can be used to estimate the degree of scatter in the data relative to the line of best fit. This can then be compared to the reproducibility estimates for each analyst or method in order to determine how much of this scatter is explained by analytical variation. The remainder is then due to difference between the subsamples analysed and other sample/time related effects.[5]

The correlation coefficient, the average X, Y values and their standard errors are often quoted as evidence of good agreement between methods or analysts. Experience indicates this to be inappropriate. Firstly, it is frequently the case that the averages are not (statistically) significantly different, and yet the slope is not unity and therefore the intercept is not zero. Secondly, the correlation coefficient (r) is only meaningful when it is close to zero. (When comparing methods where the repeatability is small numerically relative to the range of concentration covered, r is always close to unity and tends to depend mostly on the

range and not on the amount of data or degree of fit.) Thirdly, statistical evaluation may indicate a significant difference exists which is so small that it would be essentially impossible to eliminate because of the nature of calibration reproducibility. An average bias between labs over time of less than 10% is fairly acceptable. It is very difficult to maintain bias of less than 5%.

It is interesting to note that if the difference between results is plotted versus the amount reported by one analyst, the correlation coefficient is close to zero. Usually this slope must exceed $\pm$ 0.05 before the correlation coefficient becomes large enough to suggest a difference in "recovery" between the two participants. (This is equivalent to a slope of from 0.95 to 1.05 in the Y vs X plot.) Difference plots are much more useful for evaluating the difference between methods or analysts. If r is small there is no slope difference. It is then and only then that a comparison of the averages is meaningful.[5]

Interlaboratory Studies

Interlaboratory comparisons involve many analysts in an effort to determine "accuracy by consensus". The average or the median value obtained for a given sample is used to set a reference point for identifying anomalous data. One-sample studies are essentially useless except for identifying the very worst performers. Two-sample studies promoted by Youden[6] are better in that the position of points on an X,Y plot identifies the systematic nature of bias between analysts. (If one result is low the other one will probably be low as well.) However the nature of the bias is not identified. It could be proportional to concentration (slope-related bias) or independant of the concentration (blank/intercept bias).

It is often stated that all analysts involved in such studies should employ essentially the same analytical procedure. Experience with a third type of interlab comparison suggests this is not necessary provided sufficient samples and analysts are involved. The bias introduced by a particular operator/laboratory is essentially independent of the method. (If the standards are out 10% it does not matter which method is used.) The use of several samples allows one to determine such things as average rank, following the procedure of Youden[7] and to flag data based on how far it is from the median. The criteria for flagging are set to allow some participants to escape being flagged.[8] These then are identified by their peers as

"very competent". After similar performance on several studies such participants can gain the status of "reference" labs. This type of study helps identify the current state-of-the-art performance potential and often demonstrates that different methods do produce identical data in the hands of competent analysts who otherwise have never had an opportunity to meet or compare notes.

If one wishes, it is then possible to use difference regression to evaluate the difference between each result and the sample median, for each participant. The more competent analysts, given methods with adequate sensitivity and repeatability, produce extremely well defined lines indicating the slope and intercept biases present in their data on the day of analysis. Estimates of their in-run repeatability based on deviation about this line can also be obtained.[5] Under such evaluation one can observe individual points which don't fit the pattern of bias in the rest of the data. Thus an analyst might be biased high by 10% but report one result as within 1% of the median. For that analyst this point although correct is suspicious. By evaluating the distribution of these difference-regression equations it is also possible to observe "clumping" of analysts which may or may not be related to method employed. Thus some may use the same sample preparation but different colorimetric follow-up analysis, and get the same result, or vice versa.

In any event, multi-sample studies tend to reinforce the point that the analyst has either performed very well or that problems exist. Individual points out-of-control for otherwise good analysts, as well as calibration bias, when so clearly identified, require the analyst to take action.

Recovery

One of the findings of inter-analyst study, which arises because of the use of different sample work-up procedures, is that recovery of the constituent of interest is sometimes affected by the procedure used. This cannot be assumed, however, until it is successfully demonstrated that calibration error is not involved. (Multi-sample studies which include a "blind" reference material may provide such proof.)

There is a strong tendency to misinterpret data when it comes to evaluating recovery. "Spiking" the sample with a known quantity of the analyte and then

analyzing both "spiked" and "unspiked" portions is a common approach. The uncertainty attached to the difference between two variable estimates is so great, when it comes to real samples as opposed to clean standard solutions, that whether the calculated recovery is equal to 100% or falls in the range 80% to 120% provides no proof one way or the other of either under or over-recovery. In addition an enormous error in correcting for the "blank" could occur and not be caught. In one study several years ago, three different analysts provided estimates for a drinking water spiked with 50 ug/L of lead of 0.2 and 50.3, 2 and 52, and 100 and 150 ug/L. Obviously all obtained the correct 50 ug/L recovery factor but were variously able to measure what was actually present because of changes in their sensitivity and ability to detect background contamination.

A different technique for correcting for the "recovery" factor will also detect and correct for certain types of error in defining "absolute" zero. It is proposed that it be called "Spiked standard dilution". The sample to be analysed is diluted by a factor of 2 and a factor of 4. These portions, and the original sample, are then each spiked with the same amount of standard, and analysed. The results are obtained after correction for the dilution factor, at which point, if the "spike" was 50 units, there will be apparent spikes of 50, 100 and 200 in the respective results. The results are plotted on the Y-axis versus the apparent "spike" and a line drawn through the points, with greatest emphasis on the lowest point. The true sample result, corrected for both "recovery" and "zero" error, is found at the Y-axis intercept.

This technique is intended for cases where background errors result from contamination of reagents. It will not correct for sample colour or for instrumental background problems. With modification it may be possible to detect distilled water contamination.

Reporting Results

In theory every result reported should include a statement of the method employed, its biases, and effect on recovery. The units of measure should include both the "dimensions" (i.e. mg/L) and the "scale" (i.e. as Si, as SiO_2, etc.). The test name should be non-ambiguous and preferably indicate what was measured rather than what can be inferred. (Some of the traditional names are dangerously misleading,

i.e. Free Ammonia is usually measured as Ammonia plus Ammonium. At one time "Free" meant "distillable", now it is often interpreted as "undissociated" and therefore toxic to fish. Actually, in reasonably hard, i.e. slightly alkaline, water, the porportion of Ammonia to Ammonium is quite small.) There is a move towards the use of Filtered vs Unfiltered, and Total vs Reactive in the nomenclature to clarify the fraction of sample analysed and the severity of the analytical conditions.

The analytical "repeatability" is often quoted, or at least available elsewhere for reference. The quotation of standard deviation should indicate how it was determined. The "reproducibility" is often not known, or is not under control. Statements with respect to "error" should properly identify the magnitude of error that could have been detected if in fact no statistically significant error relative to a reference value was detected.

The statement of a "confidence level" based on multiplying the standard deviation by some factor should best be left to the final user, for the simple reason that there are a multitude of questions that could be asked concerning either an individual result or an assemblage of results. The factor to be used depends on whether it is to be a one-tailed or two-tailed test and whether one requires protection against both type-I and type-II decision errors. Typical questions that could arise include:

a) are these two single estimates different?
b) is this value lower, or higher, than a guideline criterion?
c) what range of concentration could exist in this sample given this result, or average result?
d) is the constituent present in this sample?
e) how much would there have to be in the sample to ensure that it's presence could be reliably detected?

Low-Level Data

Traditionally there has been little consideration given to low results and their correct interpretation. The main question was whether such a "large" amount was present that treatment would be required. Low levels were uninteresting. Nowadays it is recognized that the traditional use of "detection limit" is wrong.[9]

The first problem lies in the definition of "detection limit". It is commonly stated to be either two or three times the standard deviation, but is frequently inflated because of known or suspected error in determining an absolute zero value. In fact the criterion for determining the level above which a result can be taken to mean that the sample probably contains the constituent is 1.64s (s = standard deviation). This is the "Criterion of Detection" with a level of significance of 5%. It is used by the analyst to qualify a result which has been reported. It protects the analyst against Type-I error. On the other hand, the client may use the factor 3.28 times standard deviation to define the point at which a reported result is large enough that a result lower than 1.64s would not likely be obtained by the analyst on a subsequent reanalysis. This represents the "Analytical Detection Limit" with a level of significance of 5% and a "power" of 95%. It protects the analyst against both Type-I and Type-II decision errors, (i.e. concluding the constituent was present when in fact it was not, or that it was absent when in fact it was present). It is to be used to qualify data. It should never have been used to prevent the reporting of measurements to the laboratory's clients.

The second problem is in the loss of the actual measurement. If the value 0.006 is obtained over several samples it is much more reliable than if obtained once only. Analytical Detection Criterion and Detection Limit are to be used by the analyst to evaluate a single piece of data. In as much as the client can calculate an average, the Detection Limit will be lowered by the square root of the number of data included in the average.

The third difficulty with the traditional approach to "detection limit" is that it gives more credence to the result (eg. 0.011) just above the in-house limit (eg. 0.010) than to the result (eg. 0.009) just below it. Firstly, 0.009 is not significantly different from 0.011, and secondly, even though 0.009 is not high enough to confirm the presence of the constituent, neither is it low enough to deny the presence of as much as perhaps 0.018 units. Thirdly, if the 0.009 is in error so is the 0.011 result.

The fourth difficulty with the "less than" (<) approach to reporting that a measurement is below the "Detection Criterion" or "Detection Limit" or in-house definition of a "Minimum Reportable Value" is that only data above the limit is eligible for inclusion in an average. Therefore such averages will be biased high and the data user will be led to make a Type-I decision error, i.e. concluding the constituent is present when it probably is not.

Summary

Credibility involves presenting both sides of the coin. As analysts we too often try to prove how good our data is. We downplay the possibility of error. We sometimes unwittingly mislead some of our clients by failing to provide complete statements to qualify results in terms of nomenclature, recovery, error and/or reproducibility.

The more routine our work is, the more reliable the data produced becomes, but also the more mechanical. Credibility requires us not only to do things well, but also to do the right thing at the right time. A good quality control program on the bench supported by a formal protocol for documenting success and failure, provides others with assurance that we are doing our job to the best of our ability, and thereby provides us with the credibility we need and desire.

In the final analysis however the data produced by even the most credible laboratory is not better than the validity of the sample submitted for measurement. Quality assurance activity must be extended beyond the laboratory to include program design and field operations. Credibility is enhanced by our ability to solve problems, not by ability to produce numbers.

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D E F I N I T I O N S

CREDIBILITY: FACT OR FANCY

- THE TRUTH AS I PERCEIVE IT!
- LIES IN THE EYE OF THE BEHOLDER?

INCREDIBILITY: CREDIBILITY AT ITS WORST?!

- SELF CONFIDENCE WITHOUT PROOF!

QUALITY CONTROL: DATA AND OBSERVATIONS.

- ARE THINGS O.K. TODAY?
- TASKS PERFORMED BY TECHNICAL STAFF.

QUALITY ASSURANCE: PROTOCOLS AND EVALUATIONS

- HOW WERE THINGS LAST MONTH?
- TASKS PERFORMED BY SUPERVISORY STAFF.

CREDIBILITY: THE NATURAL OUTCOME OF

- A WELL DESIGNED QC PROTOCOL IMPLEMENTED DAILY
- DATA RECORDED AND ORGANIZED FOR REGULAR RETRIEVAL
- DOCUMENTED PROCEDURES FOR QA
- DOCUMENTED QA EVALUATIONS

MEASUREMENT

PRECISION:

- ABILITY OF A METHOD TO PRODUCE THE SAME ANSWER WITHIN A NARROW RANGE.

ACCURACY:

- ABILITY OF A METHOD TO OBTAIN THE 'CORRECT' ANSWER AS DEFINED BY AN INTERNATIONAL STANDARD.

REPEATABILITY:

- ABILITY OF AN ANALYST TO PRODUCE THE SAME ANSWER, WITHIN A NARROW RANGE, UNDER THE SAME CALIBRATION CONDITIONS.

REPRODUCIBILITY:

- ABILITY OF AN ANALYST TO PRODUCE THE SAME ANSWER, WITHIN A NARROW RANGE, UNDER DIFFERENT CALIBRATION CONDITIONS.

BIAS:

- DIFFERENCE BETWEEN ANSWERS AS PRODUCED BY DIFFERENT ANALYSTS USING THE SAME OR DIFFERENT METHODS.

ERROR:

- DIFFERENCE BETWEEN THE AVERAGE ANSWER AND THE 'CORRECT' VALUE AS DEFINED BY AN INTERNATIONAL STANDARD.

CREDIBILITY FACTORS TO BE CONSIDERED WHEN IMPLEMENTING A FIELD PROGRAM

1. RATIONALE
   - WHY ARE WE OUT THERE?

2. RESOURCES
   - WHAT SPECIAL FIELD OR LAB CAPABILITIES ARE NEEDED? DO WE HAVE THEM?

3. SAMPLING DESIGN
   - ARE WE SAMPLING THE RIGHT SPOTS?

4. FIELD ACTIVITIES
   - WHAT HAS TO BE DONE IN THE FIELD TO MAKE THE SAMPLES WORTH THE COST OF ANALYSIS?

5. SAMPLE IDENTITY AND INTEGRITY
   - ARE THE SAMPLES CORRECTLY AND SUFFICIENTLY IDENTIFIED?

6. DATA INTERPRETATION
   - WILL OUR CONCLUSIONS BE SUPPORTED BY THE DATA PRESENTED?
   - HOW GOOD IS THE DATA?

7. LABORATORY CREDIBILITY.

CREDIBILITY FACTORS CONSIDERED IN THE LABORATORY

1. PHYSICAL STRUCTURE
   - CLEANLINESS, SAFETY, SPACE
   - AIR CONDITIONING, DUST CONTROL (TEMPERATURE, HUMIDITY, ETC.)
   - STORAGE FACILITIES

2. MECHANICAL CONDITION OF EQUIPMENT
   - MAINTENANCE PROGRAM
   - SAFELY CONNECTED.

3. SOURCE AND QUALITY OF REAGENTS
   - SAFETY AND STORAGE
   - DISTILLED WATER AND SOLVENTS

4. DOCUMENTED ANALYTICAL PROCEDURES
   - RELATED TO SAMPLE TYPES BEING ANALYSED
   - SUITABLY PRECISE AND ACCURATE FOR MEETING CLIENT NEEDS.

5. DOCUMENTED QA AND QC PROTOCOLS.

6. ANALYST EXPERIENCE AND PROFICIENCY
   - TRAINED TO RECOGNIZE SPECIAL CASES.

7. CALIBRATION MATERIALS AND PROCESSES
   - SOURCE AND RELIABILITY
   - PREPARATION AND MAINTENANCE
   - EXTERNAL REFERENCES

QUALITY ASSURANCE

DOCUMENTATION

1. DAILY QUALITY CONTROL PROTOCOL
   - FACTORS TO BE CONTROLLED, LIMITS
   - RECORDS TO BE MAINTAINED
   - PROCEDURE FOR VERIFYING CONTROL
   - ACTION TO BE TAKEN IF OUT-OF-CONTROL

2. QUALITY ASSURANCE PROCEDURES
   - DATA EVALUATION TECHNIQUES
   - LONG-TERM ASSESSMENT

3. SUPERVISORY RESPONSIBILITIES
   - DAILY VERSUS LONG-TERM
   - FREQUENCY OF QC DATA EVALUATION
   - ACTION TO BE TAKEN TO DETERMINE PERFORMANCE AND IMPROVE IT.

4. SUMMARIES OF DAILY QC SUCCESS
   - HOW WELL WAS THE SYSTEM RUN
   - FREQUENCY OF FAILURE, ACTION TAKEN
   - SIGNIFICANCE TO CLIENT

5. DATA COMPARABILITY
   - FREQUENCY OF AND WITH WHOM WERE EXTERNAL COMPARISONS MADE.

6. HISTORICAL RECORDS.
   - PREVIOUS PROCEDURES, RELIABILITY
   - DEVELOPMENT OF CURRENT METHOD
   - SUITABILITY TO SAMPLE TYPES BEING ANALYSED.

QUALITY CONTROL

1. CONTROL MUST BE ESTABLISHED BEFORE IT CAN BE MAINTAINED!

2. A SYSTEM IS OUT-OF-CONTROL IF IT PRODUCES UNEXPECTED DATA MORE THAN ONCE IN TWENTY TO TWENTY-FIVE RUNS!

3. CONTROL LIMITS TEND TO TIGHTEN WITH TIME AS CONTROL PROTOCOL IS ESTABLISHED.

4. ACCEPTANCE LIMITS, LOOSER THAN CONTROL LIMITS, PERMIT RELEASE OF DATA TO CLIENTS WHO DO NOT REQUIRE PERFORMANCE EQUAL TO THE SYSTEM CAPABILITY.

5. BOTH COMPONENT AND PRODUCT CONTROLS ARE REQUIRED TO MINIMIZE SYSTEM FAILURE. PREVENTION RATHER THAN CURE!

6. CONTROL CHARTING PERMITS TREND ANALYSIS!

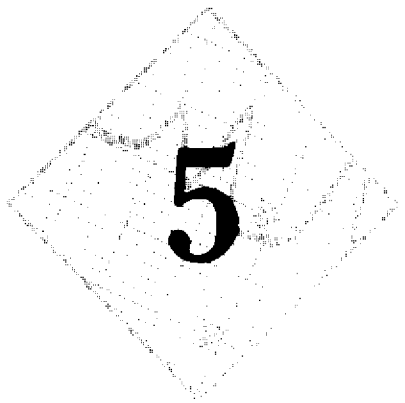
7. DON'T FIDDLE OR TRENDS, WHICH COULD IDENTIFY CAUSE, WILL BE DESTROYED!

8. CONTROL IS REQUIRED OVER

   A) REPEATABILITY

   B) REPRODUCIBILITY

   C) BIAS/ERROR/RECOVERY



High-Resolution PCB Analysis: Synthesis and Chromatographic Properties of All 209 PCB Congeners

Michael D. Mullin, Cynthia M. Pochini, Shella McCrindle, Marjorie Romkes, Stephen H. Safe and Lorna M. Safe

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High-Resolution PCB Analysis: Synthesis and Chromatographic Properties of All 209 PCB Congeners

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■ This paper reports the synthesis and spectroscopic properties of all the mono-, di-, tri-, tetra-, penta-, hexa-, and heptachlorobiphenyls and completes the synthesis of all 209 polychlorinated biphenyls (PCBs). The retention times and molar response factors of the 209 PCBs were determined relative to a reference standard, octachloronaphthalene. The retention times for these compounds generally increased with increasing chlorine content, and it was apparent that within a series of isomers there was an increase in retention time with increasing meta and para and decreasing ortho substitution. By use of a 50-m narrow bore fused silica capillary column coated with SE-54, it was possible to separate 187 PCB congeners, and only 11 pairs of compounds were not fully resolved. With some additional analytical improvements, isomer-specific PCB analysis can be utilized to determine the composition of commercial PCBs and accurately follow the fate and distribution of these pollutants within the global ecosystem.

Introduction

PCBs have been widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, flame retardants, organic diluents, and dielectric fluids (*1*). The unusual industrial versatility of PCBs is directly related to their physical properties which include resistance to acids and bases, compatibility with organic materials, resistance to oxidation and reduction, excellent electrical insulating properties, thermal stability, and nonflammability. Unfortunately, these physical properties coupled with their widespread use, relatively low acute toxicity, and improper disposal have resulted in the contamination by PCBs of every component of the global ecosystem (*1–10*). Moreover, the lipophilic nature and persistence of PCBs also contributes to their high bioaccumulation potential and their biomagnification in higher trophic levels of the food chain. PCB residues are routinely detected in fish, wildlife, and human adipose tissue, blood and breast milk (*1, 3, 7, 9, 11–18*).

There are 209 possible PCB isomers and congeners, and most of the commercial mixtures and environmental samples exhibit a multitude of peaks (*19–27*). It has been common practice to quantitate environmental PCBs by comparing their packed column gas chromatograms with the patterns exhibited by known amounts of individual commercial PCBs or mixtures of these formulations (*23, 25*). This method relies on pattern matching using specific peaks for quantitation. If the gas chromatograms of the environmental PCB residues cannot be "matched" with an appropriate cocktail containing known amounts of the commercial formulations, then quantitation is not possible. High-resolution isomer-specific PCB analysis is now a feasible option for the identification and quantitation of the individual PCBs present in commercial mixtures and environmental samples (*13, 19, 20, 22, 26–29*). This method ultimately requires samples of all 209 PCB congeners and their separation by glass capillary gas chromatography. Preliminary studies have demonstrated the feasibility of this appraoch with the isomeric octa- and nonachlorobiphenyls (*27*). This study now reports the synthesis of all the mono- to heptachlorobiphenyls and the relative retention times and response factors of 209 PCB congeners.

Materials and Methods

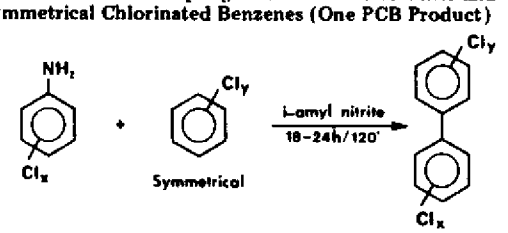
Chemicals. The 2-, 3-, and 4-chloroanilines (CA), 2,6-, 2,5-, 2,4-, 2,3-, 3,5-, and 3,4-dichloroanilines (DCA), 2,4,6-, 2,4,5-, 2,3,4-, and 3,4,5-trichloroanilines (TCA), 2,3,5,6- and

Table I. Synthesis of Mono-, Di-, and Trichlorobiphenyls[a]

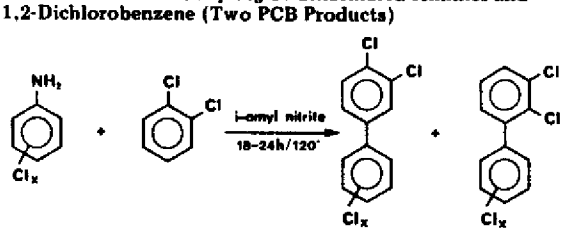
isomer no.	substitution pattern[b]	synthesis[c]	isomer no.	substitution pattern[b]	synthesis[c]
1	2^P	2-CA, benzene	21	$2,3,4^P$	2,3,4-TCA, benzene
2	3^P	3-CA, benzene	22	2,3,4′	4-CA, 1,2-DCB
3	4^P	4-CA, benzene	23	2,3,5	aniline, 1,2,4-TCB
4	$2,2'^P$	2,2′-dichlorobenzidine	24	2,3,6	aniline, 1,2,4-TCB
5	$2,3^P$	2,3-DCA, benzene	25	2,3′,4	3-CA, 1,3-DCB
6	2,3′	2-CA, MCB	26	$2,3',5^P$	3-CA, 1,4-DCB
7	$2,4^P$	2,4-DCA, benzene	27	2,3′,6	3-CA, 1,3-DCB
8	2,4′	4-CA, MCB	28	2,4,4′	4-CA, 1,3-DCB
9	$2,5^P$	aniline, 1,4-DCB	29	$2,4,5^P$	2,4,5-TCA, benzene
10	$2,6^P$	2,6-DCA, benzene	30	$2,4,6^P$	2,4,6-TCA, benzene
11	$3,3'^P$	3,3′-dichlorobenzene	31	$2,4',5^P$	4-CA, 1,4-DCB
12	$3,4^P$	3,4-DCA, benzene	32	2,4′,6	4-CA, 1,3-DCB
13	3,4′	4-CA, MCB	33	2′,3,4	2-CA, 1,2-DCB
14	$3,5^P$	3,5-DCA, benzene	34	2′,3,5	2-CA, 1,3-DCB
15	$4,4'^P$	Aldrich	35	3,3′,4	3-CA, 1,2-DCB
16	2,2′,3	2-CA, 1,2-DCB	36	3,3′,5	3-CA, 1,3-DCB
17	2,2′,4	2-CA, 1,3-DCB	37	$3,4,4'^P$	4-CA, 1,2-DCB
18	$2,2',5^P$	2-CA, 1,4-DCB	38	$3,4,5^P$	3,4,5-TCA, benzene
19	2,2′,6	2-CA, 1,3-DCB	39	3,4′,5	4-CA, 1,3-DCB
20	2,3,3′	3-CA, 1,2-DCB			

[a] The number of each PCB in Tables I–V is according to the system of Ballschmiter and Zell (*20*). [b] Superscript P indicates that it was isolated as a pure compound. [c] Isomers which are isolated as mixtures contain the following PCB impurities (indicated in the parentheses): 6(8,4); 8(13,15); 13(8,15); 16(33); 17(19,34); 19(17,34); 20(35); 22(37); 23(24,29); 24(23,29); 25(27,36); 27(25,36); 28(32,39); 32(28,39); 33(16); 34(17,19); 35(20); 36(25,27); 39(28,32).

Scheme I. Dizao Coupling of Chlorinated Anilines and Symmetrical Chlorinated Benzenes (One PCB Product)

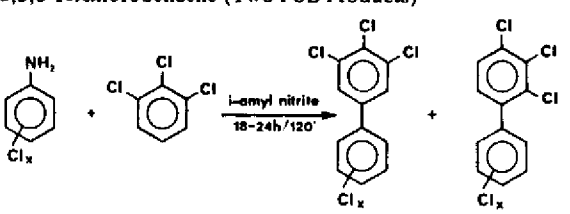


Scheme II. Diazo Coupling of Chlorinated Anilines and 1,2-Dichlorobenzene (Two PCB Products)

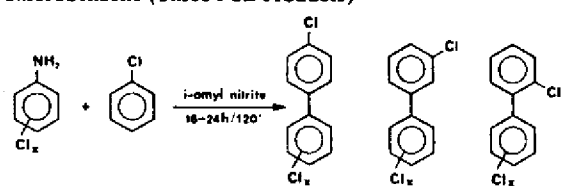


2,3,4,5-tetrachloroanilines (TCA), and pentachloroaniline (PCA) were purchased from Aldrich Chemical Co. 2,3,4,6-Tetrachloroaniline and 2,3,5-trichloroaniline were prepared by the chlorination of 2,4,6-trichloroaniline and 3,5-dichloroaniline as described (*27*). Benzene, chlorobenzene, 1,4-, 1,3-, and 1,2-dichlorobenzene, 1,2,4-, 1,2,3-, and 1,3,5-trichlorobenzene, 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene, and pentachlorobenzene were purchased from Aldrich Chemical Co. Isoamyl nitrite was obtained from Eastman Organic Chemicals; 2,2,5,5′-tetrachlorobenzidine and 3,3′- and 2,2′-dichlorobenzidine were purchased from Pfaltz & Bauer Chemical Co. Most of the PCB isomers and congeners were synthesized via the Cadogan coupling (*30*) of a chlorinated aniline (10–15) mmol) in excess of an appropriate chlorinated benzene (100–200 mmol) as indicated in Schemes I–V. Isoamyl nitrite (20–25 mmol) was added to the stirred solution of the reactants at 120–130 °C over a period of 60 min, and the reaction was continued for an additional 18 h. After

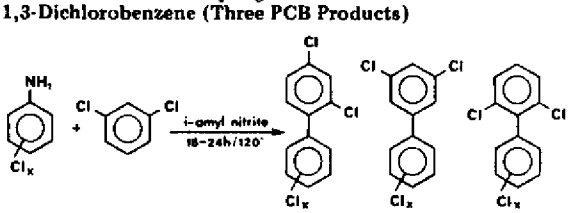
Scheme III. Diazo Coupling of Chlorinated Anilines and 1,2,3-Trichlorobenzene (Two PCB Products)



Scheme IV. Diazo Coupling of Chlorinated Anilines and Chlorobenzene (Three PCB Products)



Scheme V. Diazo Coupling of Chlorinated Anilines and 1,3-Dichlorobenzene (Three PCB Products)



removal of the excess unreacted chlorinated benzene, the crude product was adsorbed onto neutral alumina or silicic acid and added to a column of Florisil with a top layer of neutral alumina, and the PCB product was eluted with petroleum spirit (bp 30–60 °C, 300–400 cm^3). The eluate was concentrated and the residue further purified by preparative thin-layer chromatography (TLC) on silica gel HF_{254} plates (0.9-mm thickness, Merck). All the symmetrical coupling products (see Scheme I) and some of the unsymmetrical coupling products were obtained as pure

Table II. Synthesis and ^{1}H NMR Spectra of the Tetrachlorobiphenyls

isomer no.	substitution pattern[c]	synthesis[b]	^{1}H NMR spectra[a]
40	2,2',3,3'^{P}	2,3-DCA, 1,2-TCB	7.33 (H-6,6', dd, J = 1.8, 7.8 Hz), 7.48 (H-5,5', t, J = 7.9 Hz), 7.68 (H-4,4', dd, J = 1.8, 7.8 Hz)
41	2,2',3,4	2-CA, 1,2,3-TCB	7.34–7.76 (m)
42	2,2',3,4'^{P}	2,4-DCA, 1,2-DCB	7.4–7.71 (m)
43	2,2',3,5	2-CA, 1,2,4-TCB	7.52–7.82 (m)
44	2,2',3,5'^{P}	2,3-DCA, 1,4-DCB	7.25–7.82 (m)
45	2,2',3,6	2-CA, 1,2,4-TCB	7.52–7.82 (m)
46	2,2',3,6'	2,6-DCA, 1,2-DCB	7.28–7.74 (m)
47	2,2',4,4'^{P}	2,2'-tetrachlorobenzidine	7.38 (H-6,6', d, J = 8.4 Hz), 7.50 (H-5,5', dd, J = 8.4, 2.1 Hz), 7.65 (H-3,3', d J = 2.1 Hz)
48	2,2',4,5	2-CA, 1,2,4-TCB	7.25–7.82 (m)
49	2,2',4,5'^{P}	2,4-DCA, 1,4-DCB	7.38–7.67 (m)
50	2,2',4,6^P	2-CA, 1,3,5-TCB	7.65 (H-3,5, s), 7.30–7.65 (m)
51	2,2',4,6'	2,4-CA, 1,3-DCB	7.4–7.65 (m)
52	2,2',5,5'^{P}	2,5-DCA, 1,4-DCB	7.44–7.62 (m)
53	2,2',5,6'^{P}	2,6-DCA, 1,4-DCB	7.40–7.67 (m)
54	2,2',6,6'^{P}	2,6-dichloroiodobenzene	7.48–7.65 (m)
55	2,3,3',4	3-CA, 1,2,3-TCB	7.28–7.92 (m)
56	2,3,3',4'^{P}	3,4-DCA, 1,2-DCB	7.35–7.71 (m)
57	2,3,3',5	3-CA, 1,2,4-TCB	7.67 (Hz, d, J = 2.4 Hz), 7.21–7.66
58	2,3,3',5'	3,5-DCA, 1,2-DCB	7.19–7.91 (m)
59	2,3,3',6	3-CA, 1,2,4-TCB	7.53 (H-5, d, J = 8.6 Hz), 7.63 (H-4, d, J = 8.6 Hz), 7.21–7.66 (m)
60	2,3,4,4'^{P}	4-CA, 1,2,3-TCB	7.40 (H-2',6', d, J = 8.4 Hz), 7.47 (H-6, d, J = 8.4 Hz), 7.54 (H-5, d, J = 8.4 Hz), 7.66 (H-3',5', d, J = 8.4 Hz)
61	2,3,4,5^P	2,3,4,5-TCA, benzene	7.75 (H-6, s), 7.70–7.74 (m)
62	2,3,4,6^P	aniline, 1,2,3,5-TCB	7.83 (H-5, s), 7.25–7.60 (m)
63	2,3,4',5	4-CA, 1,2,4-TCB	7.30–7.81 (m)
64	2,3,4',6	4-CA, 1,2,4-TCB	7.36–7.81 (m)
65	2,3,5,6^P	aniline, 1,2,4,5-TCB	7.92 (H-4, s), 7.27–7.60 (m)
66	2,3',4,4'^{P}	2,4-DCA, 1,2-DCB	7.40–7.71 (m)
67	2,3',4,5	3-CA, 1,2,4-TCB	7.60 (H-6, s), 7.74 (H-3, s), 7.21–7.66 (m)
68	2,3',4,5'	2,4-DCA, 1,3-DCB	7.40–7.65 (m)
69	2,3',4,6^P	3-CA, 1,3,5-TCB	7.64 (H-3,5, s), 7.36–7.53 (m)
70	2,3',4',5^P	3,4-DCA, 1,4-DCB	7.42–7.71 (m)
71	2,3',4',6	2,6-DCA, 1,2-DCB	7.28–7.74 (m)
72	2.2',5,5'^{P}	3,5-DCA, 1,4-DCB	7.40–7.65 (m)
73	2,3',5',6	3,5-DCA, 1,3-DCB	7.35–7.78 (m)
74	2,4,4',5	4-CA, 1,2,4-TCB	7.30–7.81 (m)
75	2,4,4',6^P	4-CA, 1,3,5-TCB	7.33 (H-2',6', d, J = 8.5 Hz), 7.56 (H-3',5', d, J = 8.5 Hz), 7.64 (H-3.5, s)
76	2',3,4,5	2-CA, 1,2,3-TCB	7.65 (H-2,6), 7.34–7.65 (m)
77	3,3',4,4'^{P}	3,4-DCA, 1,2-DCB	7.24–7.42 (m)
78	3,3',4,5	3-CA, 1,2,3-TCB	7.28–7.92 (7)
79	3,3',4,5'	3,5-DCA, 1,2-DCB	7.16–7.91 (m)
80	3,3',5,5'^{P}	3,5-DCA, 1,3-DCB	7.46 (H-2,2',6,6', d, J = 2.0 Hz), 7.77 (H-4,4', t, J = 2.0 Hz)
81	3,4,4',5^P	4-CA, 1,2,3-TCB	7.53 (H-2',6', d, J = 8.8 Hz), 7.78 (H-3',5', d, J = 8.8 Hz), 7.87 (H-2,6, s)

[a] m = unresolved multiplet. [b] Isomers which are isolated as mixtures contain the following PCB impurities (indicated in parentheses): 41(76); 43(45,48); 45(43,48); 46(71); 48(43,45); 51(47,68); 55(78); 57(59,67); 58(79); 59(57,67); 63(64,74); 64(63,74); 67(57,59); 68(51,47); 71(46); 73(68,80); 74n64,63); 76(41); 78(55); 79(58). [c] Superscript P indicates that it was isolated as a pure compound.

PCB congeners (See Tables I–V) after TLC or repeated TLC and crystallization from methanol. The composition of the PCB reaction mixtures was determined by GLC and their proton magnetic resonance (^{1}H NMR) spectra as previously described (*27*). 2,2'-Dichloro- and 2,2',4,4'-tetrachlorobiphenyl were prepared from 2,2'-dichlorobenzidine; 2,2',5,5'-tetrachloro- and 2,2',4,4',5,5'-hexachlorobiphenyl were prepared from 2,2',5,5'-dichlorobenzidine (*30*). 3,3'-Dichlorobiphenyl and 2,2',6,6'-dichlorobiphenyl were gifts from Dr. O. Hutzinger. The ^{1}H NMR spectra were recorded on a Bruker multinuclear 60 MHz or Varian XL-200 ^{1}H NMR spectrometer in acetone-d_6 and are summarized in Tables II–V.

Chromatography. The high-resolution capillary gas chromatography was performed on a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector. A 50-m fused siliica capillary column (0.2 mm i.d.) coated with SE-54 (Hewlett-Packard) was used to separate the PCB isomers and congeners. The oven temperature was programmed at a rate of 1.0 °C min^{-1} from 100 to 240 °C. The injector and detector temperatures were 270 and 330 °C, respectively. Sample volume, 6.0 μL, was injected by using an automatic sampler with splitting in the injector (10:1 split ratio, vented from 0.75 to 1.75 min). The hydrogen carrier gas was held at a constant pressure of 2.25 kg cm^{-2} to give the optimized linear velocity ($\bar{u}$) at 100 °C of 45 cm s^{-1}. The retention times (RT) of the PCBs were expressed relative to the octachloronaphthalene (OCN; RT = 124.9 min) standard; the response factors (by weight) for the PCBs were expressed relative to OCN (R_f = 1.0 for 1 ng of OCN) by using integrated peak areas.

Results and Discussion

Synthesis and ^{1}H NMR Data. The synthetic strategies used for the preparation of all 209 PCB isomers and congeners are summarized in Schemes I–VI. Schemes I–V utilize comparable reaction conditions in which chlorinated

Table III. Synthesis and ^{1}H NMR Spectra of the Pentachlorobiphenyls

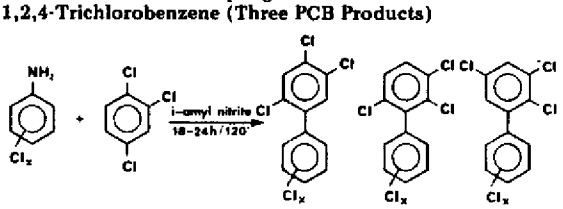
isomer no.	substitution pattern[c]	synthesis[b]	^{1}H NMR spectra[a]
82	2,2′,3,3′,4^P	2,3-DCA, 1,2,3-TCB	7.41–7.68 (m)
83	2,2′,3,3′,5	2,3-DCA, 1,2,4-TCB	7.49 (H-6, d, J = 2.4 Hz), 7.79 (H-4, d, J = 2.4 Hz), 7.30–7.70 (m)
84	2,2′,3,3′,6^P	2,3-DCA, 1,2,4-TCB	7.59 (H-5, d, J = 8.7 Hz), 7.77 (H-4, d, J = 8.7 Hz), 7.40–7.73 (m)
85	2,2′,3,4,4′^{P}	2,4-DCA, 1,2,3-TCB	7.36 (H-6, d, J = 8.8 Hz), 7.71 (H-5, d, J = 8.8 Hz), 7.31–7.79 (m)
86	2,2′,3,4,5^P	2-CA, 1,2,3,4-TCB	7.61 (H-6, s), 7.35–7.61 (m)
87	2,2′,3,4,5′^{P}	2,3,4-TCA, 1,4-DCB	6.73 (H-6, d, J = 8.8 Hz), 7.36 (H-5, d, J = 8.8 Hz), 7.49–7.58 (m)
88	2,2′,3,4,6^P	2-CA, 1,2,3,5-TCB	7.88 (H-5, s), 7.35–7.63 (m)
89	2,2′,3,4,6′	2,6-DCA, 1,2,3-TCB	7.36 (H-6, d, J = 8.5 Hz), 7.77 (H-5, d, J = 8.5 Hz), 7.49–7.62 (m)
90	2′,2,3,4′,5	2,4-DCA, 1,2,4-TCB	7.43 (H-6, d, J = 2.4 Hz), 7.79 (H-4, d, J = 2.4 Hz), 7.46–7.73 (m)
91	2,2′,3,4′,6^P	2,4-DCA, 1,2,4-TCB	7.65 (H-5, d, J = 8.5 Hz) 7.76 (H-4, d, J = 8.5 Hz) 7.31–7.73 (m)
92	2,2′,3,5,5′^{P}	2,5-DCA, 1,2,4-TCB	7.45 (H-6, d, J = 2.4 Hz), 7.80 (H-4, d, J = 2.4 Hz), 7.47–7.65 (m)
93	2,2′,3,5,6^P	2-CA, 1,2,4,5-TCB	7.99 (H-4, s), 7.35–7.65 (m)
94	2,2′,3,5,6′	2,6-DCA, 1,2,4-TCB	7.45 (H-6, d, 2.4 Hz), 7.80 (H-4, d, J = 2.4 Hz), 7.54–7.64 (m)
95	2,2′,3,5′,6	2,5-DCA, 1,2,4-TCB	7.69 (H-5, d, J = 8.6 Hz), 7.73 (H-4, d, J = 8.6 Hz), 7.47–7.65 (m)
96	2,2′,3,6,6′^{P}	2,6-DCA, 1,2,4-TCB	7.62 (H-5, d, J = 8.9 Hz), 7.80 (H-4, d, J = 8.9 Hz), 7.55–7.65 (m)
97	2,2′,3′,4,5	2,3-DCA, 1,2,4-TCB	7.65 (H-6, s), 7.84 (H-3, s), 7.30–7.80 (m)
98	2,2′,3′,4,6^P	2,3-DCA, 1,3,5-TCB	7.67 (H-3,5, s), 7.23–7.85 (m)
99	2,2′,4,4′,5	2,4-DCA, 1,2,4-TCB	7.64 (H-6, s), 7.85 (H-3, s), 7.34–7.64 (m)
100	2,2′,4,4′,6^P	2,4-DCA, 1,3,5-TCB	7.69 (H-3,5, s), 7.30–7.77 (m)
101	2,2′,4,5,5′^{P}	2,4,5-TCA, 1,4-DCB	7.65 (H-6, s), 7.84 (H-3, s), 7.47–7.64 (m)
102	2,2′,4,5,6′	2,6-DCA, 1,2,4-TCB	7.65 (H-6, s), 7.85 (H-3, s), 7.54–7.64 (m)
103	2,2′,4,5′,6^P	2,4,6-TCA, 1,4-DCB	7.67 (H-3,5, s), 7.42–7.64 (m)
104	2,2′,4,6,6′^{P}	2,6-DCA, 1,3,5-TCB	7.73 (H-3,5, s), 7.23–7.57 (m)
105	2,3,3′,4,4′^{P}	2,3,4-TCA, 1,2-DCB	7.13 (H-5′, d, J = 8.2 Hz), 7.20 (H-6′, dd, J = 2.0, 8.2 Hz), 7.43, 7.50 (H-5,6, d, J = 8.2 Hz), 7.46 (H-2′, d, J = 2.0 Hz)
106	2,3,3′,4,5^P	3-CA, 1,2,3,4-TCB	7.67 (H-6, s), 7.49–7.56 (m)
107	2,3,3′,4′,5	3,4-DCA, 1,2,4-TCB	7.30–7.81 (m)
108	2,3,3′,4,5′	3,5-DCA, 1,2,3-TCB	complex multiplet
109	2,3,3′,4,6^P	3-CA, 1,2,3,5-TCB	7.85 (H-5, s), 7.14–7.63 (m)
110	2,3,3′,4′,6	3,4-DCA, 1,2,4-TCB	7.30–7.81 (m)
111	2,3,3′,5,5′	3,5-DCA, 1,2,4-TCB	7.75 (H-4 or -6, d, J = 2.4 Hz) 7.44–7.65 (m)
112	2,3,3′,5,6^P	3-CA, 1,2,4,5-TCB	7.95 (H-4, s), 7.19–7.67 (m)
113	2,3,3′,5′,6^P	3,5-DCA, 1,2,4-TCB	7.54 (H-5, d, J = 8.8 Hz), 7.75 (H-5, d, J = 8.8 Hz), 7.36–7.82 (m)
114	2,3,4,4′,5^P	4-CA, 1,2,3,4-TCB	7.25 (H-2′,6′, d, J = 8.3 Hz), 7.30 (H-6, s), 7.37 (H-3′,5′, d, J = 8.0 Hz)
115	2,3,4,4′,6^P	4-CA, 1,2,3,5-TCB	7.33 (H-2′,6′, d, J = 8.7 Hz), 7.60 (H-3′,5′, d, J = 8.7 Hz), 7.94 (H-5, s)
116	2,3,4,5,6^P	PCA, benzene	7.21–7.60 (m)
117	2,3,4′,5,6^P	4-CA, 1,2,4,5-TCB	7.33 (H-2′,6′, J = 8.8 Hz), 7.61 (H-3′,5′, d, J = 8.8 Hz), 7.94 (H-4, s)
118	2,3′,4,4′,5^P	2,4,5-TCA, 1,2-DCB	7.24 (H-6′, dd, J = 8.0, 2.0 Hz), 7.41 (H-6, s), 7.49 (H-2′, d, J = 2.0 Hz), 7.51 (H-5′, d, J = 8.0 Hz), 7.61 (H-3, s)
119	2,3′,4,4′,6^P	3,4-DCA, 1,3,5-TCB	7.31 (H-6′, dd, J = 8.4, 2.0 Hz), 7.57 (H-2′, d, J = 2.0 Hz), 7.65 (H-3,5, s), 7.75 (H-5′, d, J = 8.4 Hz)
120	2,3′,4,5,5′	3,5-DCA, 1,2,4-TCB	7.71 (H-6, s), 7.82 (H-3, s), 7.44–7.65 (m)
121	2,3′,4,5′,6^P	3,5-DCA, 1,3,5-TCB	7.36 (H-2′,6′, d, J = 2.0 Hz), 7.60 (H-4′, t, J = 2.0 Hz), 7.65 (H-3,5, s)
122	2′,3,3′,4,5	2,3-DCA, 1,2,3-TCB	7.67 (H-2,6, s), 7.25–7.75 (m)
123	2′,3,4,4′,5^P	2,4-DCA, 1,2,3-TCB	7.51–7.63 (H-3′,5′,6′, m), 7.66 (H-2,6, s)
124	2′,3,4,5,5′^{P}	3,4,5-TCA, 1,4-DCB	7.70 (H-2,6, s), 7.53–7.60 (m)
125	2′,3,4,5,6′	2,6-DCA, 1,2,3-TCB	7.57 (H-2,6, s), 7.49–7.62 (m)
126	3,3′,4,4′,5^P	3,4,5-TCA, 1,2-DCB	ref 33
127	3,3′,4,5,5′	3,5-DCA, 1,2,3-TCB	complex multiplet

[a] m = unresolved multiplet. [b] Isomers which are isolated as mixtures contain the following PCB impurities (indicated in parentheses): 83(84,97); 89(125); 90(91,99); 92(95,101); 94(96,102); 95(92,101); 97(83,84); 99(90,91); 102(94,96); 107(110,118); 108(127); 110(107,118); 111(113,120); 120(111,113); 122(82); 125(89); 127(108). [c] Superscript P indicates that it was isolated as a pure compound.

anilines are coupled with chlorinated benzenes by using an excess of the latter reactant which also serves as the solvent media. The coupling reaction proceeds smoothly after the addition of isoamyl nitrite, and the PCB product(s) is (are) readily isolated after a series of chromatographic procedures (27). All the chlorinated benzenes used in these reactions and all but two of the chlorinated anilines are commercially available. 2,3,4,6-Tetrachloro- and 2,3,5-trichloroaniline were prepared by the chlorination of 2,4,6-trichloro- and 3,5-dichloroaniline, respectively (27).

Scheme I represents the reaction of the anilines with a symmetrical chlorinated benzene (1,4-dichloro-, 1,3,5-trichloro-, 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachloro-, and pentachlorobenzene) or benzene to yield a single PCB reaction product. These congeners were readily purified and characterized. The reaction of chlorinated anilines

Scheme VI. Diazo Coupling of Chlorinated Anilines and 1,2,4-Trichlorobenzene (Three PCB Products)



with 1,2-dichlorobenzene or 1,2,3-trichlorobenzene gives both 2,3- and 3,4-dichloro- and 2,3,4- or 3,4,5-trichloro-substituted products, respectively (schemes II and III). Some of these mixtures were separable by repeated TLC on silica gel impregnated with charcoal. Schemes IV–VI summarize the reactions in which the chloro-, 1,3-dichloro-,

Table IV. Synthesis and ^{1}H NMR Spectra of the Hexachorobiphenyls

isomer no.	substitution pattern[c]	synthesis[b]	^{1}H NMR data[a]
128	2,2′,3,3′,4,4′^{P}	2,3,4-TCA, 1,2,3-TCB	7.39 (H-6,6′, d, J = 8.3 Hz), 7.74 (H-5,5′, d, J = 8.3 Hz)
129	2,2′,3,3′,4,5^P	2,3-DCA, 1,2,3,4-TCB	7.40 (H-6′, dd, J = 1.8, 8.0 Hz), 7.51 (H-5′, t, J = 8.0 Hz), 7.68 (H-6, s), 7.73 (H-4′, dd, J = 1.8, 8.0 Hz)
130	2,2′,3,3′,4,5′	2,3,4-TCA, 1,2,4-TCB	7.40 (H-5, d, J = 8.2 Hz), 7.47 (H-6′, d, J = 2.4 Hz), 7.79 (H-6, d, J = 8.2 Hz), 7.80 (H-4′, d, J = 2.4 Hz)
131	2,2′,3,3′,4,6^P	2,3-DCA, 1,2,3,5-TCB	7.90 (H-5, s), 7.27–7.85 (m)
132	2,2′,3,3′,4,6′	2,3,4-TCA, 1,2,4-TCB	7.40 (H-5, d, J = 8.2 Hz), 7.59 (H-4′, d, J = 8.3 Hz), 7.78 (H-5′, d, J = 8.3 Hz), 7.80 (H-6, d, J = 8.2 Hz)
133	2,2′,3,3′,5,5′	2,3,5-TCA, 1,2,4-TCB	7.51 (H-6,6′, d, J = 2.4 Hz), 7.81 (H-4,4′, d, J = 2.4 Hz)
134	2,2′,3,3′,5,6^P	2,3-DCA, 1,2,4,5-TCB	8.02 (H-4, s), 7.26–7.86 (m)
135	2,2′,3,3′,5,6′	2,3,5-TCA, 1,2,4-TCB	7.52 (H-6′, d, J = 2.5 Hz), 7.64 (H-5, d, J = 8.6 Hz), 7.76 (H-4, d, J = 8.0 Hz), 7.87 (H-4′, d, J = 2.5 Hz)
136	2,2′,3,3′,6,6′	2,3,6-TCA, 1,2,4-TCB	ref 34
137	2,2′,3,4,4′,5^P	2,4-DCA, 1,2,3,4-TCB	7.65 (H-6, s), 7.33–7.70 (m)
138	2,2′,3,4,4′,5′^{P}	2,4,5-TCA, 1,2,3-TCB	7.40 (H-6, d, J = 8.5 Hz), 7.67 (H-6′, s), 7.73 (H-5′, d, J = 8.5 Hz), 7.85 (H-3′, s)
139	2,2′,3,4,4′,6^P	2,4-DCA, 1,2,3,5-TCB	7.44 (H-6′, d, J = 8.0 Hz), 7.59 (H-5′, dd, J = 2.2, 8.0 Hz), 7.73 (H-3′, d, J = 2.0 Hz), 7.91 (H-5, s)
140	2,2′,3,4,4′,6′^{P}	2,3,4-TCA, 1,3,5-TCB	7.40 (H-6, d, J = 8.4 Hz), 7.71 (H-3′,5′, s), 7.77 (H-5, d, J = 8.4 Hz)
141	2,2′,3,4,5,5′^{P}	2,5-DCA, 1,2,3,4-TCB	7.52–7.51 (H-3′,4′,6′, m), 7.69 (H-6, s)
142	2,2′,3,4,5,6^P	2-CA, PCB	7.35–7.68 (m)
143	2,2′,3,4,5,6′^{P}	2,6-DCA, 1,2,3,4-TCB	7.55–7.64 (H-3′,4′,5′, m), 7.70 (H-4, s)
144	2,2′,3,4,5′,6^P	2,5-DCA, 1,2,3,5-TCB	7.90 (H-5, s), 7.35–7.68 (m)
145	2,2′,3,4,6,6′^{P}	2,6-DCA, 1,2,3,5-TCB	7.57 (H-5, s), 7.12–7.42 (m)
146	2,2′,3,4′,5,5′	2,4,5-TCA, 1,2,4-TCB	7.49 (H-6′, d, J = 2.4 Hz), 7.70 (H-6′, s), 7.80 (H-4′, d, J = 2.4 Hz), 7.85 (H-3, s)
147	2,2′,3,4′,5,6^P	2,4-DCA, 1,2,4,5-TCB	8.01 (H-4, s), 7.35–7.76 (m)
148	2,2′,3,4′,5,6′	2,4,6-TCA, 1,2,4-TCB	7.49 (H-6, d, J = 2.4 H), 7.75 (H-3′,5′, s) 7.83 (H-4, d, J = 2.4 Hz)
149	2,2′,3,4′,5′,6	2,4,5-TCA, 1,2,4-TCB	7.59 (H-5, d, J = 8.8 Hz), 7.70 (H-6′, s), 7.78 (H-4, d, J = 8.8 Hz), 7.91 (H-3′, s)
150	2,2′,3,4′,6,6′^{P}	2,4,6-TCA, 1,2,4-TCB	7.67 (H-5, d, J = 8.6 Hz), 7.76 (H-3′,5′, s), 7.79 (H-4, d, J = 8.6 Hz)
151	2,2′,3,5,5′,6^P	2,3,5,6-TCA, 2,5-DCB	7.49–7.64 (H-3′,4′,6′, m), 8.01 (H-4, s)
152	2,2′,3,5,6,6′^{P}	2,6-DCA, 1,2,3,5-TCB	7.55–7.69 (H-3′,4′,5′, m), 8.07 (H-4, s)
153	2,2′,4,4′,5,5′^{P}	2,2′,5,5′-tetrachloro-benzidine	7.70 (H-6,6′, s), 7.91 (H-3,3′, s)
154	2,2′,4,4′,5,6′^{P}	2,4,5-TCA, 1,3,5-TCB	7.68 (H-6, s), 7.74 (H-3′,5′, s), 7.90 (H-3, s)
155	2,2′,4,4′,6,6′^{P}	2,4,6-TCA, 1,3,5-TCB	8.12 (H-3′,5′,3,5, s) in $CDCl_3$
156	2,3,3′,4,4′,5^P	3,4-DCA, 1,2,3,4-TCB	7.22 (H-6′, q, J = 8.3, 2.3 Hz), 7.37 (H-6, s) 7.48 (H-2′, d, J = 2.3 Hz), 7.53 (H-5′, d, J = 8.3 Hz)
157	2,3,3′,4,4′,5′^{P}	3,4,5-TCA, 1,2,3-TCB	7.09 (H-6, d, J = 8.0 Hz), 7.35 (H-2′,6′, s), 7.40 (H-5, d, J = 8.0 Hz) in $CDCl_3$
158	2,3,3′,4,4′,6^P	3,4-DCA, 1,2,3,5-TCB	7.31 (H-6′, q, J = 8.4 Hz), 7.61 (H-2′, d, J = 2.0 Hz), 7.78 (H-5′, d, J = 8.4 Hz), 7.86 (H-5, s)
159	2,3,3′,4,5,5′^{P}	3,5-DCA, 1,2,3,4-TCB	7.51 (H-2′,6-, d, J = 1.9 Hz), 7.60 (H-4′, t, J = 1.9 Hz) 7.72 (H-6, s)
160	2,3,3′,4,5,6^P	3-CA, PCB	7.21–7.65 (m)
161	2,3,3′,4,5′,6^P	3,5-DCA, 1,2,3,5-TCB	7.41 (H-2′,6′, d, J = 1.9 Hz), 7.63 (H-4′, t, J = 1.9 Hz), 7.87 (H-5, s)
162	2,3,3′,4′,5,5′	3,4,5-TCA, 1,2,4-TCB	7.54 (H-6, d, J = 1.9 Hz), 7.72 (H-2′,6′, s), 7.80 (H-4, d, J = 1.9 Hz)
163	2,3,3′,4′,5,6^P	3,4-DCA, 1,2,4,5-TCB	7.33 (H-6′, q, J = 8.3, 1.9 Hz), 7.61 (H-2′, d, J = 1.9 Hz), 7.78 (H-5′, d, J = 8.3 Hz) 7.95 (H-4, s)
164	2,3,3′,4,5′,6^P	3,4,5-TCA, 1,2,3,5-TCB	7.43 (H-5, d, J = 8.4 Hz), 7.81 (H-4, d, J = 8.4 Hz), 7.92 (H-2′,6′, s)
165	2,3,3′,5,5′,6^P	3,5-DCA, 1,2,4,5-TCB	7.42 (H-2′,6′, d, J = 1.9 Hz), 7.64 (H-4′, t, J = 1.9 Hz), 7.97 (H-4, s)
166	2,3,4,4′,5,6^P	4-CA, PCB	7.01 (H-2′,6′, d, J = 8.8 Hz), 7.35 (H-3′,5′, d, J = 8.8 Hz)
167	2,3′,4,4′,5,5′^{P}	2,4,5-TCA, 1,2,3-TCB	7.76 (H-2-,6-, s), 7.76 (H-6′, s), 7.85 (H-3′, s)
168	2,3′,4,4′,5′,6^P	3,4,5-TCA, 1,3,5-TCB	7.59 (H-2′,6′, s), 7.66 (H-3,5, s)
169	3,3′,4,4′,5,5′^{P}	3,4,5-TCA, 1,2,3-TCB	7.53 (H-2′,6′,2,6, s) in $CDCl_3$

[a] m = unresolved multiplet. [b] Isomers which are isolated as mixtures contain the following PCB impurities (indicated in parentheses): 130(132,138); 132(130,138); 133(135,146); 135(133,146); 146(149,153); 148(150,154); 149(146,153); 162(164,167). [c] Superscript P indicates that it was isolated as a pure compound.

Table V. Synthesis and ^1H NMR Spectra of the Heptachlorobiphenyls

isomer no.	substitution pattern[c]	synthetic[b] precursors	^1H NMR data[a]
170	2,2′,3,3′,4,4′,5[P]	2,3,4-trichloroaniline, 1,2,3,4-tetrachlorobenzene	7.44 (H-6′, d, J = 8.4 Hz), 7.71 (H-6, s), 7.76 (H-5′, d, J = 8.4 Hz)
171	2,2′,3,3′,4,4′,6[P]	2,3,4-trichloroaniline, 1,2,3,5-tetrachlorobenzene	7.41 (H-6′, d, J = 8.8 Hz), 7.79 (H-5′, d, J = 8.8 Hz), 7.91 (H-5, s)
172	2,2′,3,3′,4,5,5′	2,3,4,5-tetrachloroaniline, 1,2,4-trichlorobenzene	7.53 (H-6′, d, J = 1.2 Hz), 7.73 (H-6, s), 7.82 (H-4′, d, J = 1.2 Hz)
173	2,2′,3,3′,4,5,6[P]	2,3-dichloroaniline, pentachlorobenzene	7.30–7.87 (m)
174	2,2′,3,3′,4,5,6′	2,3,4,5-tetrachloroaniline, 1,2,4-trichlorobenzene	7.51 (H-6, s), 7.60 (H-5′, d, J = 8.5 Hz), 7.81 (H-4′, d, J = 8.5 Hz)
175	2,2′,3,3′,4,5′,6	2,3,4,6-tetrachloroaniline, 1,2,4-trichlorobenzene	7.52 (H-6′, d, J = 2.4 Hz), 7.86 (H-4′, d, J = 2.4 Hz), 7.91 (H-5, s)
176	2,2′,3,3′,4,6,6′	2,3,4,6-tetrachloroaniline, 1,2,4-trichlorobenzene	7.66 (H-5′, d, J = 9.0 Hz), 7.91 (H-4′, d, J = 9.0 Hz), 7.97 (H-5, s)
177	2,2′,3,3′,4′,5,6[P]	2,3,4-trichloroaniline, 1,2,4,5-tetrachlorobenzene	7.43 (H-6′, d, J = 8.4 Hz), 7.81 (H-5′, d, J = 8.4 Hz), 8.02 (H-4, s)
178	2,2′,3,3′,5,5′,6	2,3,5,6-tetrachloroaniline, 1,2,4-trichlorobenzene	7.56 (H-6′, d, J = 2.4 Hz), 7.87 (H-4, d, J = 2.4 Hz), 8.03 (H-4, s)
179	2,2′,3,3′,5,6,6′	2,3,5,6-tetrachloroaniline, 1,2,4-trichlorobenzene	7.57 (H-5′, d, J = 8.3 Hz), 7.85 (H-4′, d, J = 8.3 Hz), 8.08 (H-4, s)
180	2,2′,3,4,4′,5,5′[P]	2,4,5-trichloroaniline, 1,2,3,4-tetrachlorobenzene	7.72, 7.76, 7.87 (H-6′, H-3′, H-6, s)
181	2,2′,3,4,4′,5,6[P]	2,4-dichloroaniline, pentachlorobenzene	7.36–7.77 (m)
182	2,2′,3,4,4′,5,6′[P]	2,4,6-trichloroaniline, 1,2,3,4-tetrachlorobenzene	7.71 (H-3′, H-5′, s), 7.72 (H-6, s)
183	2,2′,3,4,4′,5′,6[P]	2,4,5-trichloroaniline, 1,2,3,5-tetrachlorobenzene	7.73, 7.77, 7.91 (H-6′, H-3′, H-5, s)
184	2,2′,3,4,4′,6,6′[P]	2,4,6-trichloroaniline, 1,2,3,5-tetrachlorobenzene	7.76 (H-3′, H-5′, s), 7.97 (H-5, s)
185	2,2′,3,4,5,5′,6[P]	2,5-dichloroaniline, pentachlorobenzene	7.35–7.91 (m)
186	2,2′,3,4,5,6,6′[P]	2,6-dichloroaniline, pentachlorobenzene	7.67–7.71 (m)
187	2,2′,3,4′,5,5′,6[P]	2,4,5-trichloroaniline, 1,2,4,5-tetrachlorobenzene	7.75, 7.93, 8.03 (H-3′, H-5′, H-4, s)
188	2,2′,3,4′,5,6,6′[P]	2,4,6-trichloroaniline, 1,2,4,5-tetrachlorobenzene	7.38 (H-3′, H-5′, s), 7.69 (H-4, s)
189	2,3,3′,4,4′,5,5′[P]	3,4,5-trichloroaniline, 1,2,3,4-tetrachlorobenzene	7.35 (H-6, s), 7.40 (H-2′,6′, s) in CCl_4
190	2,3,3′,4,4′,5,6[P]	3,4-dichloroaniline, pentachlorobenzene	7.36 (H-6′, dd, J = 8.4, 1.9 Hz), 7.63 (H-2, d, J = 1.9 Hz), 7.79 (H-5′, d, J = 8.4 Hz)
191	2,3,3′,4,4′,5′,6[P]	3,4,5-trichloroaniline, 1,2,3,5-tetrachlorobenzene	7.63 (H-2′, H-6, s), 7.88 (H-5, s)
192	2,3,3′,4,5,5′,6[P]	3,5-dichloroaniline, pentachlorobenzene	7.44 (H-2′, H-6′, d, J = 1.9 Hz), 7.65 (H-4, t, J = 1.9 Hz)
193	2,3,3′,4′,5,5′,6[P]	3,4,5-trichloroaniline, 1,2,4,5-tetrachlorobenzene	7.76 (H-2′, H-6′, s), 8.07 (H-4, s)

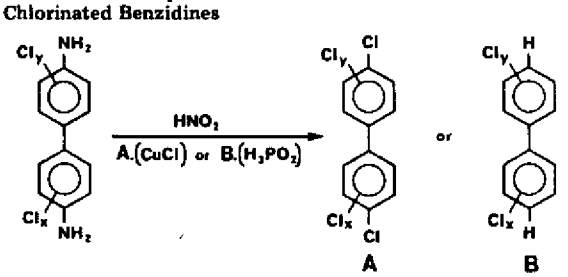
[a] m = unresolved multiplet. [b] Isomers which are isolated as mixtures contain the following PCB impurities (indicated in parentheses): 172(174,180); 174(172,180); 175(176,183); 176(175,183); 178(179,183); 179(178,183). [c] Superscript P indicates that it was isolated in the pure form.

Table VI. Relative Retention Times and Response Factors for 209 PCB Congeners

isomer no.	relative retention time	relative response factor	isomer no.	relative retention time	relative response factor	isomer no.	relative retention time	relative response factor	isomer no.	relative retention time	relative response factor
0	0.0997	0.0251	53	0.4187	0.3606	106	0.668	1.0046	157	0.8184	1.1965
1	0.1544	0.0393	54	0.38	0.3643	107	0.6628	0.8183	158	0.7429	1.132
2	0.1937	0.04[a]	55	0.5562	0.829	108	0.6626	1.0654	159	0.7655	0.9934
3	0.1975	0.0193	56	0.5676	0.829	109	0.6016	0.9625	160	0.7396	1.1914
4	0.2245	0.0374	57	0.5155	0.6[a]	110	0.6314	0.65[a]	161	0.6968	0.9672
5	0.2785	0.119	58	0.5267	0.609	111	0.6183	0.6601	162	0.7737	1.0322
6	0.2709	0.38	59	0.486	0.6[a]	112	0.5986	0.8286	163	0.7396	0.9976
7	0.2566	0.69	60	0.5676	1.0164	113	0.5862	0.604	164	0.7399	0.9848
8	0.2783	0.206	61	0.5331	1.2227	114	0.6828	1.0261	165	0.692	1.0777
9	0.257	0.388	62	0.4685	1.1478	115	0.6171	1.1328	166	0.7572	1.0421
10	0.2243	0.262	63	0.529	0.728	116	0.6132	1.3987	167	0.7814	1.0658
11	0.3238	0.0449	64	0.4999	0.607	117	0.615	0.8895	168	0.7068	0.8375
12	0.3298	0.179	65	0.4671	0.8408	118	0.6693	0.87	169	0.8625	0.8355
13	0.3315	0.2[a]	66	0.5447	0.646	119	0.5968	0.8239	170	0.874	0.75
14	0.2973	0.3047	67	0.5214	0.6[a]	120	0.6256	0.7444	171	0.8089	1.1712
15	0.3387	0.107	68	0.504	0.726	121	0.5518	0.7659	172	0.8278	1.172
16	0.3625	0.447	69	0.451	0.8024	122	0.6871	0.7247	173	0.8152	2.044
17	0.3398	0.412	70	0.5407	0.658	123	0.6658	0.6645	174	0.7965	0.806
18	0.3378	0.313	71	0.4989	0.468	124	0.6584	0.848	175	0.7611	0.381
19	0.3045	0.3037	72	0.4984	0.5515	125	0.6142	0.556	176	0.7305	1.0589
20	0.417	0.7238	73	0.4554	0.5805	126	0.7512	0.4757	177	0.8031	1.009
21	0.4135	1.0598	74	0.5341	0.671	127	0.7078	0.5834	178	0.7537	0.621
22	0.4267	1.0935	75	0.4643	0.6461	128	0.7761	1.188	179	0.7205	0.8237
23	0.377	0.5[a]	76	0.5408	0.5795	129	0.7501	0.997	180	0.8362	1.295
24	0.3508	0.793	77	0.6295	0.3812	130	0.7284	0.952	181	0.7968	1.6046
25	0.3937	0.5[a]	78	0.6024	1.1151	131	0.6853	0.8492	182	0.7653	1.1272
26	0.3911	0.603	79	0.5894	0.881	132	0.7035	0.7303	183	0.772	0.976
27	0.3521	0.495	80	0.5464	0.7278	133	0.6871	1.148	184	0.7016	1.0046
28	0.4031	0.854	81	0.6149	0.7159	134	0.6796	0.7331	185	0.7848	1.437
29	0.382	0.6339	82	0.6453	0.773	135	0.6563	0.7031	186	0.7416	1.2236
30	0.3165	0.8202	83	0.6029	0.6339	136	0.6257	0.444	187	0.7654	1.122
31	0.4024	0.562	84	0.5744	0.386	137	0.7329	1.112	188	0.692	0.7337
32	0.3636	0.278	85	0.6224	0.7396	138	0.7403	0.827	189	0.9142	1.5091
33	0.4163	0.447	86	0.6105	0.7968	139	0.6707	0.7219	190	0.874	1.31
34	0.3782	0.6092	87	0.6175	1.021	140	0.6707	0.6732	191	0.8447	1.4741
35	0.4738	0.3746	88	0.5486	0.6892	141	0.7203	1.352	192	0.8269	1.599
36	0.4375	0.2948	89	0.5779	0.561	142	0.6848	1.218	193	0.8397	1.4167
37	0.4858	0.58	90	0.5814	0.611	143	0.6789	0.7088	194	0.962	1.868
38	0.4593	0.4698	91	0.5549	0.571	144	0.6563	0.8764	195	0.9321	0.415
39	0.4488	0.347	92	0.5742	0.5375	145	0.6149	0.6789	196	0.8938	1.2321
40	0.5102	0.722	93	0.5437	0.6676	146	0.6955	0.728	197	0.8293	0.9522
41	0.499	0.5469	94	0.5331	0.4514	147	0.6608	0.6[a]	198	0.8845	1.07
42	0.487	0.792	95	0.5464	0.443	148	0.6243	0.554	199	0.8494	1.1508
43	0.4587	0.503	96	0.5057	0.4308	149	0.6672	0.572	200	0.8197	0.369
44	0.4832	0.524	97	0.61	0.631	150	0.5969	0.5676	201	0.8875	0.803
45	0.4334	0.54	98	0.5415	0.6246	151	0.6499	0.785	202	0.8089	1.165
46	0.445	0.468	99	0.588	0.613	152	0.6062	0.5235	203	0.8938	1.629
47	0.4639	0.848	100	0.5212	0.5871	153	0.7036	0.688	204	0.8217	0.8034
48	0.4651	0.556	101	0.5816	0.668	154	0.6349	0.57	205	0.9678	1.406
49	0.461	0.648	102	0.5431	0.4561	155	0.5666	0.586	206	1.0103	1.673
50	0.4007	0.6817	103	0.5142	0.6068	156	0.8105	1.389	207	0.9423	1.3257
51	0.4242	0.6[a]	104	0.4757	0.4561				208	0.932	1.1756
52	0.4557	0.418	105	0.7049	0.94				209	1.0496	1.139

[a] Estimated relative response factor based on other isomeric PCBs.

Scheme VII. Preparation of PCBs from Chlorinated Benzidines



and 1,2,4-trichlorobenzenes are used to give three PCB isomers per reaction. With the exception of several 1,2,4-trichlorobenzene-chlorinated aniline coupling product mixtures, the PCBs formed via Schemes IV and V were not separable. In some cases the ^{1}H NMR spectra of the individual compounds in these mixtures were apparent and are reported in the tables. The following PCB congeners, 2,2′,4,4,5,5-hexa-, 3,3′-dichloro-, 2,2′-dichloro-, and 2,2′,4,4′-tetrachlorobiphenyl, were prepared via diazotization of commercially available benzidines followed by Cl replacement or deamination as described (*30*) (Scheme VII). By use of the synthetic pathways noted in the

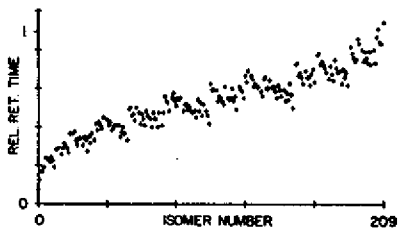


Figure 1. Plot of relative retention times of PCB congeners vs. isomer number (*20*).

schemes, a total of 142 pure PCB congeners were prepared [note, this includes the octa-, nona-, and decachlorobiphenyls previously reported (*27*)].

The ^{1}H NMR spectra of the synthetic tetra-, penta-, hexa-, and heptachlorobiphenyls are summarized in Tables II–V. The congeneric PCBs are numbered by using IUPAC nomenclature rules (*20*). It was apparent that as the degree of chlorination of these compounds decreased, the complexity of the ^{1}H NMR spectra increased. The ^{1}H NMR spectra of 20 of the 24 heptachlorobiphenyls were first order, and the chemical shift data and coupling constants were readily assigned. In contrast this assignment could only be made for 6 of the 42 tetrachlorobiphenyls. Therefore, the spectra of the mono-, di-, and trichlorobiphenyls were not reported due to their complexity. The proton chemical shift data and coupling constants were consistent with results previously reported for the octa- and nonachlorobiphenyls (*27*). The 2,2′,6 and 6′ protons were the most shielded hydrogens and exhibited the lowest chemical shifts; the protons at the para positions gave the highest chemical shift values, and the meta hydrogens (3,3′,5 and 5′) tended to give chemical shifts between those observed for the ortho and para protons.

Gas Chromatographic Properties. Table VI summarizes the GLC retention times and response factors relative to the standard, octachloronaphthalene (OCN), for 209 PCB isomers and congeners. Figure 1 summarizes a plot of RRT vs. isomer groups and illustrates the overall increase in RRT with increasing PCB chlorine content. This is consistent with several previous reports (*1*, *30*). However, a close inspection of the data reveals that the RRT values for isomeric homologues are highly dependent on structure. For example, (1) for the monochlorophenyl-substituted PCB homologues the RRT values increase in the order 2- < 3- < 4- with the following observed ΔRRT values: 0.04–0.05 (3-chloro- to 2-chloro-) and 0.004–0.013 (4-chloro- to 3-chloro-). (2) For the dichlorophenyl-substituted PCB homologues, the RRT values increase in the order 2,6- < 2,5- < 2,4- < 2,3- < 3,5- < 3,4-, and the observed ΔRRT values were 0.03–0.04 (2,5-dichloro- to 2,6-dichloro-), 0.004 (2,4-dichloro- to 2,5-dichloro-), 0.022 (2,3-dichloro- to 2,4-dichloro-), 0.02–0.40 (3,5-dichloro- to 2,3-dichloro-), and 0.035–0.045 (3,4-cichloro- to 3,5-dichloro-). (3) For the trichlorophenyl substituted PCB homologues the RRT values increase in the order 2,4,6- < 2,3,6- < 2,3,5- < 2,4,5- < 2,3,4- < 3,4,5-, and the observed ΔRRT values were 0.027–0.033 (2,3,6-trichloro- to 2,4,6-tichloro-), 0.027–0.033 (2,3,5-trichloro- to 2,3,6-trichloro-), 0.0077–0.01 (2,4,5-trichloro- to 2,3,5-trichloro-), 0.036 (2,3,4-trichloro- to 2,4,5-trichloro-), and 0.036–0.045 (3,4,5-trichloro- to 2,3,4-trichloro-). (4) For the tetrachlorophenyl-substituted PCB homologues the RRT values increase in the order 2,3,5,6- < 2,3,4,6- < 2,3,4,5-, and the observed ΔRRT values were 0.0068 (2,3,4,6-tetrachloro- to 2,3,5,6-tetrachloro-) and 0.064 (2,3,4,5-tetrachloro- to 2,3,5,6-tetrachloro-). It was also apparent from the results that within each series of isomers

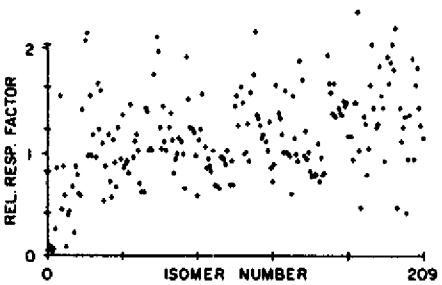


Figure 2. Plot of the relative response factors for PCB congeners vs. isomer number (*20*).

there was generally an increase in retention time with a decrease in the number of *o*-chloro substituents.

Figure 2 summarizes the relative response factors of the 209 PCBs. There is a broad trend that suggests an increase in the GC response with increasing chlorine. However, it is evident that within each isomer group there are large differences in the magnitude of their GC response. The rationale for these differences is not apparent.

A close inspection of the data reveals that 11 pairs of isomers or congeners, including 94/61, 70/76, 95/80, 60/56, 145/81, 144/135, 140/139, 133/122, 163/160, 202/171, and 203/196 exhibit similar or identical retention times. The remaining 187 PCB congeners can be separated on the fused silica capillary column coated with SE-54; moreover, preliminary GLC analysis of reconstituted mixtures of congeners and commercial PCB formulations indicates that some of the 11 pairs of congeners are also resolved by high-resolution capillary GLC. For example, by use of the SE-50-coated capillary column, the chromatogram of the commercial Aroclor 1260 resolves peaks 203/196 despite their similar retention times. We are currently assessing other capillary columns and chromatographic conditions and increased integrator resolution which will further resolve the problem of coelution of individual PCB compounds.

Summary. The synthesis and chromatographic properties of all 209 PCB congeners will lead to a more comprehensive understanding of the ecodynamics of PCBs in the environment. This work, coupled with the identification of the more toxic PCB components (*31–35*), will permit a more rational assessment of the enironmental and human health effects of these compounds since it will now be possible to quantitate the major toxic PCB congeners which bioconcentrate in wildlife and human tissues.

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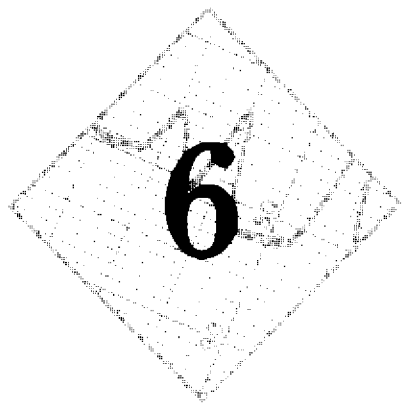

Interpretation of Percent Recovery Data

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Interpretation of percent recovery data

IN SPIKED (FORTIFIED) sample studies, known amounts of a compound or compounds of interest are added to aliquots of a sample, and the percentage of analyte recovered by a test method is used to evaluate the performance of that method. The Environmental Protection Agency (EPA), for example, uses spiking studies in method development (e.g., Ref. 1) and has proposed the use of spiked samples in quality control programs under National Pollutant Discharge Elimination System (NPDES) permits.[2] Thus, the proper conduct and interpretation of spiking programs are critical to the development and implementation of the analytical methods upon which important environmental programs are based.

Spiking is particularly useful in wastewater analyses because the variety of sample matrices and the number of analytes of interest in each sample make realistic standard reference materials difficult to produce. Spiking permits flexibility in the choice of sample matrix and in the combinations and levels of analytes that can be evaluated. The usefulness of spiked-sample analyses is not limited to wastewater or environmental samples, however, and proper interpretation of data from such analyses (percent recovery data) is important whatever the application.

This paper will describe statistical properties of percent recovery data when analytical bias and precision are proportional to sample concentration. The impact of the presence of the analyte of interest in the unspiked sample (i.e., nonzero background concentration) will be examined, and some of the potential pitfalls in the interpretation of percent recovery data in method development and quality control applications will be discussed.

Assumptions

In investigating the statistical properties of percent recovery data, we assume that the expected value of a concentration measurement (X) for a sample with true concentration B is

$$E(X) = pB \tag{1}$$

where 100 p is the mean percent recovery of the method. If $p = 1$, the method is unbiased; otherwise, its absolute bias is proportional to true concentration. We also assume that the variance of a sample with concentration B is

$$\mathrm{Var}(X) = (pB)^2C^2 \tag{2}$$

where 100 C is the coefficient of variation of the method. That is, analytical precision is proportional to concentration; the smaller C, the more precise the method (on an absolute basis).

It is important to keep in mind that p and C, the parameters that characterize the bias and precision of the analytical method, are assumed constant with respect to concentration. This is a realistic assumption for many methods within their ranges of applicability. However, the values of p and C sometimes depend on the sample matrix involved, and the value of C sometimes increases at low concentrations. These possible departures from the simple properties assumed above often are investigated in method development studies through statistical analyses of percent recovery data (Ref. 1, for example).

To estimate the mean and variance of percent recovery for a test method at a particular concentration, one typically analyzes n aliquots of a sample spiked at that level and computes

$$\bar{P} = n^{-1}\Sigma P_i \tag{3}$$

and

$$s^2 = (n-1)^{-1}\Sigma(P_i - \bar{P})^2 \tag{4}$$

(e.g., Ref. 2), where P_i denotes the observed percent recovery for the ith aliquot. The statistical proper-

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ties of $\overline{P}$ and s^2 are described separately below for the cases of zero and nonzero background concentration.

Zero background

If the sample background concentration is known to be zero ($B = 0$), the percent recovery is defined as

$$P = 100\,Y/T \tag{5}$$

where T is the spike level and Y is the measured concentration for a spiked aliquot. The most common case in which background concentration is known to be zero is when spikes are added to distilled water. It can be shown using the assumed properties of the analytical method that in this case the mean and variance of $\overline{P}$ [defined in Eq. (3)] are

$$E(\overline{P}) = 100\,p \tag{6}$$

and

$$\mathrm{Var}(\overline{P}) = (100\,pC)^2/n \tag{7}$$

Thus the sample average percent recovery ($\overline{P}$) is an unbiased estimator of the mean percent recovery of the method. It also can be shown that the mean of s^2 [defined in Eq. (4)] is

$$E(s^2) = (100\,pC)^2 \tag{8}$$

that is, s^2 is an unbiased estimator of the variance of percent recovery for the method.

Nonzero background

If the sample background concentration is nonzero ($B>0$), percent recovery may be defined as

$$P = 100\,(Y - \overline{X})/T \tag{9}$$

where T is the spike level, Y is the measured concentration of a spiked aliquot, and $\overline{X}$ is an estimate of the background concentration based on the mean of measurements on m unspiked aliquots [e.g., Eq. (3)]. If n aliquots of the same sample are spiked at level T and analyzed, $\overline{P}$ and s^2 can be computed as described in Eqs. (3) and (4) and used to estimate the mean and variance of percent recovery for the analytical method. It can be shown in this case that the mean and variance of $\overline{P}$ are

$$E(\overline{P}) = 100\,p \tag{10}$$

and

$$\mathrm{Var}(\overline{P}) = \frac{(100\,pC)^2}{n}\left[\left(1+\frac{1}{k}\right)^2 + \frac{n}{mk^2}\right] \tag{11}$$

where $k = T/B$ (k may be termed the spike/background ratio). These results show that $\overline{P}$ also is an unbiased estimator of mean percent recovery in this case, but that the variance of $\overline{P}$ is greater in the zero-background case by a factor that depends on

Table 1 Impact of spike-to-background rates on variability of percent recoveries

Spike-to-background ratio (k)	$\mathrm{Var}(\overline{P})$ from Eq. (11), with $m = 1, n = 1$	Expected range in % recoveries* $p = 1.0, C = 0.1$	$p = 1.0, C = .2$	$p = .5, C = .2$
Zero background	$(100\,pC)^2$	(80,120)	(60,140)	(30,70)
100	$1.02\,(100\,pC)^2$	(80,120)	(60,140)	(30,70)
50	$1.04\,(100\,pC)^2$	(80,120)	(59,141)	(30,70)
10	$1.22\,(100\,pC)^2$	(78,122)	(56,144)	(28,72)
5	$1.48\,(100\,pC)^2$	(76,124)	(51,149)	(26,74)
1	$5.00\,(100\,pC)^2$	(55,145)	(10,190)	(5,95)
0.5	$13.0\,(100\,pC)^2$	(28,170)	(−44,240)	(−22,122)
0.1	$221\,(100\,pC)^2$	(−200,400)	(−500,700)	(−247,347)
0.05	$841\,(100\,pC)^2$	(−480,680)	(−1100,1300)	(−530,630)
0.01	$20{,}200\,(100\,pC)^2$	(−2700,2900)	(−5600,5800)	(−1400,1500)
0.005	$80{,}400\,(100\,pC)^2$	(−5600,5800)	(−11,200,11,000)	(−5600,5700)

*95% tolerance interval for percent recoveries with assumed values for p and C [tolerance limits = $100\,p \pm 1.96\sqrt{\mathrm{Var}(\overline{P})}$].

the numbers of spiked and unspiked aliquots analyzed (n and m) and the ratio of spike and background concentrations (k). As the spike/background ratio decreases, it can be seen from Eq. (11) that $\mathrm{Var}(\overline{P})$ increases, and $\overline{P}$ becomes a poorer estimator of analytical percent recovery.

The consequence of this result is easily illustrated by some examples. *Table 1* shows the impact of k on $\mathrm{Var}(\overline{P})$ and the expected range in recoveries for three cases with only one spiked and one unspiked aliquot analyzed ($m = n = 1$). The expected range in recovery is based on a 95% tolerance interval for a normal distribution:

$$[100p \pm 1.96\sqrt{\mathrm{Var}(\overline{P})}]$$

As can be seen from Table 1, when $k = 1$, $\mathrm{Var}(\overline{P})$ is five times the zero-background value; when $k = 0.1$, $\mathrm{Var}(\overline{P})$ is about 221 times the zero-background value.

It can also be shown that the mean of s^2 in the nonzero-background case is

$$E(s^2) = (100pC)^2(1 + 1/k)^2 \qquad (12)$$

This is greater than the result for $B = 0$ by a factor that depends once again on k. For example, when spike and background levels are equal ($k = 1$), $E(s^2)$ in Eq. (12) is four times the zero-background value; when $k = 1/5$, $E(s^2)$ is 36 times the zero-background value. Thus, s^2 is a biased estimator of the variance of percent recovery, and s^2 overestimates that variance to a greater extent the smaller the spike/background ratio.

Alternate definitions for percent recovery

The definitions above are not the only ones used for percent recovery. One alternative definition (based on expressing recovery as a percentage of the total spiked sample concentration) is

$$P = \frac{100\,Y}{T + \overline{X}}$$

Another alternative (applicable when the spike level is a multiple, h, of the estimated background concentration) is

$$P = \frac{100(Y - \overline{X})}{h\overline{X}}$$

Regardless of how percent recovery is defined, it can be shown that percent recovery data tend to be unreliable when the spike/background ratio is small.

Interpreting percent recovery data

Two issues were investigated in the method evaluation studies for all of EPA's 600 series methods:

1. Does method performance depend on the sample matrix involved; e.g., do p and C values differ for distilled, natural water, and wastewater samples?
2. Does performance depend on the sample concentration; e.g., is C larger at lower concentrations?

These questions were investigated by analyzing spiked aliquots of both distilled and natural water samples and by spiking aliquots of given samples at different levels (e.g., Ref. 1).

We have shown that for samples with nonzero background, such as wastewater samples, $\mathrm{Var}(\overline{P})$ is large when k is small. Thus the estimate of p for a wastewater sample with $B>0$ and k small may differ greatly from the true mean percent recovery of the method and, therefore, may appear to differ from the corresponding estimate for a distilled water sample. That is, the different statistical properties of percent recovery data in the zero- and nonzero-background cases may mislead one to conclude that matrix differences affect mean analytical percent recovery.

We also have shown that for samples with nonzero background, s^2 tends to overestimate the variance of percent recovery to a greater extent the smaller the spike/background ratio. Thus, if s^2 values from different spike levels are compared when $B>0$, it is likely to appear that relative precision is poor at lower concentrations even when it is not.

The discussion above shows two misconceptions that can arise in method development due to misinterpretation of percent recovery data. The statistical properties of such data may also lead to misdirection in analytical quality control programs. For example, EPA's *Handbook for Analytical Quality Control in Water and Wastewater Laboratories* suggests that when analytical precision varies with concentration, separate control charts should be kept for different concentration ranges.[3] If the relationship of precision to concentration is investigated by estimating variances of percent recovery data at different spike levels, one may erroneously conclude (if $B>0$) that separate charts are needed for low concentrations. This would increase the cost of process control activities unnecessarily. In fact, in the QA appendix to the proposed 600 series

methods,[2] EPA recommends spiking at levels equal to 1, 9, and 99 times background, computing $\bar{P}$ and s^2 at each level, and comparing these estimates for different levels. Results at the lowest spike level may well appear to be more variable than results at higher levels because of the dependence of $E(s^2)$ on the spike/background ratio.

An example of the importance of knowing the statistical properties of percent recovery data is illustrated in a report by the Chemical Manufacturers Association (CMA) on results of a joint CMA/EPA study of the quality of wastewater from five organic chemicals plants.[4] One objective of the study was to characterize the mean and standard deviations of percent recovery for the analytical methods used to measure organic priority pollutants in the industry's wastewater. The report generally was critical of the capabilities of the analytical methods used. However, in planning the study it was decided that the spike level should approximately equal the background concentration; thus, overestimation of percent recovery variability was built into the study. Furthermore, spike/background ratios less than one sometimes were employed. The consequences were reflected most dramatically in results for acrylonitrile: the 12 influent percent recovery results for this compound ranged from −7000% to 400% and had mean and standard deviation of −465 and 2060, respectively, due to the two extreme results. The estimated spike/background ratios for the aliquots with −7000% and 400% recovery were 10,000/890,000 = 0.011 and 10,000/210,000 = 0.048, respectively. By Eq. (11), it can be seen that the standard deviation of $\bar{P}$ is about 14,200 *pC* for $k = 0.01$ and $m = n = 1$; thus, a result of −7000% is not surprising when k is this small. Proper consideration of the statistical properties of percent recovery data would have led to the choice of a higher spike/background ratio in planning the study and to the exclusion from summary statistics of those values made meaningless by the use of too small spike/background ratios.

Another example of potential misinterpretation of data from a spiking study can be found in Ref. 5. As described, spiking studies were used to assess the performance of laboratories. The authors concluded that overall performance by the five laboratories in the study was poor. In one test, an unknown freshwater sample was analyzed with and without spikes of various minerals. The estimated spike/background ratios for the six minerals were as follows: 0.14, 1.1, 0.21, 5.0, 0.71, and 5.0. Some of the variability in recoveries attributed to poor laboratory performance may have been due to the statistical properties of recoveries with low spike/background ratios.

It should be noted that these examples of problems in the interpretation of percent recovery data from the area of wastewater analysis were selected because this is the application with which the authors are most familiar. There is no reason to doubt that similar examples could be found in other areas such as clinical and agricultural chemistry.

Summary

The statistical properties of percent recovery data are important to consider when interpreting results of analytical studies. When background quantities of the spiked analyte are present, percent recoveries can be highly variable and estimates of analytical precision can be biased.

In designing method evaluation studies, spike levels should first be chosen to cover the range of concentration of interest. Once these levels are determined, then sample matrices with background levels that are small compared to these spiking levels should be chosen for the study. If this is done, then the statistical properties of percent recovery data will not affect the evaluation of method bias and precision.

In some situations (e.g., studying the properties of a method near the detection limit), it may be difficult to obtain low background levels in the sample matrix of interest. If it then becomes necessary to perform spiking studies with a low spike/background ratio, the statistical properties of the recoveries should be considered in interpreting the results and in comparing them to results at other concentrations or in other matrices.

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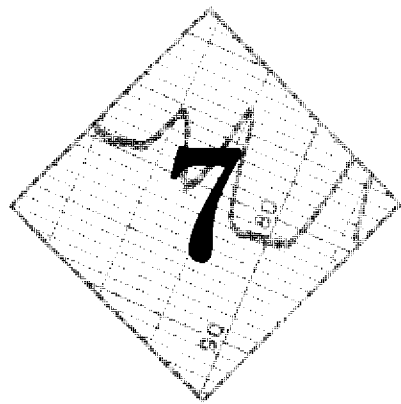

Laboratory Data Base for Isomer-Specific Determination of Polychlorinated Biphenyls

Ted R. Schwartz, Randolph D. Campbell, David L. Stalling,
Robert L. Little, Jimmie D. Petty, James W. Hogan and Edwin M. Kaiser

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Laboratory Data Base for Isomer-Specific Determination of Polychlorinated Biphenyls

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A computer-assisted technique for quantitative determination of polychlorinated biphenyl isomers is described. PCB isomers were identified by use of a retention index system with *n*-alkyl trichloroacetates as retention index marker compounds. A laboratory data base system was developed to aid in editing and quantitation of data generated from capillary gas chromatographic data. Data base management was provided by computer programs written in DSM-11 (Digital Standard MUMPS) for the PDP-11 family of computers.

Polychlorinated biphenyls (PCBs) constitute a complex heterogeneous group with 209 theoretically possible isomers. Identification and quantitation of the single components that constitute environmentally degraded PCB formulations are difficult. In spite of the concern about PCB contamination, relatively little is known about metabolic fates of PCBs as a total class of compounds. This general lack of knowledge about metabolic fate is due in part to the complexity of the chromatographic profile obtained when PCBs are subjected to high-resolution capillary gas chromatography (GC) and, in part, to the inability to identify specific isomers in the chromatogram. The problem is compounded by the massive amounts of data generated when technical PCB mixtures are analyzed for differences in isomer distribution and relating these data to the isomer distribution after the PCBs have been exposed to abiotic degradation and biotransformation processes. Assessment of the biological effects of PCBs ultimately requires correlation of the changes in isomer distribution to levels of biological or toxological effects of specific PCB isomers.

Of the 209 possible isomers, relatively few are available as analytical standards. In this study we used a retention index system to identify the PCB isomers that were observed when PCB technical formulations (or environmental samples contaminated by PCBs) were chromatographed (*1*).

The main criticism of PCB analyses in which electron capture (EC) detection is used, when analytical standards are not available for specific isomers, has been the variation in the detector response as a function of the number and position chlorine atoms on the biphenyl nucleus (*2, 3*). Regardless of this difficulty, GC/EC analysis is the most sensitive method for detecting trace residues of PCBs. In the case where individual isomers of known structure are unavailable, the disproportionality arising from EC detection sensitivity need not be an insurmountable analytical barrier since the isomers present in Aroclor mixtures show nearly the same molar response on a flame ionization detector (FID) (*4, 5*). A calibration factor applied to the integrated response of the EC will allow the data to be quantitated in terms of concentration of isomers.

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The most efficient means of manipulating and managing the massive amount of data generated when analyzing Aroclors or environmental samples for PCB isomer distribution is an interactive data base system. The programming language selected for the isomer specific analysis of PCBs was DSM-11 (Digital Standard MUMPS) for the PDP-11 family of computers. The PCB analysis system developed with DSM-11 is a menu-driven software package incorporating helpful and easily understood error messages as a guide to the user. In addition, default values are provided to reduce key strokes and errors by the operator. Figure 1 shows the basic functions presented to the operator of the PCB analysis system.

EXPERIMENTAL SECTION

A Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used for sample analysis. The data sampling and gas chromatography (GC) program operations were controlled by a Varian Autosampler Model 8000, which also delivered a calibrated amount of sample to the GC injection port. Chromatographic conditions were similar for all sample analysis: initial temperature, 120 °C, programmed at 2 °C/min to a final temperature of 255 °C; detector temperature, 320 °C; and injector temperature, 220 °C. The PCB congeners were separated by using a glass capillary chromatographic column (30 m × 0.25 mm i.d.) coated with C87 stationary phase (Quadrex Corp., New Haven, CT). Hydrogen, linear velocity 32 cm/min, was used as the carrier gas, and nitrogen was delivered at 15 mL/min as the detector makeup gas. The parameters used for the flame ionization detector to determine the molar response of the isomers were similar to those previously given for the ECD. An operational amplifier was interfaced with the GC to amplify the signal from the ECD, which was then collected by a Digital Equipment Corp. (DEC) PDP-11/34 minicomputer. The data were processed by a specialized laboratory data collection software system, PEAK-11, under the DEC RT-11 operating system. The raw data were organized, with appropriate sample identification, into a series of files on hard disk media and transferred off-line to a TM-11 (800 bpi, 9 track) compatible magnetic tape utilizing DEC RT-11 operating system file structure. The data were then read from the tape into the PCB analysis system described later where a tree structured disk file was created. The highest node of the file contained the sample identification information, and the second level nodes, each representing an absolute retention time of an individual peak in the chromatogram, contained the peak area and peak height.

RESULTS AND DISCUSSION

More than 100 components were separated from a 1:1:1:1 mixture of Aroclors 242, 1248, 1254, and 1260. A chromatogram of this mixed Aroclor standard is shown in Figure 2. The method of peak identification was the retention index system first proposed by Kovats (*6*). This identification system depends upon the approximately linear relationship that exists between the retention time of a compound and the theoretical carbon number of the retention index marker compound (*7*), according to

$$I = 100z + 100(t_{Rx} - t_{R(z+1)}/t_{R(z+1)} - t_{Rz}) \qquad (1)$$

where I is the calculated retention index, z is the number of carbon atoms in the alkyl chain, t_{Rx} is the retention time of

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                         POLY 3
             CHFRL Chemical Analysis System
     Friday          18-Mar-83        2:14 PM

                    DATA INPUT/OUTPUT
 0. Read Peaks Data From Tape           1. Output Concentrations To Tape

          PEAK RESOLUTION AND ANALYTICAL PROCEDURES
 2. Match Peaks Stand. To FID Standards   3. Match Samples To Peaks Standards
 4. Calculate RRT's / RI's                5. Calculate Masses Of Isomers

                  REPORTING PROCEDURES
 6. Ambiguities In Standards Match-Ups    7. Summary Of Standards Match-Ups
 8. Ambiguities In Sample Match-Ups       9. Summary Of Sample Match-Ups
10. Report On Calculated Isomer Masses   11. Generate Homolog Analysis Report
12. Summary Of Linear Regression Analysis 13. PCB Run Status Report

                   EDITING PROCEDURES
14. Edit The ART's                       15. Edit The RRT's / RI's
16. Edit Standard Match-Up Files         17. Edit Sample Match-Up Files
18. Create / Edit  FID/IUPAC File        19. Edit Calculated Isomer Masses
20. Combine / Split Analysis Runs

               Procedure Number:
```

Figure 1. Users menu for PCB analysis system.

a substance (x) whose retention index is to be determined, and t_{R_r} and $t_{R(r+1)}$ are the retention times of the retention index standards bracketing substance x. In our study the retention index marker compounds were a homologous series of n-alkyl trichloroacetates synthesized specifically for retention index determination by using EC detection. The utility, synthesis, and chromatography of these compounds are described in a previous publication (8). Three n-alkyl trichloroacetates (decyl, pentadecyl, and dodecyl) were added to the PCB samples prior to their analysis by GC at a concentration of 0.3 ng/L. The retention index program was written in MUMPS with a general format that allows the operator to use the retention index marker compounds as coinjected reference markers or as external markers when the retention index compounds are not added to the sample. Both identification options allow the operator to select a retention window near which the marker compound is located. Once the retention index marker compound has been identified the I values for all peaks in all chromatograms identified by the operator are calculated according to eq 1. Following the identification of the PCB congeners using retention indexes, the peak area must then be converted to concentrations.

The gram formula weights of each resolved PCB isomer for which we had no available reference standard were determined by GC/MS. When used in conjunction with the relative peak area measured by FID, this information provides a method of calibration in the absence of pure isomer standards. The relative peak area of a given congener is determined by dividing the measured area of the congener by the total area of the mixture. This relative peak area is proportional to the congener's mole fraction as determined by FID analysis. In PCBs where the relative degree of chlorination is known, the isomer mass fraction of each congner, F_i, can be expressed as

$$F_i = A_i M_i / \sum_i A_i M_i \tag{2}$$

where A_i is the integrated peak area and M_i is the molecular mass of the ith congner. When applied to a known standard PCB mixture, a series of F_i values can be generated that correspond to the individual chlorobiophenyls of the mixture (9). Using the proper F_i value, one can determine the specific mass, m_i, of a chlorobiphenyl i from the equation

$$m_i = m_t F_i \tag{3}$$

where m_t is the mass of standard (normally expressed in nanograms) injected into the GC column. Chromatographing this same standard under similar GC conditions by EC detection gives similar (and for all practical purposes the same)

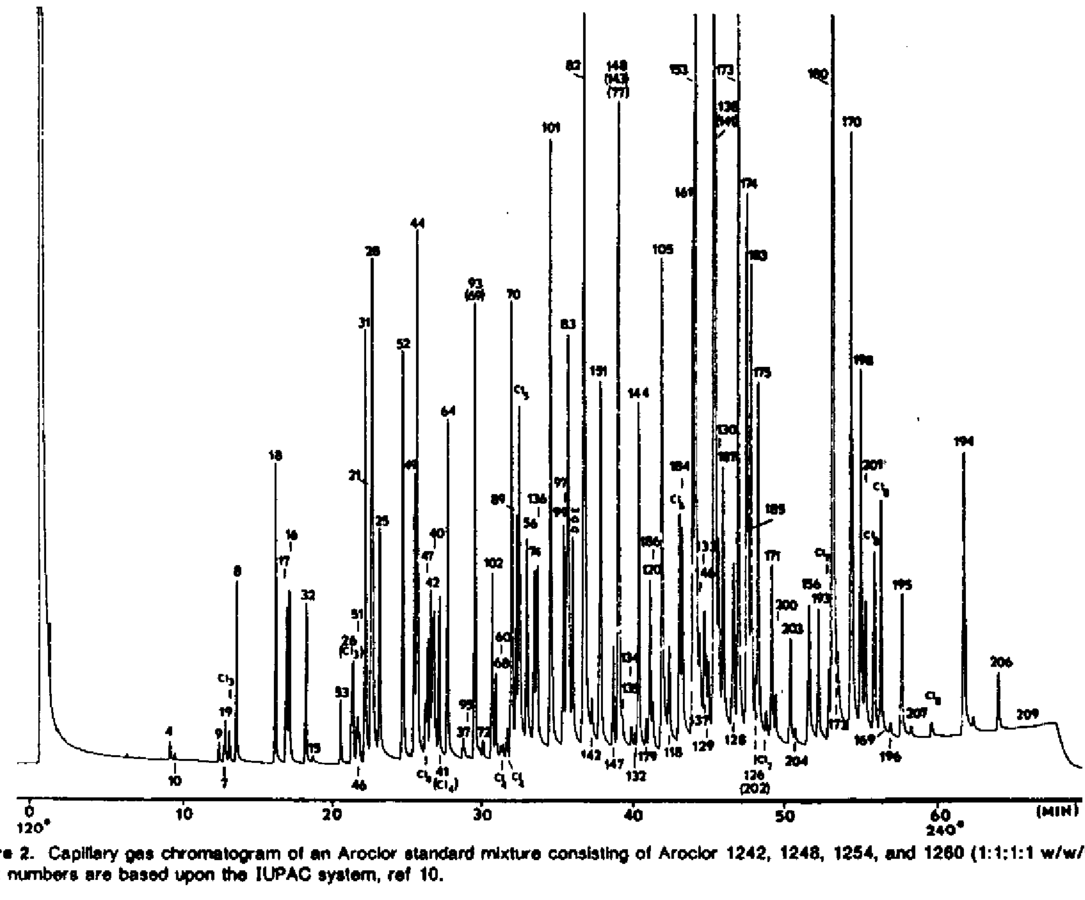

Figure 2. Capillary gas chromatogram of an Aroclor standard mixture consisting of Aroclor 1242, 1248, 1254, and 1260 (1:1:1:1 w/w/w/w). Peak numbers are based upon the IUPAC system, ref 10.

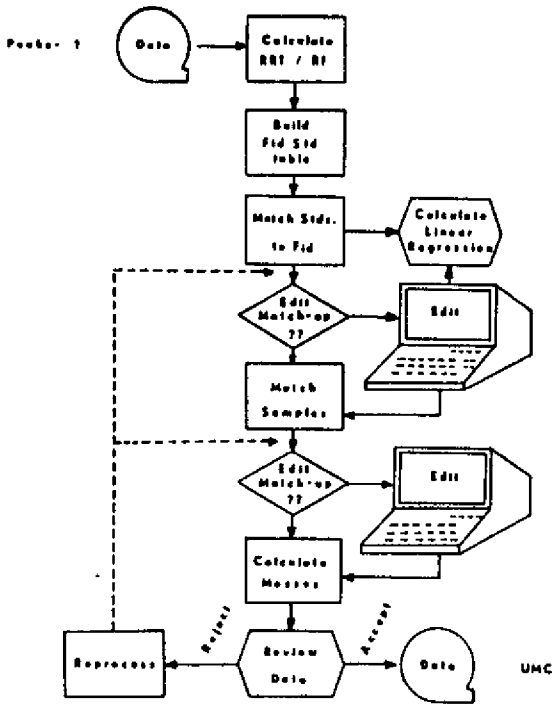

Figure 3. PCB analysis system flow diagram.

congener separation but with different response characteristics. The EC response can be expressed as

$$E_i = A'_i/m_i \quad (4)$$

where A'_i refers to the integrated area of the ith component detected by the EC detector and E_i is the EC response. The use of eq 4 presents a problem since E_i is dependent on the operational parameters that affect A'_i, whereas F_i in eq 2 is internally consistent and independent of the absolute FID response. Therefore the calibration utility of E_i is limited. This problem can be overcome by injecting several standards (m_i) and generating linear regression plots of integrated area vs. total mass of standard injected. For each chlorobiphenyl for which F_i has been determined the mass of a congener, m_i, can then be calculated from the integrated area, the equation of the appropriate line, and eq 3.

The following discussion describing the PCB analysis system refers to the flow diagram shown in Figure 3 and the analysis menu shown in Figure 1. The FID response factor table is generated by selecting a representative Aroclor standard within the data set being analyzed and assigning the known isomeric structure corresponding to the calculated retention index. Once identified, this standard will serve as the master standard to which all subsequent standards and samples are compared for identification of peak structures. Peaks are matched by specifying a retention index search window in which a positive identification is to be made. The search window selected may vary as a function of the complexity of the chromatogram. For the chromatogram shown in Figure 2, a retention search window of 1.5 proved sufficient for peak identification. A positive peak identification may be based on several characteristics such as peak area, peak height, or distance from the center of the search window.

Ambiguities may occur in peak matching when more than one peak occurs in a retention search window. Ambiguities that result in matching analytical standards to the FID master file must be resolved before the regression analysis can be performed. The user is provided with a printed sumary that

Two Incidents of a Single FID Standard Peak Matched to Multiple Standard (Sample) Peaks

ACTION REQUIRED

FID STANDARD PEAK		STANDARD PEAK		
Peak	RI	RI	area	height
49	1401.33	*1401.83	819581	5110.8
		1402.42	998	159.32
50	1403.86	1402.42	998	159.32
		*1403.86	2.34E+06	15815.5

One Incident of Multiple FID Standard Peaks Matched to One Standard (Sample) Peak

ACTION REQUIRED

FID STANDARD PEAK		STANDARD PEAK		
Peak	RI	RI	area	height
49	1401.33	1402.42	998	159.32
50	1403.86			

Figure 4. Ambiguity report indicating peaks which require operator interaction in the standard match up.

identifies the chromatogram number and peak number and classifies the ambiguity into one of three categories that require varying degress of operator interaction. The editing procedure can best be described with the example given in Figure 4. The first few lines of information contain the sample identification and corresponding data set in which the sample is located. The first editing category indicates that, within a specified retention index window of 1.5 units, two peaks match one FID standard peak. The second editing category indicates that the peak with the retention index value of 1402.42 has been matched to two structural isomers in the FID file. In both of these categories the ambiguity must be resolved by the operator before quantitation programs can be executed. This is accomplished through the editing procedure number 16 or 17 (see Figure 1) where the operator selects the correct peak for matching or rejects the peaks as positive matchup. In the present case if the peak occurring at RI of 1401.83 were selected by the operator as the appropriate match to FID standard peak of 1401.33, the peak occurring at 1402.42 would not be used in the quantitation routines. The significance of the asterisk next to retention indexes in the action required categories designates the peak having the retention index nearest to the standard FID value. If the operator determines that the asterisked retention indexes are indeed the correct match, an automation routine will select those peaks thus limiting operator key strokes. The final category summarizes the peaks that could not be matched to any of the structures in the FID standard file. This category is designed only for the operator's information; no action by the operator is required.

Once peak matching is complete and all ambiguities are resolved, a linear regression analysis is executed on the standards needed for quantitation. For the EC detector, the nonlinear behavior over a large concentration range is determined to be best described by a logarithmic function: therefore, if the resulting correlation coefficient for any individual peak in a standard is less than 0.9500, a logarithmic transformation of the peak area is executed and the regression analysis is repeated. Quantitation is then based on the regression line having a correlation coefficient closest to 1.0. A printed copy of the regression analysis summarized the slope, intercept, correlation coefficient, and standard concentration for all the regression lines.

The sample peaks are then matched to the FID file according to their retention indexes. Ambiguities are resolved in a manner similar to that used for the standards previously

Table I. Isomer Specific Report Obtained on Completion of Analysis

peak	(IUPAC#)	RRT(RI)	Chlorines total	Chlorines ortho	structure	Fi	area	Mt	Mi ng	Ci ug/g
1	(4)	894.80	2	2	2,2'	6.10E-03	29253	6.64E+00	4.05E-02	4.05E-02
2	(10)	902.46	2	2	2,6	5.80E-04	4818	5.99E+00 L	3.48E-03	3.47E-03
3	(9)	957.85	2	1	2,5	8.10E-04	39206	6.29E+00	5.10E-03	5.10E-03
4	(7)	963.89	2	1	2,4	2.60E-04	12734	6.81E+00	1.77E-03	1.77E-03
5	(19)	966.29	3	3	2,2',6	3.30E-03	106363	6.43E+00	2.12E-02	2.12E-02
6	(-)	971.47	3	-	3Cl	2.40E-03	90813	6.35E+00	1.52E-02	1.52E-02
7	(8)	979.68	2	1	2,4'	1.50E-02	550360	6.23E+00	9.85E-02	9.85E-02
8	(18)	1029.37	3	2	2,2',5	3.19E-02	1002510	6.24E+00	1.99E-01	1.99E-01
9	(17)	1042.63	3	2	2,2',4	1.08E-02	400754	7.65E+00 H	8.26E-02	8.26E-02
10	(16)	1046.88	3	2	2,2',3	1.19E-02	430001	7.15E+00	8.51E-02	8.51E-02
11	(32)	1068.27	3	2	2,4',6	6.90E-03	518994	6.22E+00	4.29E-02	4.29E-02
12	(15)	------	2	0	4,4'	Not Detected in FID Standard				
13	(33)	1112.96	4	3	2,2',5,6'	4.50E-03	200609	6.39E+00	2.87E-02	2.87E-02
.					.					.
.					.					.
.					.					.
97	(-)	1806.92	8	-	8CL	2.90E-03	900826	6.44E+00	1.86E-02	1.86E-02
98	(169)	------	6	0	3,3',4,4',5,5'	Not Detected in FID Standard				
99	(196)	------	8	3	2,2',3,3',4,4',5',6	Not Detected in FID Standard				
100	(195)	1833.54	8	3	2,2',3,3',4,4',5,6	2.00E-03	598223	6.45E+00	1.29E-02	1.29E-02
101	(207)	1845.20	9	4	2,2',3,3',4,4',5,6,6'	5.00E-05	18596	6.00E+00	3.00E-04	3.00E-04
102	(-)	1872.71	8	-	8Cl	1.20E-04	98417	6.72E+00	8.04E-04	8.04E-04
103	(194)	1914.04	8	2	2,2',3,3',4,4',5,5'	4.30E-03	1498830	6.39E+00	2.75E-02	2.75E-02
104	(206)	1958.13	9	3	2,2',3,3',4,4',5,5',6	6.00E-04	291939	7.06E+00	4.23E-03	4.23E-03
105	(209)	------	10	4	2,2',3,3',4,4',5,5',6,6'	Not Detected in FID Standard				
								Total		
								unwindowed	6.31E+00	6.33E+00
								windowed	6.31E+00	6.33E+00

described. The sample peaks are then matched to the appropriate regression line and the mass of the corresponding area is calculated, eq 3, and adjusted to a component concentration as dictated by

$$C_i = \frac{m_i V_s (\mathrm{DF})}{(\mathrm{Mass}) V_{inj}} \qquad (5)$$

where C_i is the calculated isomer concentration in parts per million, V_s is the sample volume (milliliters), DF represents any appropriate dilution facotrs, Mass (grams) is the original sample mass, and V_{inj} (microliters) is the sample volume delivered by the autosampler.

Final concentration reports are formated to present either isomer specific or homologue reports. An example of the isomer specific report is illustrated in Table I for an Aroclor standard consisting of equal weights of Aroclor 1242, 1248, 1254, and 1260 (1:1:1:1 w/w/w/w). The report header (not

Table II. PCB Homologue Report Obtained by Grouping of Appropriate Isomers (Concentrations in Micrograms/Gram [% of Total Residue])

	Ortho Chlorines						
Cl #	0	1	2	3	4	other	Total Cl (ug/g)
1							0
					.4[17.1]		
4		.425	.911	.0636		.0187	1.41
		[6.7]	[14.4]	[1.0]		[.3]	[22.4]
5	.0256	.21	.818	.288		.174	1.51
	[.4]	[3.3]	[12.9]	[4.6]		[2.7]	[24.0]
6		.0221	.675	.481	.085	.0748	1.33
		[.4]	[10.7]	[7.6]	[1.3]	[1.2]	[21.2]
7			.275	.248	.0575	.00338	.584
			[4.4]	[3.9]	[.9]	[.1]	[9.2]
8			.0275	.168	.00355	.0344	.233
			[.4]	[2.7]	[.1]	[.5]	[3.7]
9				.00423	.0003		.00453
				[.1]	[.0]		[.1]
10							0
							[0.0]
	.0605	1.35	3.16	1.27	.146	.333	6.33
	[1.0]	[21.4]	[50.0]	[20.2]	[2.3]	[5.3]	[100.0]

shown) indicates the sample set, quantiation factors used for concentration calculation, sample control, and laboratory numbers. The report body indicates the PCB peak elution number and also, parenthetically, the IUPAC structural number (*10*). Numbers in the second row are the calculated retention index values from eq 1. The next three rows of numbers indicate peak structure information. The F_i values from eq 2 are indicated in the next row, followed by the integrated peak area. The column labeled M_t indicates the appropriate mass value for a given response and indicates whether the peak area was below (L) or above (H) the calibration range of the particular isomer in the Aroclor standard. The final column expresses the calculated isomer concentration in terms of wet sample mass. The isomer concentrations are then totaled to give a final concentration of PCB in terms of only those isomers which were within the calibration range (windowed) and all isomers quantitated (unwindowed). An alternate report indicating the homologue distribution can also be obtained. This report is useful in presenting the analyst with an overview of changes in the homologue distribution and is shown in Table II.

A review of programming languages for the development of a multiuser data base system prompted our selection of DSM-11. The DSM-11 system is a multiuser, time sharing operating system that also functions as a programming language. As an operating system, DSM-11 supports a variety of peripheral devices; as a language it is high level, self-documenting, and interpretively executed and provides features to directly implement data base management.

Many features of DSM-11 contributed to its selection for implementation of the PCB analysis system. Listed here are five of the most important: (1) ease of program development and maintenance, (2) dynamic run-time allocation of data elements, (3) sparse array structure of files, (4) extensive capabilities for the processing of variable length string data, and (5) multilevel security protection of data and programs.

During the process of software design and implementation, the problems of verification and validation were continually addressed. Verification techniques were designed to determine the logical consistency of the software design whereas validation techniques determined the ability of the design to function according to the stated user's requirements (*11*).

To ensure verification of the software design, our laboratory implemented the PCB system in two phases. The first phase was the design and implementation of a baseline system, that is, a shell containing the basic functions, yet with a structure that allowed for expansion as the system was further developed. This base line system had the ability to enter, edit, and report data, as well as the ability to create new files and functions as required. The second phase (in fact an ongoing phase) involves the implementation of specific data reduction and analysis functions and their later integration into the base line system. More sophisticated editing and reporting capabilities were also added in the second phase. With this system

architecture, individual functional modules could be replaced or added with no effect on the rest of the system.

Performance and reliability of the software were ensured by using two types of validation, manual and automatic. Manual validation was the periodic recalculation by hand of results, and automatic validation involved the recalculation of computed resits by software. Automatic validation was accomplished by the implementation of separate programs (functions) which, on the basis of the computed results, recalculated the input values used to obtain those results. These recalculated inputs were compared with the actual input values to ennsure the reliability and performance of the computational programs. In addition to validation of the computatations, the results were validated after they were transferred to the destination data base. This validation also was done in two ways: (1) each data item output to tape from the PCB analysis system was attached to a special character, so that the character was always located in a predetermined column relative to the data item. At the destination site, the location of this special character was checked to detect any loss of characters or addition of noise on the tape, (2) the method for validating transferred data was the transferring of a test set of data items at the beginning and the end of the sample data items, which were checked against an existing test set at the destination.

The detail in which the data are presented, with inclusion of IUPAC peak numbers and structural assignment, makes the data valuable in determining subtle changes in isomer distribution. The information acquired from this analysis system provides a detailed account of the PCB isomer distribution in a format that lends itself to statistical treatment. An application to environmental problems utilizing this PCB analysis system in conjunction with the pattern recognition technique SIMCA-MACUP is being published elsewhere (*12*, *13*).

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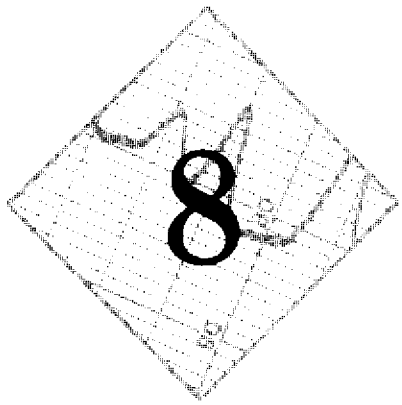

Limit of Detection: A Closer Look at the IUPAC Definition

Gary L. Long and J.D. Winefordner

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Report

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The ability to quantify a trace element or molecule in chemical and biological matrices using specific analytical methods is often viewed in terms of the limit of detection. This limit of detection is a number, expressed in units of concentration (or amount), that describes the lowest concentration level (or amount) of the element that an analyst can determine to be statistically different from an analytical blank (*1*). Although this definition seems rather straightforward, significant problems have been encountered in expressing these values because of the various approaches to the term "statistically different." The calculated limit of detection for an element can easily vary an order of magnitude through the use of different statistical approaches (*1–13*). In spite of the fact that the International Union of Pure and Applied Chemistry (IUPAC) adopted a model for the limit of detection calculations in 1975 (*1*), and the ACS Subcommittee on Environmental Analytical Chemistry reaffirmed this standard in 1980 (*2*), acceptance of this model by the general analytical community has been slow. The result of this slow acceptance has led to a great deal of uncertainty when limits of detection are used as a basis for comparison between various analytical procedures, methods, or analytical instruments. Unless the limits of detection are calculated in a consistent manner, the comparison may be meaningless.

It is the purpose of this REPORT to examine the statistical meaning of limit of detection values in a format consistent with the IUPAC definition. It is not intended to be a comprehensive review of the various methods of calculating limit of detection values. For a more complete review, the reader is referred to the excellent articles by Kaiser (*3–6*), Boumans (*7–9*), Currie (*10*), Glaser et al. (*11*), as well as available textbooks (*12, 13*). Rather, this REPORT is intended to be a simple and general discussion on methods for calculating limits of detection; it is geared to the analyst who does not have a rigorous knowledge of statistics. In this examination, the significance of the limit of detection values will be emphasized, and the possible problems encountered when using limit of detection values obtained from non-IUPAC methods as a basis for comparing methods and instruments will be discussed. Also, two methods will be introduced that permit measurement errors in the analytical sensitivity to be included in the limit of detection calculation. These two methods as well as the IUPAC method will be evaluated for their ability to incorporate these errors into the numerical limit of detection.

Definition

The IUPAC definition, adopted in 1975, states that "the limit of detection, expressed as a concentration c_L (or amount, q_L), is derived from the smallest measure, x_L, that can be detected with reasonable certainty for a given analytical procedure" (*1*). This concept is further clarified by the ACS definition, which states "the limit of detection is the lowest concentration of an analyte that an analytical process can reliably detect" (*2*). To understand what a reasonably certain measure or a reliable detection is, the method of measurement as well as the errors (including noises) associated with the measurement must be well understood.

Measurements

Generally, most analytical methods require the construction of analytical calibration curves for the determination of unknowns. These curves are generally plots of signal, x, vs. analyte concentration, c, and are represented as in Figure 1. The relationship between x and c can be obtained by performing a linear regression analysis on the data. This analytical calibration relationship can be expressed as

$$x = mc + i \qquad (1)$$

where m is the slope or analytical sensitivity and i is the intercept. When an unknown sample containing the analyte is subjected to the analytical procedure, a value, x_U, can be measured. This value may then be inserted into Equation 1 to determine the concentration of the unknown, c_U. However, the ability to solve accurately for c_U is dependent upon how well the m and i

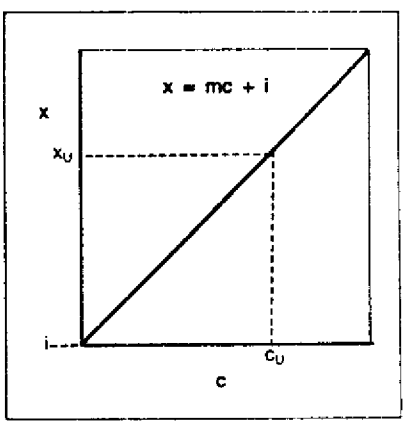

Figure 1. Analytical calibration curve of signal, x, vs. concentration, c

The unknown signal, x_U, is related to an unknown concentration, c_U, through the equation x = mc + i, where m is the slope and i is the intercept of the line

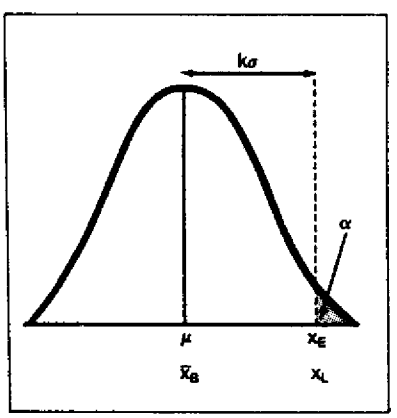

Figure 2. Normal distribution curve for a measured x variable

The shaded area, α, represents the probability that $x_E \geq (\mu + k\sigma)$ or $x_L \geq (\bar{x}_B + 3s_B)$

values are known. As long as the calibration curve is obtained in the linear response region of the method, the more points obtained in the construction of the calibration curve the better defined the m value will be. Also, if points are sampled near the origin, the i value may be better defined. However, if the m and i values are not well-defined because of nonlinearity in the calibration curve or a poor choice of calibration curve ranges, the result of the unknown determination, c_U, may be subject to considerable error.

Statistics

The amount of error associated with a measurement of x can be statistically estimated. Most measurements are subject to error that follows a normal distribution. If a sufficiently large number of observations is made, plotting the measured responses would produce a curve similar to that shown in Figure 2. The mean value of the responses, μ, occurs at the center of the curve. The curve is symmetric around μ and extends outward in units of standard deviation, σ. Since this curve includes all x values that could be obtained from the procedure for the sample, the area under the curve can be expressed in terms of probability, P (i.e., there is a 100% chance, P = 1, that a measured x value would fall somewhere under the curve).

The relationship between area and probability can be measured to estimate the chance that a newly measured x value, x_E, would be a certain number of standard deviation units away from the mean response, μ. In Figure 2, x_E is shown to the right of μ and can be measured to be $k\sigma$ away from μ. By dissecting the curve with a line drawn at this x value, the area to the right of the line, α, is the probability that $x_E \geq (\mu + k\sigma)$. This chance, which is represented by the pink shaded area, can be determined from

$$\text{area} = \frac{1}{2\pi}\int_k^\infty \exp\left(\frac{-k^2}{2}\right)dk \quad (2)$$

where the x_E value is $k\sigma$ away from μ, (i.e., $(x_E - \mu)/\sigma = k$).

This illustration can also be used to aid in the explanation of the smallest detectable signal, x_L, in the IUPAC definition. When the determination of a limit of detection is performed, blank measurements, x_B, are normally taken. The question, however, is "how well are these x_B values known?" A mean value of the blank responses, $\bar{x}_B$, can be calculated as

$$\bar{x}_B = \frac{\sum_{j=1}^{n_B} x_{Bj}}{n_B} \quad (3)$$

and the standard deviation as

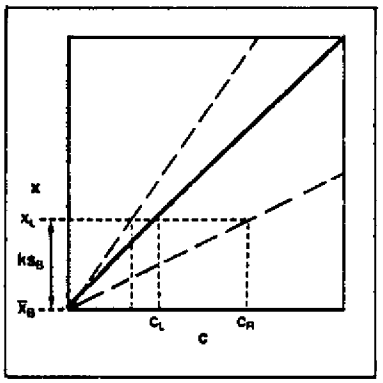

Figure 3. Analytical calibration curve of signal, x, vs. concentration, c, showing the relationship of ks_B to the limit of detection, c_L

$$s_B^2 = \frac{\sum_{j=1}^{n_B} (x_{Bj} - \bar{x}_B)^2}{(n_B - 1)} \quad (4)$$

for n_B observations. Because a finite small number of blank readings is normally taken, e.g., $n_B = 20$ or greater, s_B must be used instead of σ_B. If a sample of this size is used, s_B can be considered to be a reasonable indicator of σ_B. If the random error follows a normal distribution, a plot of these responses (frequency of occurrence vs. x_B values) would resemble Figure 2. The probability that the smallest discernible analytical signal, x_L, can be measured and not be a random fluctuation of the blank is dependent upon how many standard deviation units x_L is from $\bar{x}_B$. If x_L is $3s_B$ away from $\bar{x}_B$, the area to the right of x_L is no less than 0.0013. Thus, there is a 0.13% chance that a signal measured at x_L or greater would be the result of a random fluctuation of the blank signal. This small chance of error can then fulfill the requirement of a reasonably certain signal.

In defining c_L, IUPAC states that

$$x_L = \bar{x}_B + ks_B \quad (5)$$

where k is a numerical factor chosen in accordance with the confidence level desired. The c_L is a function of x_L and therefore

$$c_L = \frac{(x_L - \bar{x}_B)}{m} \quad (6)$$

where m is the analytical sensitivity. Because the mean blank reading, $\bar{x}_B$, is not always 0, the signal must be background corrected. By substituting Equation 5 into Equation 6, Equation 7 is obtained

$$c_L = \frac{ks_B}{m} \quad (7)$$

This definition of c_L can be illustrated as shown in Figure 3. The limit of detection is found by relating ks_B to a concentration value by dividing by the slope of the calibration curve line obtained from the linear regression analysis. However, the c_L value obtained can only be a true reflection of the limit of detection when m is well-defined and i is essentially 0.

The use of k = 3 allows a confidence level of 99.86% that $x_L \geq (\bar{x}_B + 3s_B)$ for a measurement based on the error of the blank signal following a normal distribution. It must be emphasized that if x_B does not follow a normal distribution, then the probability that $x_L \geq (\bar{x}_B + 3s_B)$ would be $100(1 - 1/k^2)$, or 89% according to Tschebyscheff's inequality (*5*). Hence, *values of k < 3 should not be used* for limit of detection calculations.

Other Approaches

The majority of the other approaches to calculating c_L values are similar to the IUPAC model in that s_B and k factors are involved. However, it is because of these terms that trouble may be encountered when c_L values are used as a basis for the comparison between procedures, methods, or instruments. The most widely debated of the two factors has been the choice of a value for k. Kaiser was perhaps the first to stress the use of k = 3 for c_L values (*3, 4*). This value has also been agreed upon by other authors (*9, 13*), by IUPAC (*1, 14*), and by the ACS (*2*). A value of 2 for k had been initially suggested (*8*) but this value corresponds to a 97.7% confidence level for normal distribution and 75% for a nonnormal distribution of measurement error.

Although the use of k = 3 instead of k = 2 slightly increases the c_L value, it is clear that c_L values must differ by a factor of three for the values to be significantly different. Nevertheless, factors of less than three have been commonly used for comparison purposes. In order to minimize confusion, IUPAC suggested that x_L values be reported in all literature with their k value, $x_{L(k=3)}$. It would be extremely useful to go one step further and include the k values when c_L values are reported, $c_{L(k=3)}$. This change would be beneficial because c_L values are more commonly reported than x_L values.

A problem encountered in the comparative use of c_L values is the use of the standard deviation of the mean, $s_{\bar{B}}$ (*11*), the pooled standard deviation, s_P (*7, 12*), or the relative standard deviation (RSD) (*7, 9*). Although each of these standard deviation expressions is important and has its place in analytical chemistry, the use or misuse of these expressions in c_L calculations may result in significant deviation

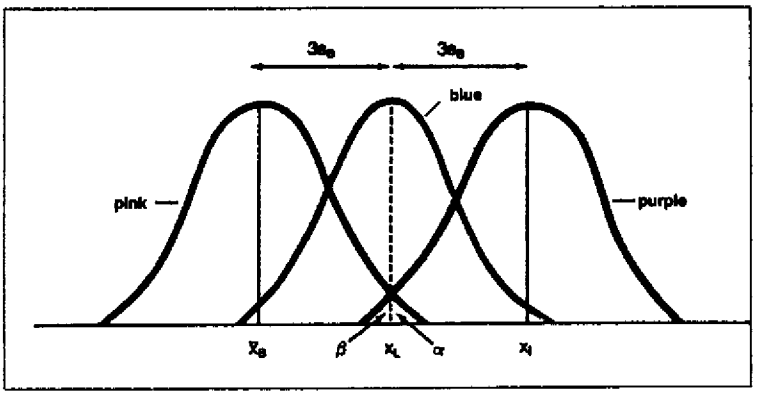

Figure 4. Normal distribution curves for the blank observation, x_B (pink line), the limit of detection, x_L (blue line), and the limit of identification, x_I (purple line)
Here all standard deviations follow a normal distribution and are equal

from the IUPAC model.
The standard deviation of the mean (or the standard error), $s_{\bar{B}}$, is expressed by

$$s_{\bar{B}} = \frac{s_B}{(n_B)^{1/2}} \qquad (8)$$

This value is calculated by dividing the standard deviation of the blank measures, s_B, by the square root of the number of blank observations, n_B. Usually when $s_{\bar{B}}$ values are used, the k factor is replaced by a t distribution value. Even though the t values reflect the problem of defining a standard deviation for a finite number of observations, $3s_B > t_\alpha s_B/(n_B)^{1/2}$ for $\alpha = 0.005$ and five observations (four degrees of freedom). This inequality greatly increases as n_B increases. If a large number of observations are taken on the blank, say 30, the use of the t and $s_{\bar{B}}$ will reduce the value of c_L by a factor of six from the IUPAC model.

The use of the pooled standard deviation includes the number of blank measurements as well as sample measurements. Generally, the pooled standard deviation, s_P, is expressed as

$$s_P = \left[\frac{s_S^2}{n_S} + \frac{s_B^2}{n_B}\right]^{1/2} \qquad (9)$$

where s_S is the standard deviation of a sample measurement and n_S is the number of sample observations. If $s_S = s_B$, then Equation 9 reduces to

$$s_P = s_B \left[\frac{1}{n_S} + \frac{1}{n_B}\right]^{1/2} \qquad (10)$$

For $n_S = 1$, s_p is essentially the same as s_B. However, if n_S and n_B are both large, $s_P < s_B$. Again, the use of a large number of observations as mentioned above would cause a significant reduction in the c_L value as compared to the IUPAC model.

Another common practice involves the use of the RSD, which is defined as

$$RSD = \frac{s_B}{\bar{x}_B} \qquad (11)$$

This relationship is useful in expressing the "concentrational limit of determination," c_D (7). Here, the measure x_D corresponding to c_D is defined as

$$x_D = k_D s_B \qquad (12)$$

where s_B is the standard deviation of the blank (not the RSD), and k_D is a confidence factor that is defined by the reciprocal of the magnitude of the RSD value. For a maximum allowed RSD of 5%, $k_D = 20$ and for a RSD of 10%, $k_D = 10$.

Another limiting expression involving RSD values involves the rearranging of Equation 7 (9). If the background signal is included in the numerator and denominator of Equation 7, then

$$c_L = k\left(\frac{s_B}{\bar{x}_B}\right)\left(\frac{\bar{x}_B}{m}\right) = k(RSD)\frac{\bar{x}_B}{m} \qquad (13)$$

In many ICP determinations, the RSD has been measured to be 0.01 (as determined by emission flicker noise). Unfortunately, many ICP c_L values in papers and current manufacturers' bulletins have been calculated with $k = 2$. Inserting these values into Equation 13 yields

$$c_L = 0.02\frac{\bar{x}_B}{m} \qquad (14)$$

A problem that may be encountered with this approach is that the RSD for some spectrometric detection systems may be >0.01. Before using this method, an analyst should reconfirm the validity of RSD ≤ 0.01. Failure to measure and correct for high RSD values could result in erroneous c_L values. An attempt to compare an ICP c_L value obtained from this method and an IUPAC c_L value for the same analysis procedure would be meaningless.

A well-based but seldom used concept in the calculation of detection limits is the limit of identification, c_I, as described by Boumans (7) or the limit of guarantee for purity, c_G, described by Kaiser (3). These concepts are essentially the same and are based on the idea that the lowest statistically discernible signal should be

$$x_I = x_L + 3s_I \qquad (15)$$

where s_I is the error associated with the measurements at the x_I level, or

$$x_I = x_L + 3s_B \qquad (16)$$

if $s_I = s_B$. This concept is illustrated in

Table I. Statistical Expressions for the Slope and Intercept

$$m = \frac{\left(n\sum_{j=1}^{n} c_j x_j\right) - \left(\left(\sum_{j=1}^{n} c_j\right)\left(\sum_{j=1}^{n} x_j\right)\right)}{\left(n\sum_{j=1}^{n} c_j^2\right) - \left(\sum_{j=1}^{n} x_j\right)^2}$$

$$I = \left(\frac{\sum_{j=1}^{n} x_j}{n}\right) - m\left(\frac{\sum_{j=1}^{n} c_j}{n}\right)$$

$$s_m = \frac{s}{(s_{cc})^{1/2}}$$

$$s_I = \frac{s\left(\sum_{j=1}^{n} c_j\right)^{1/2}}{(ns_{cc})^{1/2}}$$

$$s = \left(\frac{s_{xx} - bs_{cx}}{n-2}\right)^{1/2}$$

$$s_{cc} = \left(\sum_{j=1}^{n} c_j^2\right) - \frac{\left(\sum_{j=1}^{n} c_j\right)^2}{n}$$

$$s_{xx} = \left(\sum_{j=1}^{n} x_j^2\right) - \frac{\left(\sum_{j=1}^{n} x_j\right)^2}{n}$$

$$s_{cx} = \left(\sum_{j=1}^{n} c_j x_j\right) - \frac{\left(\sum_{j=1}^{n} c_j\right)\left(\sum_{j=1}^{n} x_j\right)}{n}$$

Figure 4. When measurements are made for a sample, the x values obtained should follow normal distribution around a mean value. If a sample were measured to have a mean at the x_L value, the distribution of these x values around x_L would resemble the blue line in Figure 4. One-half of the time the measurement would fall below the x_L value and could not be considered a true signal according to the IUPAC definition. To avoid this high probability, the limit of x_I is set at $3s_I$ away from x_L. This level is chosen so the areas α and β are equal. If $s_I = s_B$ then this level is $3s_B$ away from x_L or $6s_B$ away from $\bar{x}_B$. By using $k = 3$, the area of the x_I distribution curve below x_L, β, is no less than 0.0013. Thus, there is a 0.13% chance that an x value measured at x_I would fall below the x_L limit and not be considered as a true signal.

This idea of further statistically separating the blank measurements distributions and true signal distributions has been proposed by the ACS Subcommittee on Environmental Analytical Chemistry and has been termed the limit of quantitation (2). Since the numerical significance of the analyte concentration increases as the analyte signal increases above x_L, a minimum criterion, representing the ability to quantify the sample, can be established reasonably far way from $\bar{x}_B$. This criterion, called the limit of quantification (LOQ), is 10σ away from $\bar{x}_B$. For limit of detection work, $\sigma = s_B$. Samples that are measured as having a signal, x, where $x > 10s_B$ are termed to be in the region of quantitation while samples where $3s_B \leq x \leq 10s_B$ are termed to be in the region of detection.

By setting the quantitation level as $10s_B$ or the identification limit as $6s_B$, a much higher probability is afforded that the sample signal is not just a random fluctuation of the blank. However, when making comparisons using LOQ or c_I to IUPAC c_L values, the analyst must bear in mind the difference in the k factors for each limit.

Methods Involving Analytical Sensitivity Error

The previous models for calculating detection limits consider the error in the blank measurements. These models also consider the analytical sensitivity, m, as a well-defined value. In practice, however, m may have significant error due to nonlinearity in the calibration curve, or measurement errors.

The following proposed detection limit approaches include errors associated with measurements of the analytical sensitivity. The first method, a graphical approach, includes the standard deviation of the slope, s_m, in the c_L expression. The second method, a propagation of errors approach, considers the standard deviation of the concentration, s_c. This value is calculated by including the standard deviations of the blank, slope, and intercept in the equation. The statistical expressions for these values are listed in Table I.

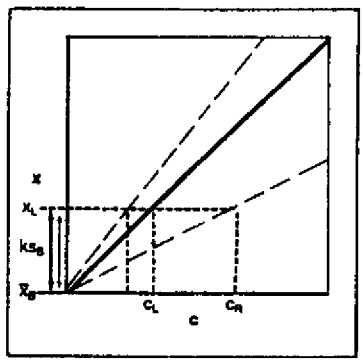

Figure 5. Analytical calibration curve of signal, x, vs. concentration, c, showing graphical approach to limit of detection calculation

dashed lines are the limit of error bars. c_L is obtained from x_L if no error occurs in slope. c_R is obtained from x_L if slope error is significant

Although these models require additional calculations, most linear regression analyses are performed using calculators or microcomputers. With additional programming, these calculations can be easily performed, allowing more accurate determinations of c_L values to be made. These values may also be used for a truer comparative look at the ability of an analytical method or instrument to quantify trace elements (or compounds) in a sample.

Graphical Approach

To obtain a more reliable c_L value, the m value should be expressed as a confidence interval $m \pm t_\alpha s_m$, where s_m is the standard deviation of the slope and t_α is a t distribution value chosen for the desired confidence level, α, and the degrees of freedom, ν. The insertion of this interval into Equation 7 produces

$$c_L = \frac{ks_B}{m \pm t_\alpha s_m} \qquad (17)$$

The effect of the inclusion of the confidence interval can best be seen by referring to Figure 5. The error bars (confidence interval) generated around the regression line are indicated as white dashed lines. Because of error in the slope, three concentration values are found for a given x_L value. When $t_\alpha s_m = 0$, (as in Equation 7), the value of c_L is obtained. However, the reduced analytical sensitivity yields a value, c_R, to the right of c_L. If $m \gg t_\alpha s_m$, then the c_R will not be statistically different from c_L. For the situation when $t_\alpha s_m$ is not sufficiently small as compared to m, a c_R may be substantially larger than c_L. Therefore, only the larger value should be used in reporting a limit of detection.

An important consideration in the use of this model is the choice of a t value. The recommended k value of 3 involves a 99.87% confidence level. Therefore, the t should reflect a similar level. An $\alpha = 0.0005$ results in a confidence level of 99.9% for a two-tailed t distribution. The degrees of freedom, ν, are $n - 2$ for a linear regression model. The n value used should be the number of points used to prepare the calibration curve, i.e., each point consists of a mean measure and a concentration value.

Propagation of Errors Approach

In the second approach used to evaluate c_L, the error in the intercept term, i, as well as the error in m are considered. To include these errors, Equation 1 must be rearranged to

$$c = \frac{x - i}{m} \qquad (18)$$

From Equation 18, any value of x can be related to a concentration using the m and i values obtained from the linear regression model. The i term is usually neglected in most cases and assumed to be 0 because the analytical measures are background subtracted. But in most linear regression analyses, $i \neq 0$. If a true reflection of the error in the determined concentration is sought, the error in m and i must be included in Equation 7.

The contribution of each term to the total error may be found by taking the first derivative of c with respect to each term:

$$s_c^2 = \left(\frac{\partial c}{\partial x}\right)^2 s_x^2 + \left(\frac{\partial c}{\partial i}\right)^2 s_i^2 + \left(\frac{\partial c}{\partial m}\right)^2 s_m^2 \qquad (19)$$

Taking the designated derivatives and the square root gives

$$s_c = \left[\left(\frac{1}{m}\right)^2 s_x^2 + \left(\frac{1}{m}\right)^2 s_i^2 + \left(-\frac{x - i}{m^2}\right)^2 s_m^2\right]^{1/2} \qquad (20)$$

and finally combining like terms yields

$$s_c = \frac{\left[s_x^2 + s_i^2 + \left(\frac{i - x}{m}\right)^2 s_m^2\right]^{1/2}}{m} \qquad (21)$$

Table II. ICP-Excited ICP Fluorescence Data

(A) Ca(II)

c (ppm)	X
0	0
0.1	402
1	6750
10	67000
m = 6710	s_i = 101
s_m = 20	s_B = 5.4
I = −79	

(B) Ca(II)

c (ppm)	X
0	0
10	67000
100	718000
1000	7600000
m = 7610	s_i = 13700
s_m = 27	s_B = 5.4
I = 16600	

(C) Cu(I)

c (ppm)	X
0	0
1	55
10	1810
100	34500
m = 350	s_i = 545
s_m = 10	s_B = 3.5
I = −628	

(D) Co(II)

c (ppm)	X
0	0
1	796
10	2800
100	60700
m = 614	s_i = 1200
s_m = 24	s_B = 97.6
I = −980	

Table III. $c_{L(k=3)}$ Values (ppm)

	$\frac{3s_B}{m}$	$\frac{3s_B}{m - t_\alpha s_m}$	$\frac{3\left(s_B^2 + s_i^2 + \left(\frac{i}{m}\right)^2 s_m^2\right)^{1/2}}{m}$
(A) Ca(II)	0.002_4	0.002_7	0.05
(B) Ca(II)	0.002_1	0.002_4	5
(C) Cu(I)	0.03	0.3	5
(D) Co(II)	0.5	−2	6

Equation 21 allows the determination of standard deviation in a c value calculated from any x value.

In the case of limits of detection, the c_L value is actually a confidence value expression of how well the blank is known. If Equation 7 is reconsidered, it can be written as

$$c_L = ks_c \qquad (22)$$

where the s_B/m term describes the error in terms of c (if $c \sim c_L$). Equation 21 could now be used to evaluate s_c where x is the blank signal, $\bar{x}_B$, and s_B is substituted for s_x. By measuring $\bar{x}_B$, s_B, and calculating m, i, s_m, and s_i, the value of s_c can be determined.

In most determinations, the data are background corrected, that is, $\bar{x}_B = 0$. Substituting the above measured and calculated values in Equations 21 and 22, the expression for c_L is further simplified to

$$c_L = \frac{k\left[s_B^2 + s_i^2 + \left(\frac{i}{m}\right)^2 s_m^2\right]^{1/2}}{m} \qquad (23)$$

In the event that no significant error occurred in the slope, Equation 21 reduces to

$$c_L = \frac{k[s_B^2 + s_i^2]^{1/2}}{m} \qquad (24)$$

If the error in the intercept, s_i, is sufficiently small, Equation 23 reduces to Equation 7, which is the IUPAC definition of the limit of detection.

Evaluation of Approaches

The IUPAC, graphical, and propagation of errors models will be applied to four different sets of experimental data to show the effect of certain experimental conditions on the estimation of c_L values (Table II). The data in Table II have been taken from a recent paper on ICP-excited ICP fluorescence detection limits (*15*). The four sets of typical experimental conditions are: A, Ca(II) fluorescence data which have well-defined m and i values; B, Ca(II) fluorescence data where s_B is essentially the same as in A, but the calibration curve data are taken far away from c_L, resulting in a poorly defined i value and a well-defined m value; C, Cu(I) fluorescence data where there is nonlinearity in the calibration curve resulting in ill-defined m and i values; and D, Co(II) fluorescence data where extreme nonlinearity in the calibration curve results in severe errors in both m and i values. The limit of detection values, $c_{L(k=3)}$, for the three methods are tabulated in Table III. Here the $c_{L(k=3)}$ values are reported only to one significant figure, as all c_L values should be. The subscript numbers representing the second significant digit in cases A and B are included only for comparative purposes.

For case A, the IUPAC model and the graphical approach agree well. Only the propagation of errors model shows a significantly higher $c_{L(k=3)}$ value; this deviation is the result of the error associated with the intercept value.

The values obtained in case B emphasize the problem of an ill-defined, nonzero intercept. This problem is the direct result of constructing calibration curves for detection limits when the lowest point of the calibration curve data is considerably removed from the $c_{L(k=3)}$ value. Only the propagation of errors model accounts for this error, while the other two methods indicate an erroneously low $c_{L(k=3)}$ value. Although there are no set guidelines for constructing calibration curves, the third approach clearly illustrates the problem of sampling too far away from the limit of detection.

In some instances, calibration curves may not be linear. Although the linear regression procedure will fit a line through the data, the resulting m value is by no means a "true" representation of the analytical sensitivity at all concentrations. Nonlinear calibration curves generally produce significant s_m, s_i, and i values. Case C represents such conditions. The IUPAC model results in a $c_{L(k=3)}$ of 0.03 ppm. Repeating the c_L calculation using the graphical approach results in a value of 0.3 ppm. Finally allowing m and i errors to be included results in a $c_{L(k=3)}$ of 5 ppm for the propagation of errors method. The propagation of errors c_L value is 170 times the IUPAC $c_{L(k=3)}$ value.

The problem of nonlinearity can be further emphasized by considering case D. Here, the errors associated with m and i are greater; however, the large value of s_m results in a special problem with the graphical approach. If $t_\alpha s_m > m$, then the concentration value for the limit of detection can even be negative. Such negative values are the direct result of the graphical model not being statistically valid. Although the graphical model is easier to

use than the propagation of errors approach, the former may give erroneous results as seen in Case D. Thus, the graphical method could be used only for approximating c_L values. The detection limit using the propagation of errors approach is calculated to be 6 ppm. Correspondingly, the detection limit for the IUPAC model is 0.5 ppm, differing by a factor of 12 between the two methods.

Conclusions

Based on the above considerations, the graphical approach to c_L should not be used. The IUPAC approach is valid only if the major source of error is in the blank, i.e., $s_B^2 \gg s_i^2$ or s_m^2. Therefore, the IUPAC approach in most cases gives artificially low values of c_L. The propagation of errors approach is certainly the most liberal approach and will give values of c_L consistent with the reliability of the blank measures and the signal measures of the standards.

We recommend that analysts report limits of detection using the IUPAC approach with $k = 3$ ($c_{L(k=3)}$). The use of the propagation of errors approach is also recommended because errors in the analyte measurements can be incorporated into the c_L value. By adopting these approaches, meaningful comparisons of analytical methods and instruments based on c_L values can be made.

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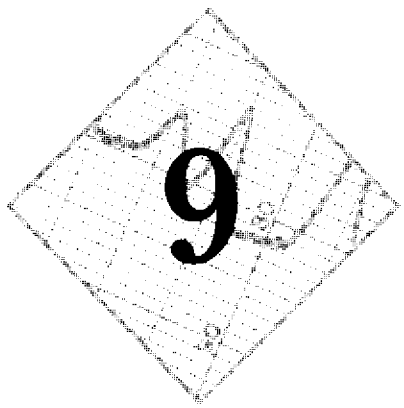

Long-Term Stability of Organochlorine Residues in Frozen Fish Tissue

R.J. Hesselberg and D.A. Nortrup

Long-Term Stability of Organochlorine Residues in Frozen Fish Tissue

by
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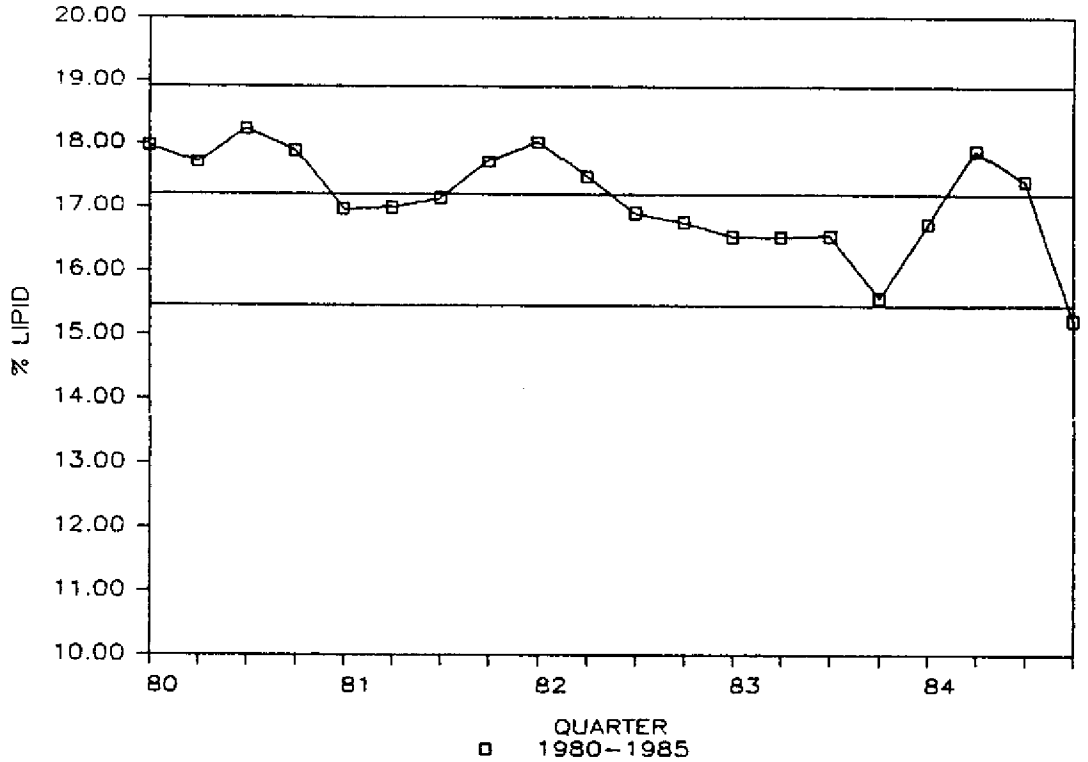
In 1980, as part of a routine quality assurance program, the Great Lakes Fishery Laboratory began using performance samples prepared from a composite of lake trout collected near Saugatuck, Lake Michigan, in 1979. Initial mean concentrations were determined by repetitive analyses for lipids, DDT monologs, dieldrin and PCBs. The performance sample was then reanalyzed with sets of unknown samples to monitor method performance and help maintain quality control. After pre-weighing a sufficient number of samples to last for about a year, they were put into glass sample jars with screw caps, and the bulk supply and the samples were stored at -30°C. For each succeeding year, the bulk supply of fish tissue (stored at -30°C in 4-mil plastic bags) was rethawed, additional samples were prepared, and the samples plus remaining bulk refrozen.

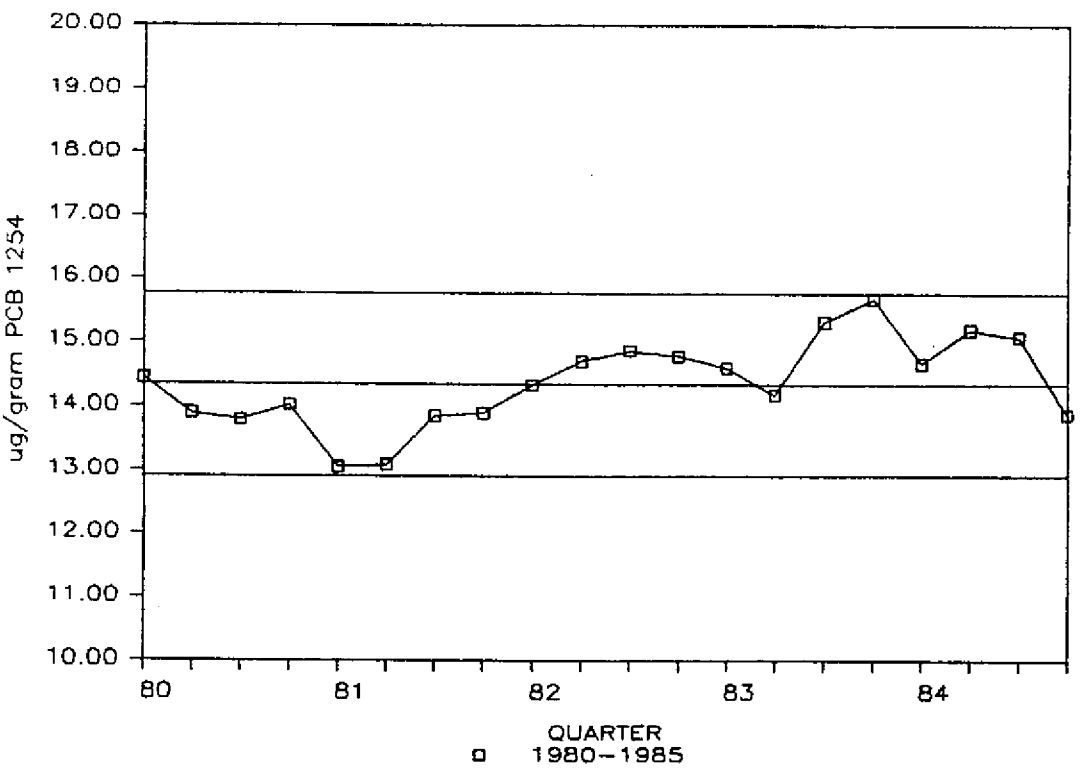
Results from repetitive analyses of the performance samples change gradually over a five-year period. Lipids decreased at about 1.5 percent per year, while organochlorine concentrations (DDT monologs, dieldrin and PCBs) increased the same amount. Decreases in lipid content suggest that the tissue was slowly degrading, possibly as a result of the periodic thawing of the bulk supply mentioned above. Increases in contaminants concentration over the five-year period were likely the result of slow moisture loss due to storing the bulk reference material in a plastic bag. Moisture content was not measured. The gradual changes that occurred in the samples were within normal analytical error per year and could most likely be reduced further by pre-weighing sufficient performance samples to last for the duration of the intended use.

Performance samples thus prepared and maintained provide a useful addition to routine quality assurance programs as a measure of method performance over time on environmental samples.

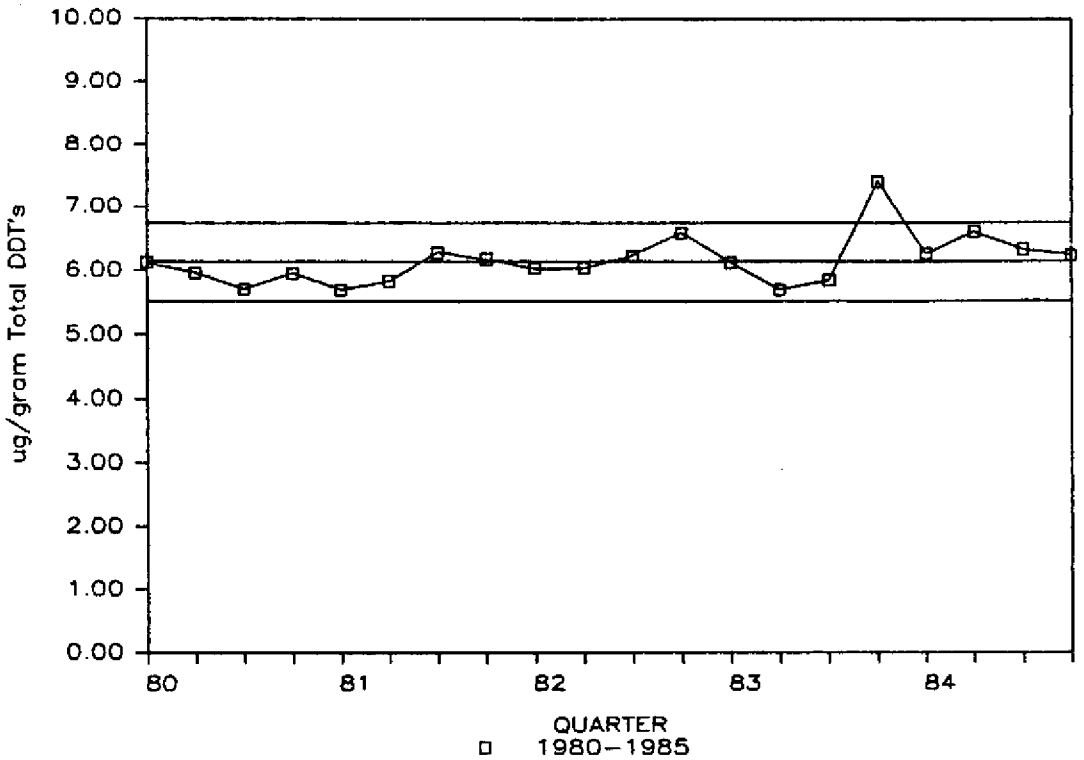
CHECK 79 % LIPID



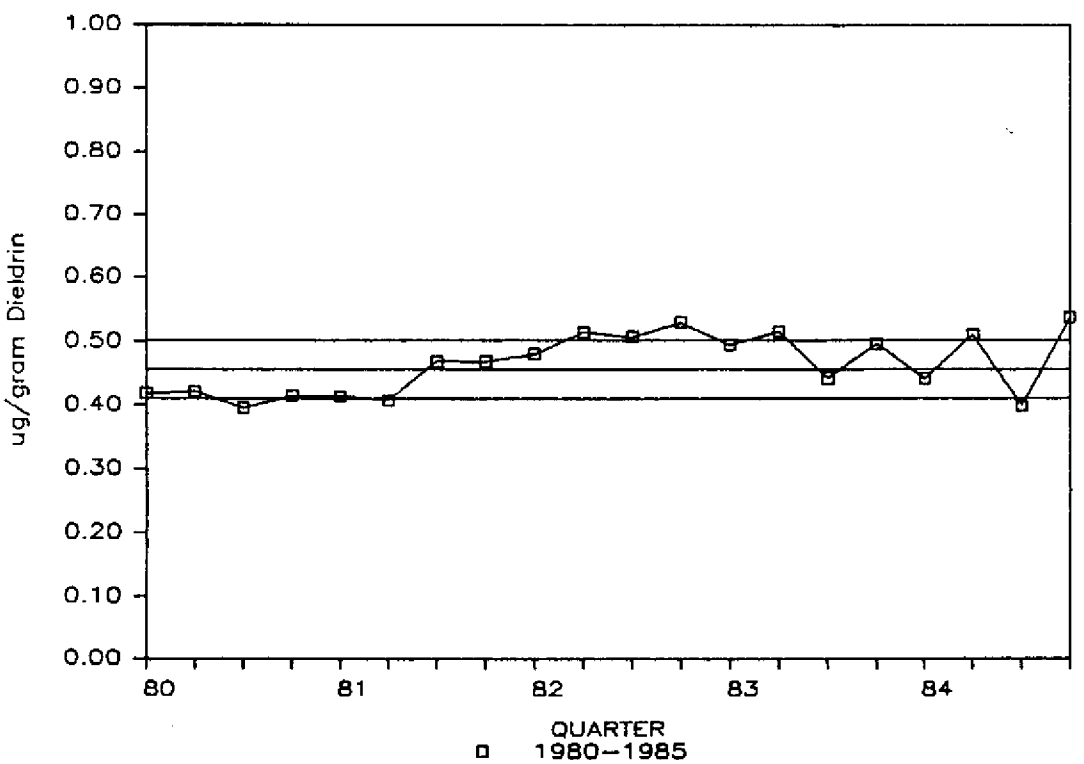
CHECK 79 ug/gram PCB 1254

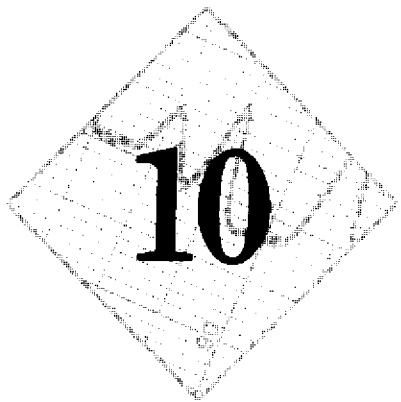


CHECK 79 ug/gram TOTAL DDT's



CHECK 79 ug/gram DIELDRIN





Minimizing Effects of Closure on Analytical Data

Erik Johansson, Svante Wold and Kristina Sjödin

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Minimizing Effects of Closure on Analytical Data

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Closure, or the constant sum, is a well-known problem in geology and geochemistry but has apparently been neglected in analytical chemistry. Closure affects the data, and thereby the results, each time there is a normalization (scaling) of the data sample (object). Chromatography and mass spectrometry are examples of methods where the closure problem is frequent. The effect of closure on two chromatographic data sets is demonstrated and commented upon. A discussion is given of how to predict the influence of closure and how to avoid or minimize the problems.

Analytical chemistry is becoming increasingly multivariate (*1–4*). In many situations where multivariate analytical data are measured, the data are normalized to a constant sum which makes the data "closed". This closure introduces a dependence between the variables so that if one large variable goes up, the others automatically go down because their sum is fixed. The effect of closure might or might not be crucial to the results of the investigation.

In chromatography the data are often closed. The value of each constituent is often expressed as a percentage of the total amount to compensate for variation in the amount of sample injected or pyrolyzed (*5*). In mass spectrometry the fragment peaks m/z are presented with the largest fragment peak set to 100 and the other fragment peaks expressed as percentages of the largest peak (*6*). This is a type of sample normalization which affects both comparisons of mass spectra and library searches based on similarities.

The consequence of expressing all variables as percentages of the total is that "the sum of covariances of each variable will be exactly equal to its variance and opposite in sign" (*7*). This means there will be a considerable risk for spurious negative correlations between major variables (variables that have a high percentage of the total) and also a risk for spurious positive correlations between minor variables (*8–11*).

Much effort has been spent on estimating the amount of closure on already closed data sets. Eventhough it may be possible to remove some of the trivial correlations that are introduced by normalization, it should be noted that, if data have gone through a closure inducing normalization, no method exists that can "open up" such data (*11*, *12*). In most analytical chemical treatments, fortunately, the raw data are available. This makes it possible to either keep the trivial correlations small or to some extent predict the influence of closure.

THEORY

To demonstrate the effect of closure on two chromatographical data sets we use principal components (PC) analysis, a common method for getting a view of multivariate data (*13–15*). PC analysis has been described in detail elsewhere (*16*); hence only a short presentation of pertinent features will be given.

The data set, see Figure 1, is modeled by the PC expression in eq 1.

$$x_{ki} = \bar{x}_i + \sum_{a=1}^{A} t_{ka} b_{ai} + e_{ki} \qquad (1)$$

Here x_{ki} represents the observed value of variable i (height or integral of peak i) of object k (chromatogram k). The parameters $\bar{x}_i$ are averages of the variables i. The PC parameters b_{ai} and t_{ka} are computed so that the residuals e_{ki} are minimized in the least-squares sense. The smaller the residuals, the better the fit of the model, i.e., the more of the variance in the data is explained. The number A of statistically significant components (product terms) is determined by cross validation (*20*). PC based models are sensitive to the

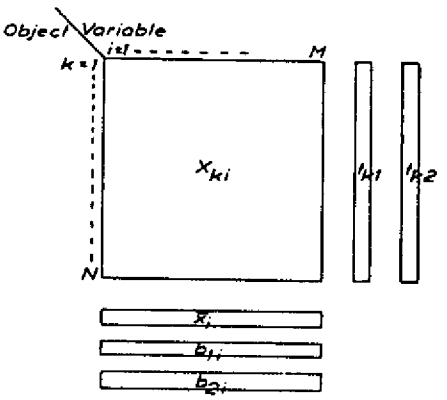

Figure 1. Data matrix X with elements x_{ki}.

Table I. Results of the Different Normalization Procedures[a]

				normalization
a	6 var.	1	44%	
b	6 var.	1	47%	Norm (6) = 100
c	5 var.	(1)	(33%)	Norm (5) = 100
d	6 var.	(1)	(27%)	Norm high = 100

[a] a, b, c, and d are defined in the text. The components in parentheses are not significant according to cross validation. Percentages, e.g., 44%, are the amount of variance explained by the PC model.

variance of the variables. In all of the following calculations, all variables are therefore scaled to the same variance (1.0).

We emphasize that closure is a general problem that affects *the data* and hence any multivariate data analytic method applied to it, e.g., factor analysis (*17*), KNN (*18*), LDA (*19*), and ALLOC (*20*). It also affects ordinary variable by variable plots.

EXPERIMENTAL SECTION

The first data set is a small pilot study of the amounts of monoterpenes in spruce and their relation to the geographical origin of the spruce. Needles from spruce were collected and a headspace sample was injected on a 2.0-m glass column (5% Dow 11 on Chromosorb W-AW-DMCS 100–120 mesh). The amounts of monoterpenes were estimated as the peak height of each peak. Part of this data set (one geographical origin) was selected for the present study.

Closure can also be a problem in large data sets with many variables. This is shown by using part of the GC data from a study on volatile constituents of pine wood. Differences in the composition of volatile constituents between healthy and weakened trees were investigated. Weakened trees are more susceptible to insect infestation and odor signals may be a significant factor in guiding the insects to suitable trees. Headspace analyses of wooden samples of pines were performed on a 50-m OV-225 glass capillary column. A full description of both the analytical procedures and the multivariate analysis and the biological significance of the results for this second data set will be published later.

RESULTS

Spruce Data with Six Variables. The test data contained 15 chromatograms (objects) each with six constituents (variables). To study the effect of closure, we performed PC analyses on the nonnormalized raw data and on data normalized in three different ways. The following PC analyses were made: (a) on the raw data; (b) on data summed to 100 for each object over all six variables; (c) on data summed to 100 for each object for the first five variables; (d) on data normalized as in MS, i.e., the largest peak set to 100 and the remaining expressed as percentages of this peak.

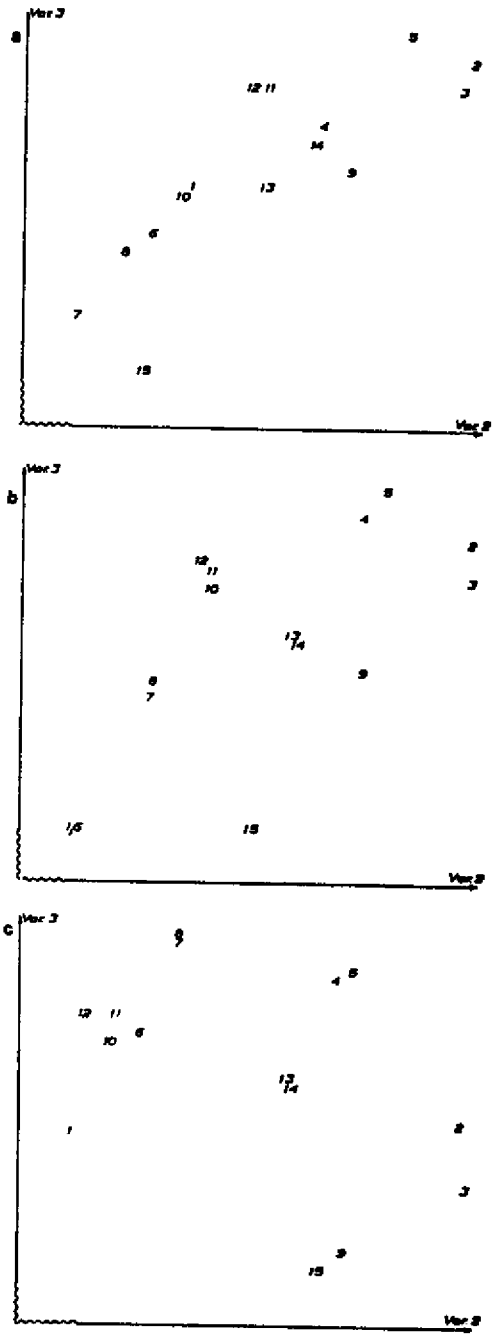

Figure 2. Plots of the relation between variables 2 and 3 for three different data treatments: (2a) nonclosed; (2b) closed over 6 variables; (2c) closed over 5 variables.

As can be seen from Table I the difference between (a) and (b) is minor and the effect of closure is small. To demonstrate how sensitive to normalization a data set with few variables can be, one variable, the last, was deleted and a new round of PC analyses were performed. The PC results on five nonnormalized variables are similar to that of six nonnormalized and not included in Table I. The data were thereafter normalized over five variables and a PC analysis was performed. The result of this normalization is considerable and no statistically significant component is found.

Another way of demonstrating the effect of closure is variable by variable plots. The two largest variables, 2 and

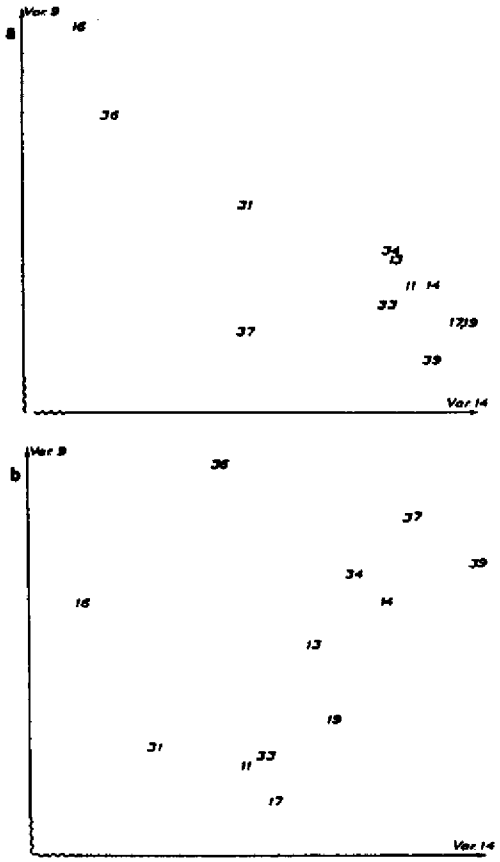

Figure 3. Plot of the correlation between variables 9 and 14 for data closed over all variables (a) and the selectively closed data (b).

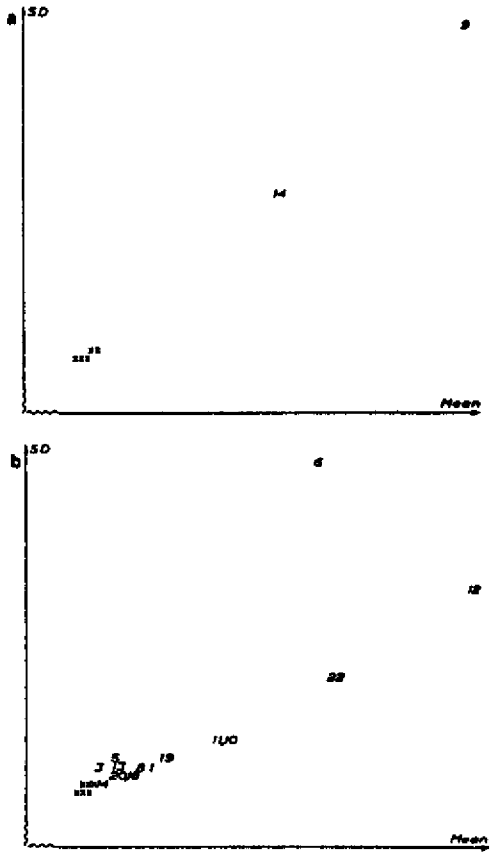

Figure 4. Plots of mean value vs. standard deviation for the variables (crosses indicates many numbers on top of each other).

3, are plotted against each other in Figure 2. It can be seen that Figure 2a and Figure 2b are similar while Figure 2c is considerably different.

The normalization commonly used in mass spectrometry (d) affects the data strongly and gives a loss of information. No significant component is obtained and the calculated model describes less variance than all other models. This result indicates that the common habit in mass spectrometry of closing the largest variable to a constant (100) diminishes the possibility of extracting relevant information from mass spectral data.

Pine Data with 31 Variables. One group of trees, weakened, giving a total of 12 samples were subjected to two types of closure. The first normalization was the traditional, i.e., to sum all 31 variables to 100. The second normalization was the new proposed type (see below) where a homogeneous set of variables was selected and their sum normalized to 100 by a separate normalization factor for each object. Thereafter this normalization factor was used to scale all variables for this object. The selection of a homogeneous set of variables will be commented upon in the Discussion.

The effect of the closure procedures was most profound for the two largest GC peaks. These are plotted against each other in Figure 3a ($r = -0.85$) and Figure 3b ($r = 0.27$). The strong spurious negative correlation stems from the fact that if variables 9 and 14 have to occupy the same space, it is impossible that there exist a case after closure were both 9 and 14 are large.

DISCUSSION

A number of Monte Carlo calculations of the results of closure have been presented (*8*, *21*). The conclusions are that the effect of closure is small on variables with equal mean and variance when the number of variables is larger than 8 but that the effect increases as the number of variables decreases. As demonstrated in Figure 2a and Figure 2b the effect of closure might be small even when as few as six variables are closed. That example shows that the effect of closure is dependent on the data structure of the nonclosed set.

Skala has shown that if variables have different means and/or variances the closure effect might be strong even if the number of variables is large (*21*). These effects include the presence of artificial negative correlation among the large variables and sometimes a positive correlation between the smallest of the variables. Indeed, the artificial negative correlation between the largest variables was found in our second example where the number of variables was fairly large.

Selective Closure: a Partial Solution. To avoid the most prominent closure effect, the artificial negative correlation among large peaks, we performed a selective closure of the data over a subset of the variables. The variables were selected according to the following criteria. First, the means and standard deviations of the variables should be approximately of the same size and secondly the number of variables in the selected set should be as large as possible.

The selection of variables is conveniently made from a plot of the standard deviation for each variable against the mean for each variable. In Figure 4a it is apparent that variables 9 and 14 are almost an order of magnitude larger than the others so therefore they were excluded and the remaining variables plotted again. The result as shown in Figure 4b is that the size and standard deviation of the variables are now reasonably homogeneous even if there still are differences in

variable means and standard deviations.

The 12 selected variables were normalized with a normalization factor so that for each object they had a sum of 100. The remaining variables, that is, the two large (*9*, *14*) and the small variables, were thereafter multiplied by the normalization factor associated with each object. The small variables should not be included to avoid positive correlations between them (*7*). This will have the effect that closure will be limited to the 12 included variables while the remaining variables are not included in the constant sum, and thereby they will not be directly affected by closure.

We want to stress that this selective normalization does indeed give a closure effect on the data, but due to the selection of many homogeneous variables it will hopefully be small. Our recommendation for closed GC data is therefore that variable–variable plots for a few of largest variables should be made and if a strong negative correlation exists, the selective normalization should be used instead. We do not recommend attempts to try a number of different normalization procedures since this will mean that the risk for spurious results will increase. It is also important that the same type of normalization is done on all samples included in the data set. Attempts to normalize different parts of the data in different ways will give nonsense separations between these parts.

Practical results of the use of multivariate methods in mass spectrometry have so far been meager (*22*). One reason for this may be the habit of closing the raw data by setting the largest peak to 100. This usually will mean a loss of information compared to other forms of closure. It should also be noted that on most MS systems the raw data are available and our belief is that these nonclosed data could be used instead.

CONCLUSION

If the data are closed, no method exists that can "open up" the data (*12*).

When the raw data are available there exist ways to keep the effect of closure small and thereby to allow the maximal amount of information to be extracted. The suitability of the selective closure procedure proposed in this paper, and all other closure procedures, is dependent on both the internal structure of the data and the scope of the investigation.

Our recomendation is that normalization should be used with caution. If normalization is necessary, the selection of normalization procedure should be based on the prior information about the raw data structure and the scope of the investigation.

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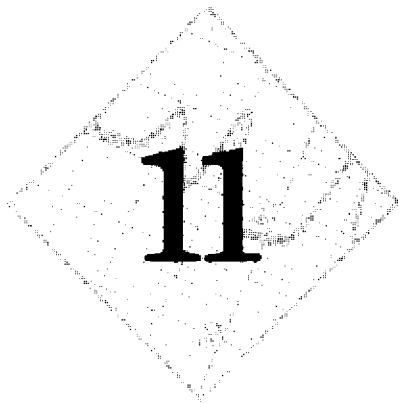

11

On the Use of Filters for Distinguishing Between Dissolved and Particulate Fractions in Natural Waters

Lars Göran Danielsson

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ON THE USE OF FILTERS FOR DISTINGUISHING BETWEEN DISSOLVED AND PARTICULATE FRACTIONS IN NATURAL WATERS

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Abstract—The influence of filtration on the colloid content of a natural sample as a function of filter load has been studied. Two membrane filters and one glass fibre filter commonly used for the filtration of natural samples have been tested.

Membrane filters are shown to be unsuitable for the separation of suspended matter unless the effects of filter load are studied. The importance of the colloidal particles passing normal filters in interpreting the transport of trace metals in the environment is stressed.

INTRODUCTION

The transformations between dissolved and particulate forms, e.g. those taking place in estuaries, have a decisive influence on the further transport into the oceans (Turekian, 1977; Sholkowitz, 1976). Studies of these and similar processes necessitates the determination of both dissolved and particulate fractions of the metals involved. Furthermore, tests of thermodynamic models of metal behaviour in natural waters also require this separation to be made.

Membrane filters have been commonly used to collect particulate matter from natural water samples. Different pore sizes are available, but most work has been done with 0.45 μm filters. Use of filters of this porosity is a standard procedure for the determination of dissolved components.

Filtration of water samples prior to the determination of trace constituents can cause errors in various ways (Riley, 1975 and references therein). Adsorption onto filters and other filtering equipment can give rise to low results (Marvin *et al.*, 1970). On the other hand, high concentrations of trace metals have been found in various filters (Robertson, 1968; Spencer & Mannheim, 1969). Increased concentrations in the filtrate due to leaching of these metals cannot be excluded. Furthermore, if trace metal concentrations in the particulate matter are to be determined, contamination can be severe. These problems have been circumvented by soaking the filters in acid for several days and then rinsing in the filtering equipment with large amounts of sample before collecting a filtrate (Florence, 1977; Mart, 1979).

The use of 0.45 μm filters for the distinction between dissolved and particulate forms has been questioned by several authors. Hem & Robertson (1967) showed that particulate aluminium could be trapped on a 0.1 μm filter from waters previously filtered through a 0.45 μm filter. Clay particles in the size range 0.1–0.5 μm were thought to be responsible for errors in determinations of dissolved Al, Fe, Mn and Ti found by Kennedy *et al.* (1974). Similar experiences were obtained for iron in river water by refiltration of previously 0.45 μm filtered water through filters with smaller pores (Boyle *et al.*, 1977). Up to 79% of the "dissolved" iron could be collected on a 0.05 μm filter. Trapping of particles smaller than the nominal pore size of the filter was shown to take place for some of the most commonly used filters. Furthermore, the effective pore size was influenced by the amount of particles on the filter (Sheldon & Sutcliff, 1969; Sheldon, 1972). The present work was undertaken in order to study the effects of filtration on colloidal matter present in natural waters.

METHODS

The sample used for this study was obtained from lake Alenäshålan in southwestern Sweden. This lake is surrounded by peat bogs and its water has a high concentration of iron and humic substances, as indicated by a high colour value. Sampling was performed by lowering a 25 l. polyethylene carboy into the water from a large stone at the shore taking care not to disturb the unconsolidated surface sediment. The samples were stored in the carboys at room temperature for various lengths of time. After some initial settling the samples were remarkably constant in composition.

Filtrations were performed with filters of either 47 or 142 mm diameter. The 47 mm filters were used in Swinnex filter holders attached to a Millipore 0.6 l. filling system. This equipment was pressurized with tank nitrogen to a pressure of 3.5 kg cm^{-2}.

The large filter (142 mm) was used in a Millipore stainless-steel filter holder. The sample was pumped through the filter with a membrane pump (Prominent, Heidelberg, F.R.G.) at a pressure not exceeding 6 kg cm^{-2}.

The various filters were rinsed with distilled water before filtration of sample. Portions of filtrate (10 ml) were taken at intervals and used for the various analyses.

Iron analyses were performed by atomic absorption spectrophotometry. Concentrations higher than 0.2 mg l^{-1}

were determined by flame nebulisation using a Perkin-Elmer 370 AAS. For samples with lower iron concentrations a Perkin-Elmer 403-HGA 2100 combination was used. Colour measurements were performed at natural pH (6.9–7.0) with a Perkin-Elmer 451 U.V.-VIS Spectrophotometer. The wavelength used was 430 nm and absorbance readings were transformed into colour units by comparison with a Pt-Co standard (APHA, 1971).

The concentration of humic substances were estimated from the fluorescence compared to a standard isolated from a Swedish river (Almgren and Josefsson, 1973).

RESULTS

Figure 1 illustrates the result of filtration with 0.45 μm Millipore and 0.40 μm Nucleopore membrane filters. In order to simplify comparison between filters of different sizes the volume filtered per unit filter area has been used. From Fig. 1(a) it can be seen that for both filters the iron concentration in the filtrate, at low load, is the same as in an unfiltered sample. At intermediate loads, 10–20 ml cm^{-2}, the Millipore filter tends to retain a slight amount of iron while the Nucleopore filter is still permeable to all the iron. On continued filtration the iron concentration of the filtrates decreases drastically. The decrease is sharper and starts earlier for the Nucleopore filter. The final concentration at loads in excess of 45 ml cm^{-2} is, however, the same for the two filters.

Results from colour measurements of the same samples as those used for iron determinations are shown in Fig. 1(b). The colour values change with load in a way similar to that of the iron concentrations. However, the residual colour at high load is about 10% of the colour in unfiltered sample. For

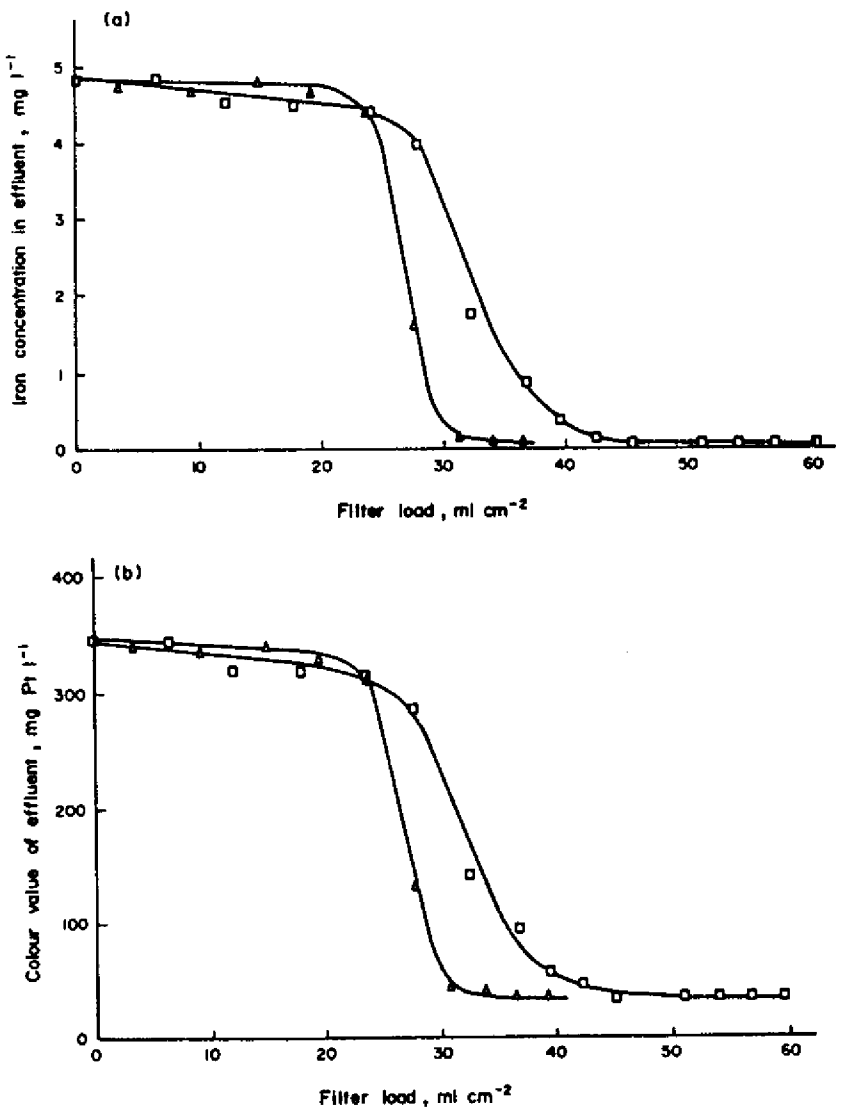

Fig. 1. Effect of filter load on the retention of colloids. □ Millipore HAWP; △ Nucleopore 0.40 μm. (a) Iron concentration in effluent, (b) Colour value of effluent.

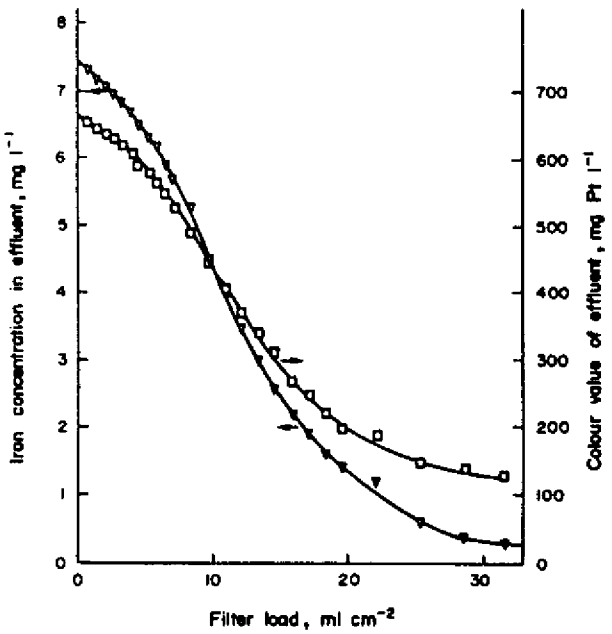

Fig. 2. Effect of filter load on the retention of colloids using a spectrograde glass fibre filter. ▽ Iron concentration in effluent. □ Colour value of effluent.

iron the residual concentration is $< 1\%$ of the original.

In order to examine the behaviour of organic substances in the sample during filtration some measurements of humic substances were made. Surprisingly very slight retention was found. For samples taken at low load the concentration of humic substances was found to be 13.5 mg l^{-1}. At high load, when the penetration of iron was very slight, the concentration had only decreased to 12.5 mg l^{-1}.

Glass fibre filters are often recommended for filtrations of particulate laden waters due to their large dirt holding capacity. Using a Whatman GF/F glass fibre filter (0.7 μm) no change in iron concentration or colour value was detected at a load of 170 ml cm^{-2} (greater values were not studied). However, with a Spectrograde glass fiber filter a completely different result was obtained as can be seen in Fig. 2. For this filter a decrease in the amount of iron in the filtrate can be seen even at very low loads.

DISCUSSION

The water used for this study is admittedly extreme in its high iron concentration and high colour value. However, the compounds responsible for these values are present in most natural waters. The nature of these compounds is probably a co-colloid of iron hydroxide and organic compounds. The organics have a strong stabilizing effect on iron as exemplified by the high iron concentration even after long time storage at natural pH under oxic conditions. If the organics are destroyed by u.v.-irradiation the iron precipitates within a few hours. However, in spite of the collection of 99% of the iron and 90% of the colour only a slight decrease in fluorescence was measured. This can be due to quenching of the fluorescence by iron hydroxide or that the organic substances stabilising the iron hydroxide colloids are non-fluorescent. A third possibility is that the association between iron hydroxide and organics is so loose that on compaction on the filter the organic part is lost and passes into the filtrate.

The two types of membrane filters used have widely different construction. Millipore filters are made of a co-polymer of cellulose nitrate and cellulose acetate forming a three-dimensional net. This means that the specified pore size is actually a mean value. Nucleopore filters have a somewhat more defined pore size as these filters are made by perforating a sheat of polycarbonate in a well-defined way. The filtration characteristics are, however, rather similar for the two filters. The more closely defined pore size of the Nucleopore filters explains the sharper breaks for this filter in the curves shown in Fig. 1. For the Millipore filter, trapping in small pores explains the slight loss at low loads. The larger load tolerated by the Millipore filter before clogging is probably due to slightly larger pore size (0.45 vs 0.40 μm). However, the different ways in which the filters are produced, giving Millipore filters some of the characteristics of a depth filter, might also have an influence.

The drastic decrease in iron concentration of the filtrate at intermediate load is caused by changes in the effective pore size of the filter. As the pores become clogged smaller particles are trapped and clogging accelerates. At this stage flowrate will of course also decrease. Finally, a state is reached where

no further clogging occurs. The reason for this may be that no particles are present that can clog the very fine pores that are still present.

Results obtained with glass fibre filters are somewhat puzzling. The large load that can be attained with the 0.7 μm Whatman filter may be explained by a combination of a large dirt-holding capacity and the small amounts of particles that are trapped with this filter. According to a company representative, the Spectrograde filter should have a mean pore size not less than 0.7 μm. The rapid clogging of this filter therefore seems astonishing. However, the Spectrograde filter is produced specifically for gas filtration and has received special treatment to avoid reactions with air pollutants. This treatment might have increased the adsorption power of the filter explaining the rapid clogging.

CONCLUSIONS

The use of filters of normal pore sizes (0.4–0.7 μm) for the separation of particulate and truly dissolved fractions of metals in natural waters is inadequate, especially for colloid-laden samples like bog waters, woodland streams and lakes and estuarine waters. Furthermore, the use of membrane filters for the removal of large particles from a water sample can introduce large errors due to the changing effective pore size. To avoid these errors careful investigation of the effects of filter load for the sample in question is recommended. If the contamination problems can be solved, glass fibre filters are helpful due to their large dirt-holding capacity. For large volume filtrations frequent filter change and/or large area filters should be used. Filter holders supplied with stirrers like those used for ultra filtration might also be beneficial, helping to avoid clogging.

The all too frequent neglect of particulate matter in the colloidal size range can lead to serious errors in estimations of the transport of trace metals in the environment (Breger, 1970). Use of the term dissolved for everything that passes through a 0.45 μm filter has impeded studies of these colloids. Hopefully, future studies of trace metals in natural waters will also include the colloidal fraction.

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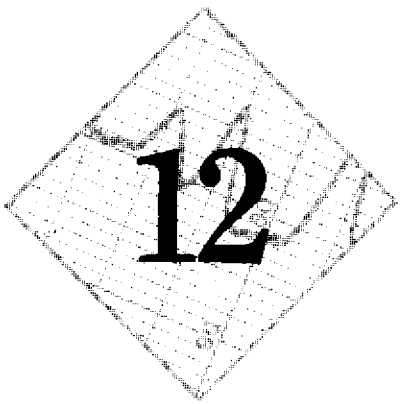

PCBQ: Computerized Quantification of Total PCB and Congeners in Environmental Samples

P.D. Capel, R.A. Rapaport, S.J. Eisenreich and B.B. Looney

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PCBQ: COMPUTERIZED QUANTIFICATION OF TOTAL PCB AND CONGENERS IN ENVIRONMENTAL SAMPLES

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ABSTRACT

Computerized methodologies for the quantification of total PCBs, PCB in Aroclor mixtures and individual PCB congeners in environmental samples are presented. The method for total PCBs is based on a multiple-linear regression analysis using data from capillary gas chromatography of Aroclor standards. PCB congeners were identified and their weight percentages determined in Aroclor mixtures by GC/MS. PCB congeners and total PCBs were accurately quantified in predetermined test data and environmental samples.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of hydrophobic organic compounds which are ubiquitous in the environment and have been a concern for more than two decades (1). They are produced by the direct chlorination of biphenyl, resulting in 209 posible congeners, of which more than 100 are observed (2). PCBs have been marketed in the United States under the trade name Aroclor (Monsanto) and contain a known weight percent of chlorine (e.g., Aroclor 1242 is 42% chlorine by weight). Most environmental PCB measurements have been reported as total PCB (3-5), many fewer as the percent of the component Aroclor mixture (6,7) and only a few as individual PCB congener concentrations (8,9). Recently, Eisenreich et al. (8) used the distribution of PCBs between Aroclor 1242 and 1254 and the behavior of individual congeners to gain a better understanding of the cycling of PCBs in large lakes. PCB congeners differ significantly in terms of their physical/chemical properties (2,10-12), persistence (13-15), and degree of bioaccumulation (16,17). Safe et al. (18) identified potentially toxic PCB congeners based on experimental data and structure-activity relationships, and suggested that the behavior of specific

congeners ought to be studied in the environment. Thus it is crucial to identify and quantify specific congeners in environmental samples. Duinker et al. (9) and Mullin et al. (19) advocate the usefulness of congener quantification towards a better understanding of environmental PCB behavior.

There have been a number of approaches to PCB quantification over the past two decades, most based on the use of packed-column gas chromatography. These include perchlorination of sample PCBs followed by measurement of decachlorobiphenyl (20), declorination of sample PCBs followed by measurement of biphenyl (21) and comparison of sample PCB gas chromatographic profiles to Aroclor standards (22,23). Duinker et al. (9) have reviewed the methods and their limitations. With the advent of routine, capillary gas chromatography (GC), an analytical tool is available to provide detailed information on total PCB and congeners in complex environmental samples.

METHODS

Congener Identification

The capillary gas chromatographic peaks in Aroclor mixtures (Figure 1) were initially identified by comparison to published Aroclor mixture chromatograms (24) and relative retention times of PCB congeners (19). Peaks were then independently confirmed using single congener standards, gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography/gas chromatography (HPLC/GC). Ballschmiter and Zell (24) have published capillary GC chromatograms in which the congener(s) in each peak are identified. Mullin et al. (19) have reported relative capillary GC retention times for all 209 PCB congeners. Chromatographic liquid phases used by Ballschmiter and Zell (24; SE-30 GC) and Mullin et al. (19; SE-54) were similiar to the one used in this study (HP Crosslinked 5% phenylmethyl silicone). This enabled the identification of PCB congeners through direct comparision of the chromatograms and relative retention times.

Twelve single congener standards, representing compounds from Aroclor 1242, 1254, and 1260 were chromatographed under identical conditions (see Figure 1). The results agreed unambiguously with mixture identifications from the literature described above (19,24).

Complete resolution by capillary GC of all 209 PCB congeners has not been demonstrated in this study or in others (19,24). Thus, some GC peaks contain two or more congeners. The dominant congener in each unresolved peak was determined by GC/MS and an HPLC technique (10). In some cases, both congeners contributed significantly to the total and both are listed in Table 1.

GC/MS (HP 5985 B) analysis was used to identify coeluting congeners with different molecular weights (i.e. chlorine number). In order to compare gas chromatography with electron capture detection (GC/EC) to GC/MS results, identical capillary columns and chromatographic conditions were employed (exception - Helium was used for GC/MS and N_2 for GC/EC; Figure 1). The MS was operated in the electron impact mode (70 eV) with an electron multiplier voltage of 2400 emf and a source temperature of 200°C. Ions were scanned from 50 to 500 amu.

The total ion spectrum of the chromatographic peaks thought to contain more than one congener was examined closely for variations in the parent ions. When the peak contained congeners of varying molecular weight, different parent ions

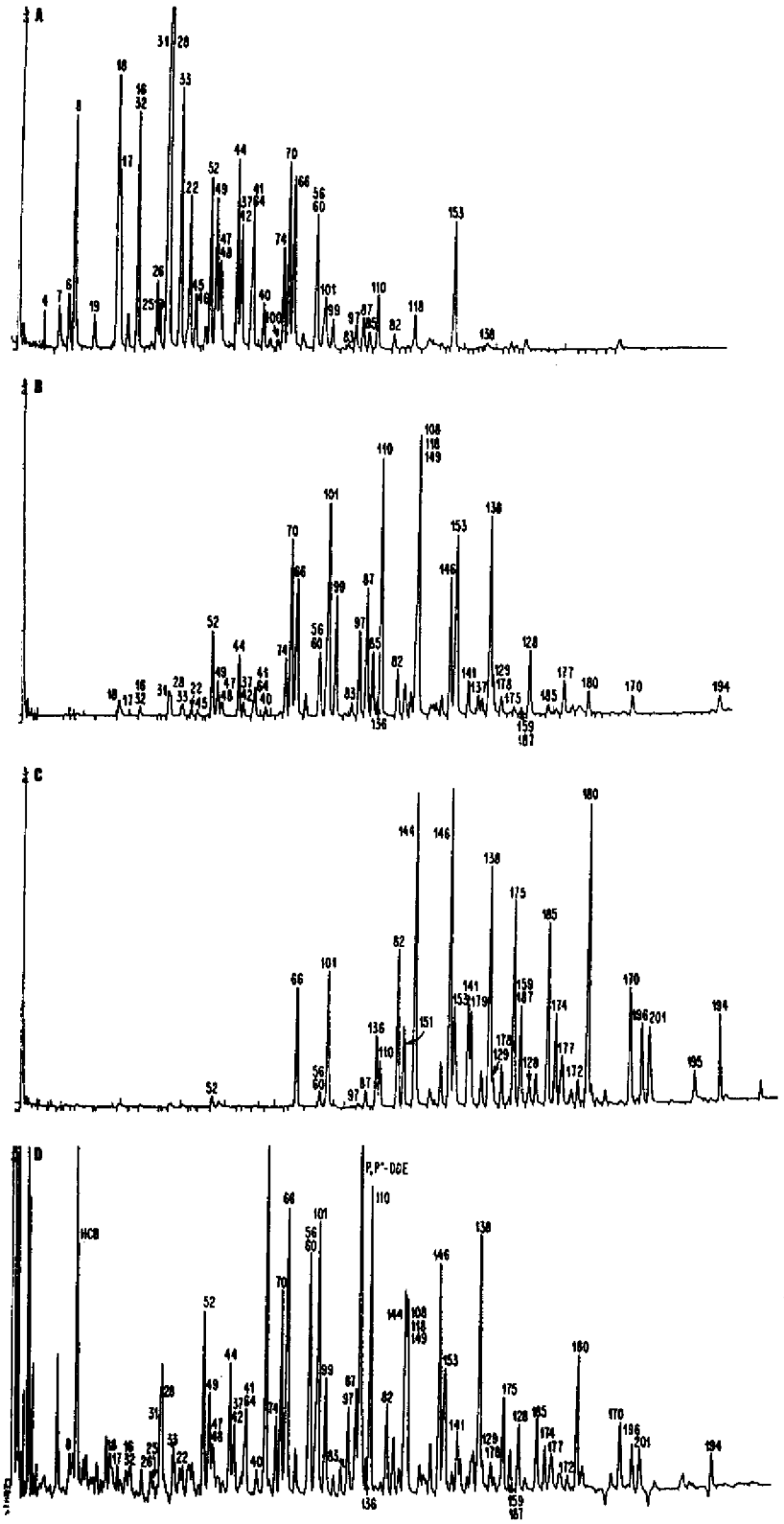

Figure 1 - Capillary column gas chromatograms of A) Aroclor 1242, B) Aroclor 1254, C) Aroclor 1260, and D) an environmental sediment sample (Lake Erie, LE-83-MS 18-20 cm). Column: HP Crosslinked 5% phenylmethylsilicone Conditions: Initial temp 150°C for 1 min, 10°/min to 175°, 1.3°/min to 250° Injector temp: 265° Detector temp: 335° Carrier gas: Nitrogen Detector: 63Ni EC. Numbers correspond to IUPAC convention (24). Structures are given in Table 1.

were observed when the peaks were examined for single ions. The single m/z values examined (188, 222, 256, 292, 326, 360 and 394) are the molecular ions for monochloro- to heptahlorobiphenyl. In these cases, total ion peaks were clearly separated into distinct single-ion peaks corresponding to specific congener molecular weights. Unless one parent ion was clearly dominant, both congeners were listed for that peak.

A technique used by Rapaport and Eisenreich (10) in their determination of octanol-water partition coefficients of PCB congeners provided additional information. Some congeners known to coelute in capillary GC can be separated using reversed-phase HPLC. Predictable differences in the HPLC retention times of these congeners, based on their molecular structure, enabled their identification. This allowed the determination of the dominant congener for peaks which contain two congeners of the same chlorine number.

Identifications of PCB congeners will be considered in four groups. The largest group are the chromatographic peaks which contain only one PCB congener (Group 1). A second group includes peaks which have two congeners with one being dominant (>90%) and are noted in Table 1 as a single congener (Group 2). The third group contains those peaks which have more than one significant congener (both >10%) (Group 3). Lastly are the peaks which contain more than one congener, but could not be further elucidated (Group 4).

Composition of Aroclor Mixtures

Having identified the PCB congeners, their weight percentages in each Aroclor mixture were determined by GC/MS. Weight percentages have been previously reported (25,26), but important discrepencies warranted new determinations. The earlier reports were based on packed-column GC and a different set of congeners were reported than the more recent studies using capillary GC (19,24).

In GC/MS operation, chromatographic peak area is proportional to the mass of the compound (27). Using the instrument as a mass detector, each chromatographic peak was integrated, then divided by the total area of the GC/MS chromatogram yielding a weight percentage for the peaks (Table 1). Because the MS is less sensitive than the electron capture detector for chlorinated compounds, some of the minor components were not detected.

Aroclor mixture percentages were determined for congeners in the four groups described above. For group 1, the percentage was determined directly. Since one congener is dominant in group 2 peaks, the entire percentage of that peak was assigned to the dominant congener. In groups 3 and 4, all congeners in a given peak share the weight percentages. In group 3 the congener with the highest mass is designated with an asterisk, although the percentage includes both congeners.

For comparison to these GC/MS values, congener percentages were also calculated from the electron capture relative response factors determined by Mullin et al. (19). The areas of each capillary GC/EC peak in this study were divided by the appropiate relative response factor(s), summed for the entire Aroclor mixture and divided by the sum to yield a percentage. The results listed in Table 1 for three Aroclor mixtures show close agreement with the GC/MS derived percentages.

Albro (28) and Streichen et al. (29) have warned that different Aroclor production lots may be variable. This variability did not seem to be significant in the work by Streichen et al. (29) or in the comparison of various

Aroclor 1242 mixtures presented in Table 2. Therefore, the percentages of the congeners listed in Table 1 can be considered representative of those Aroclor mixtures.

TABLE 1 - Weight percentages of the major PCB congeners in Aroclors 1242, 1254, and 1260

			Congener Weight Percents					
			Aroclor 1242		Aroclor 1254		Aroclor 1260	
Congener	Structure	Group	MS	RF	MS	RF	MS	RF
4	22'	2	0.64	1.37		0.00		0.00
7	24	1		0.65		0.00		0.00
6	23'	1		1.29		0.00		0.00
8+	24'	2	10.73	11.45		0.19		0.13
19	22'6	1		1.03		0.11		0.00
18+	22'5	1	11.91	9.38		0.80		0.11
17+	22'4	1	7.57	4.27		0.17		0.04
24	236	1		0.47		0.00		0.00
16*,32+	22'3	3	6.52	5.77		0.38		0.07
26	23'5	1		1.19		0.08		0.00
25	23'4	1		0.81		0.00		0.14
31+	24'5	1	10.07	6.21		0.82		0.12
28+	244'	1	10.26	5.53		0.40		0.00
33+	2'34	2	7.75	7.32		0.65		0.09
22+	234'	1	3.12	1.79		0.13		0.01
45	22'36	1		1.09		0.22		0.03
46	22'36'	1		0.45		0.06		0.00
52+	22'55'	1	4.19	4.54	3.17	3.34		0.43
49+	22'45'	1	2.89	2.67		0.86		0.06
47*,48+	22'44'	3	1.90	1.75		0.00		0.00
44+	22'35'	1	3.90	4.28		2.24		0.07
37,42+	344' 22'34'	4	3.11	2.88		0.52		0.04
41,64+	22'34 234'6	4	3.57	4.62		1.50		0.00
40	22'33'	1	0.46	0.78		0.26		0.01
100	22'44'6	1		0.19		0.10		0.00
74+	244'5	1	1.76	1.92	1.55	1.71		0.03
70+	23'4'5	1	3.93	3.68	7.55	5.52		0.08
66+	23'44'	1	3.89	4.22	6.98	6.10	3.43	3.48
60*,56+	2344'	3	2.64	2.30	2.06	1.82		0.31
101+	22'455'	1		1.27	11.32	8.42	4.47	4.04
99+	22'44'5	1		0.67	4.32	4.56		0.04
83	22'33'4	1		0.08		0.45		0.02
97+	22'3'45	1		0.53	2.40	3.06		0.13
87+	22'345'	2		0.43	4.43	3.32		0.34
85	22'344'	1		0.34	1.38	2.08		0.02
136	22'33'66'	1		0.00		0.59	1.50	3.16
110+	233'4'6	1		1.18	11.93	10.32		1.63
82+	22'33'4	1		0.31	0.61	1.73	4.34	4.53
151	22'355'6	2		0.08		1.14	1.56	2.52
144+	22'345'6	2		0.00		0.00	14.45	8.73
118*,108, 149+	23'44'5	3		0.68	15.59	11.50		0.00
146+	22'34'55'	1		0.05	4.94	4.75	18.95	10.05
153+	22'33'55'	1		0.41	8.14	3.14		1.32
141+	22'3455'	1		0.00		0.69	2.17	1.95
179	22'33'566'	1		0.00		0.00	2.57	2.58
137	22'344'5	1		0.00		0.37		0.02
138+	22'344'5'	1		0.08	9.49	9.11	11.68	8.33
129*,unk	22'33'45	3		0.00		0.78		0.63
178	22'33'55'6	1		0.00		0.80	0.20	1.65
175+	22'33'45'6	1		0.00		0.56	7.73	13.81
187*,159+	22'34'55'6	3		0.00		0.21	2.03	2.56
128	22'33'44'	1		0.00	1.26	1.66		0.38

Congener	Structure	Group	Aroclor 1242 MS	Aroclor 1242 RF	Aroclor 1254 MS	Aroclor 1254 RF	Aroclor 1260 MS	Aroclor 1260 RF
185+	22'3455'6	1		0.00		0.20	5.78	3.03
174+	22'33'456'	1		0.00		0.18	2.05	2.55
177	22'33'4'56	1		0.00	0.50	1.01	0.33	1.61
172	22'33'455'	1		0.00		0.05		0.51
180+	22'344'55'	1		0.00		0.54	14.45	6.50
170+	22'33'44'5	1		0.00		0.83	3.80	4.77
196+	22'33'44'5'6	1		0.00		0.00	1.38	1.72
201+	22'33'4'55'6	1		0.00		0.00	1.50	3.13
195	22'33'44'56	1		0.00		0.00		2.09
194	22'33'44'55'	1		0.00		0.00	0.83	0.87
Percent totals			100.83	100	94.45	100	105.20	100

Congener number based on IUPAC covention (24).
Group number 1: Single congener in chromatographic peak
2: More than one congener in chromatographic peak, but the congener listed is dominant.
3: Two or more significant congeners in chromatographic peak, all are listed. * denotes the major congener.
4: More than one congener in the chromatographic peak, but could not be further elucidated.
MS: GC/MS determined weight percentages.
RF: Weight percentages calculated from electron capture detector relative response factors (19).
+: Denotes congeners routinely used in this laboratory for quantification

TABLE 2 - Comparison of Five Aroclor 1242 Standards

Congener	A	B	C	D	E
8	.37	.35	.36	.37	.39
18	.66	.65	.65	.64	.69
16,32	.33	.32	.35	.32	.33
28,31	1.00	1.00	1.00	1.00	1.00
33	.42	.42	.43	.42	.41
22	.25	.24	.26	.25	.24
52	.22	.22	.23	.23	.21
49	.18	.18	.19	.20	.18
47,48	.18	.17	.18	.18	.17
44	.22	.22	.24	.24	.21
37,42	.20	.21	.21	.22	.19
41,64	.23	.25	.26	.27	.23
40	.07	.06	.07	.06	.05
74	.12	.13	.13	.14	.11
70	.23	.24	.24	.27	.20
66	.33	.28	.28	.31	.23
56,60	.18	.18	.19	.20	.15
79,101	.12	.06	.06	.07	.05
99	.03	.04	.04	.04	.05

Sources of Aroclor 1242 standards:
A: EPA Large Lakes Laboratory, Grosse Ile, MI
B: Nanogen
C: FDA, Minneapolis, MN
D: Applied Science
E: EPA, Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC
Relative abundances were based on the chromatographic peak containing congeners 28 and 31. These two congeners coeluted under the GC conditions used in this comparison study (Column: SP-2100).

QUANTIFICATION

Congener Quantification

Since each PCB congener is a unique compound, the ideal quantification method would employ a direct comparison between the environmental capillary GC peak containing only one congener and the corresponding standard. The synthesis of all 209 congeners recently reported by Mullin et al. (19) presents the potential for using individual congener standards. Although use of congener standards would be an excellent quantification method, the cost and required sample resolution would seem to make this method unlikely for routine use in the near future. An alternative invokes the use of the congeners contained in an Aroclor mixture as the standards. If the total concentration of the Aroclor standard is known, as well as the percent that a particular congener contributes to the total, then the concentration of that congener can be calculated and used as a standard. From the information presented in Table 1 this may easily be done for all of the major congeners in Aroclor 1242, 1254, and 1260. The congener concentration in the sample, adjusted for sample volume (or mass) and final analysis volume, can be calculated as follows:

$$[C(j)] = (EN(j)/ST(j)) \times [AR] \times AR(j) \times EC(j) \times (VVOL/SVOL)$$

where: C(j) = concentration of congener j in the environmental sample
EN(j) = environmental sample detector response to j
ST(j) = Aroclor standard detector response to j
[AR] = concentration of the Aroclor standard
AR(j) = weight fraction of j in Aroclor standard
EC(j) = factor to account for the contamination of j by another congener in the GC peak (normally equal to unity)
VVOL = final analysis volume
SVOL = sample volume (mass)

The Aroclor mixture which contains the largest percentage of the congener being quantified is used as the source of the congener standard. As an example, Table 3a presents the PCB congener concentrations that are observed in a Lake Erie sediment sample (Figure 1).

Total PCB and Aroclor Mixtures

A least-squares multiple linear regression is employed to yield the total PCB concentration and Aroclor distribution (30,31). The integrated areas for 30 to 50 chromatographic peaks comprise the independent vectors for the one or more standard Aroclor mixtures against which the environmental sample is regressed. Chromatographic peaks unique to each Aroclor mixture must be included as part of the regression vector. The dependent vector contains the areas for the identical chromatographic peaks in the environmental sample. The independent vector coefficients, adjusted for standard concentration and sample volumes are the Aroclor concentration in the environmental sample. The regression equation is forced through the origin because there is no basis for the existence of an intercept. The assumption is made that the quantified PCBs exist in the environment as relatively unaltered linear combinations of Aroclors, a valid assumption considering that PCB congeners undergo minimal environmental

transformations (13). This assumption can be checked in a semi-quantitative manner by the r^2 value of the regression equation. For many environmental samples, the r^2 is high (>.85), indicating that the sample closely matches the Aroclor mixtures. A low r^2 value indicates that the sample has undergone environmental modification.

A simple numerical example is presented in Figure 2 as an aid to understanding the calculations. A sediment sample from Lake Erie (LE-83-MS, 18-20 cm) was quantified (Figure 1b). The r^2 value (.91) indicates close agreement between the PCBs found in the sample and the distribution of the three Aroclor mixtures. The total PCB concentration and Aroclor distribution of the sediment sample is given in Table 3b.

TABLE 3 - Concentrations of total PCB and its congeners for the environmental sample presented in Figure 1 (Lake Erie sediment, site LE-83-MS, 18-20 cm)

A. From Congener Calculations - See Table 1 for congener identity.

2-Cl		3-Cl		4-Cl		5-Cl		6-Cl		7-Cl		8-Cl	
#	ng/g	#	ng/g	#	ng/g	#	ng/g	#	ng/g	#	ng/g	#	ng/g
8	2.18	16,32	1.16	41,64	2.02	82	.81	138	8.77	170	1.98	196	.85
		17	.71	44	2.44	87	2.43	141	1.28	174	.78	201	.83
		18	1.11	47,48	.73	97	1.81	144	6.36	175	3.50	Sum	1.68
		22	.66	49	1.76	99	2.83	146	5.55	180	5.93		
		28	2.61	52	3.71	101	10.01	153	9.97	185	2.14		
		31	3.43	56,60	4.81	110	9.91	159,187	.76	Sum	14.33		
		33	2.00	66	9.66	118,108	5.39	Sum	26.69				
		37,42	1.45	70	5.83	Sum	33.19						
		Sum	13.13	74	1.10								
				Sum	32.06								

Sum of the congeners = 123.2 ng/g

B. From Linear Regression Analysis

Total PCB: 125 ng/g
Aroclor Distribution: 24% Aroclor 1242, 42% Aroclor 1254, 34% Aroclor 1260
r^2: 0.91

The regression analysis for the calculation of total PCB and Aroclor distributions has been tested and validated using a number of predetermined and environmental data sets (Table 4). The results of the predetermined test data indicate that the method can accurately function over the entire range of Aroclor distribution. Samples quantified with 30, 40, and 50 peaks yielded nearly identical results. Environmental samples and a quality control standard were quantified independently by the regression method and manually by a modified version of the Webb and McCall method (22,32). Concentrations calculated by both methods agreed within one standard deviation. The quality control standard was quantified to within 5% of its known concentration by both methods.

The sum of the individual congener concentrations is in excellent agreement with the total PCB calculated by the regression analysis. In over 50 sample analyses checked for internal consistency (including water, suspended solids,

sediments and peat sections), the percentage of the sum of the congeners to total PCBs (regression) ranges from 80-110%. This variation may result from the differential fate of PCB congeners in the environment.

TABLE 4 - Validation of Regression Analysis

Predetermined Test Data: (µg/ml)

KNOWN			REGRESSION ANALYSIS			
[1242]	[1254]	% 1242	[1242]	[1254]	% 1242	r^2
0.500	0.000	100	0.500	0.000	100	1.00
0.450	0.050	90	0.450	0.050	90	1.00
0.350	0.150	70	0.350	0.150	70	1.00
0.250	0.250	50	0.250	0.251	50	1.00
0.150	0.350	30	0.150	0.351	30	1.00
0.050	0.450	10	0.050	0.451	10	1.00
0.000	0.500	0	0.000	0.500	0	1.00

Environmental data:

Lake Superior Water Samples - 1979 (ng/l)

	30 Peaks			40 Peaks			50 Peaks		
	[PCB]	%1242	r^2	[PCB]	%1242	r^2	[PCB]	%1242	r^2
Site 1	2.5	45	.87	2.5	44	.84	2.4	45	.86
Site 7	0.5	61	.78	0.5	57	.64	0.5	56	.66
Site 23	8.7	43	.96	8.5	44	.96	8.5	45	.96
Site 27	4.3	37	.95	4.3	37	.95	4.2	37	.94

Lake Superior Sediment Samples - 1982 (ng/g)

	Modified Webb and McCall			Regression Analysis			
	[A-1242]	[A-1254]	%1242	[A-1242]	[A-1254]	%1242	r^2
Bx-1-1	2.3±0.8	9.4±3.9	20	1.8	6.9	21	0.92
Bx-1-2	2.3±1.2	13.2±5.2	15	1.5	8.3	15	0.87
Bx-1-3	2.7±0.9	14.9±6.8	15	1.9	11.0	15	0.88

Quality Control Standard (µg/ml)

	[1242]	[1254]	%1242	r^2
Modified Webb and McCall	0.046±0.007	0.056±0.014	45	
Regression analysis	0.051	0.054	49	1.00
Known	0.050	0.050	50	

CONCLUSIONS

Regression analysis for total PCB and Aroclor distributions, and congener quantification based on Aroclor mixtures combine to make a useful, easy, and accurate method for quantification of PCBs in environmental samples. These methods have been written in FORTRAN for a mainframe computer and with "spreadsheet" software for a personal computer. These programs and/or instructions are available from the authors. The method is routinely used in this laboratory to quantify PCBs in a variety of environmental matrices, including sediments, water, air, and peat, and has proved extremely useful in assisting the interpretation of the fate and transport of these environmental pollutants.

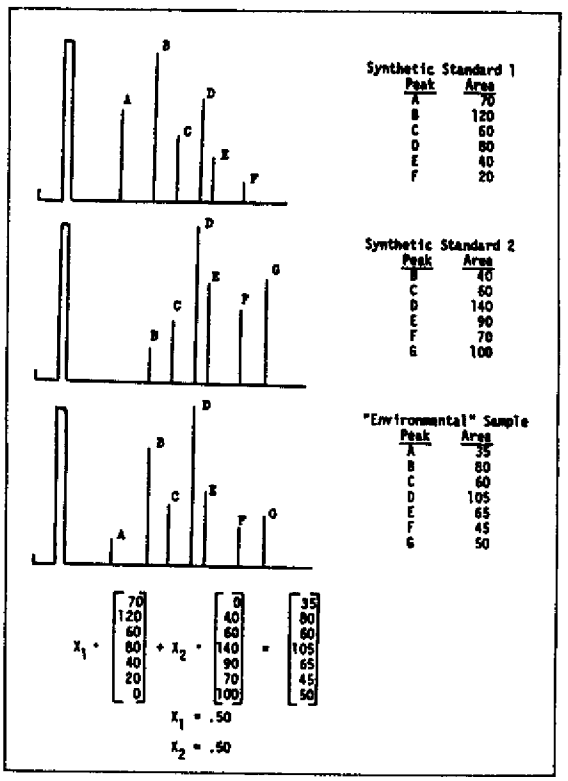

FIGURE 2 - A simple numerical example of the regression technique.

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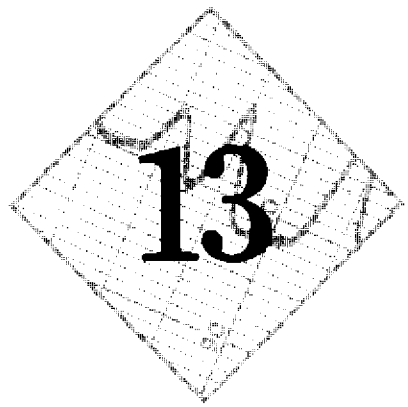

Pattern Recognition for Classification and Determination of Polychlorinated Biphenyls in Environmental Samples

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Pattern Recognition for Classification and Determination of Polychlorinated Biphenyls in Environmental Samples

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A technique is proposed for the quantitative determination of constituents in complex mixtures characterized by gas chromatography data. The technique is partial least squares in latent variables (PLS) and the data to which it is applied are gas chromatograms of the Aroclors 1242, 1248, 1252, and 1260. In addition the problem is formulated as one of classification and calibration and a sample of used transformer oil from a waste dump is classified as to its origin.

Polychlorinated biphenyls (PCBs) are some of the most widely distributed of environmental pollutants (*1–6*). These substances, formed by the extensive chlorination of biphenyl, were commercially marketed and widely distributed. The commercial names for the significant formulations in the United States are Aroclor 1242, 1248, 1254, and 1260, where the last two digits in the name designate the percentage of chlorine by weight in the formation. The Aroclors are very complex, but relatively invariant in composition mixtures, and are obtained by fractionation of the extensively chlorinated mixture. There are 209 possible isomers of PCBs of which about 125 are encountered in Aroclor technical materials. Recently, essentially all of these have been synthesized or identified and chromatographically characterized (*7*). Each Aroclor is characterized by a different distribution of isomers having a characteristic chromatographic profile of approximately 70–100 of the isomers (*8, 9*).

Due to the wide distribution of these compounds in the environment and the mounting evidence that these substances exert toxic effects to biological systems (*10, 11*), it is important to be able to quantitatively compare the composition of PCB

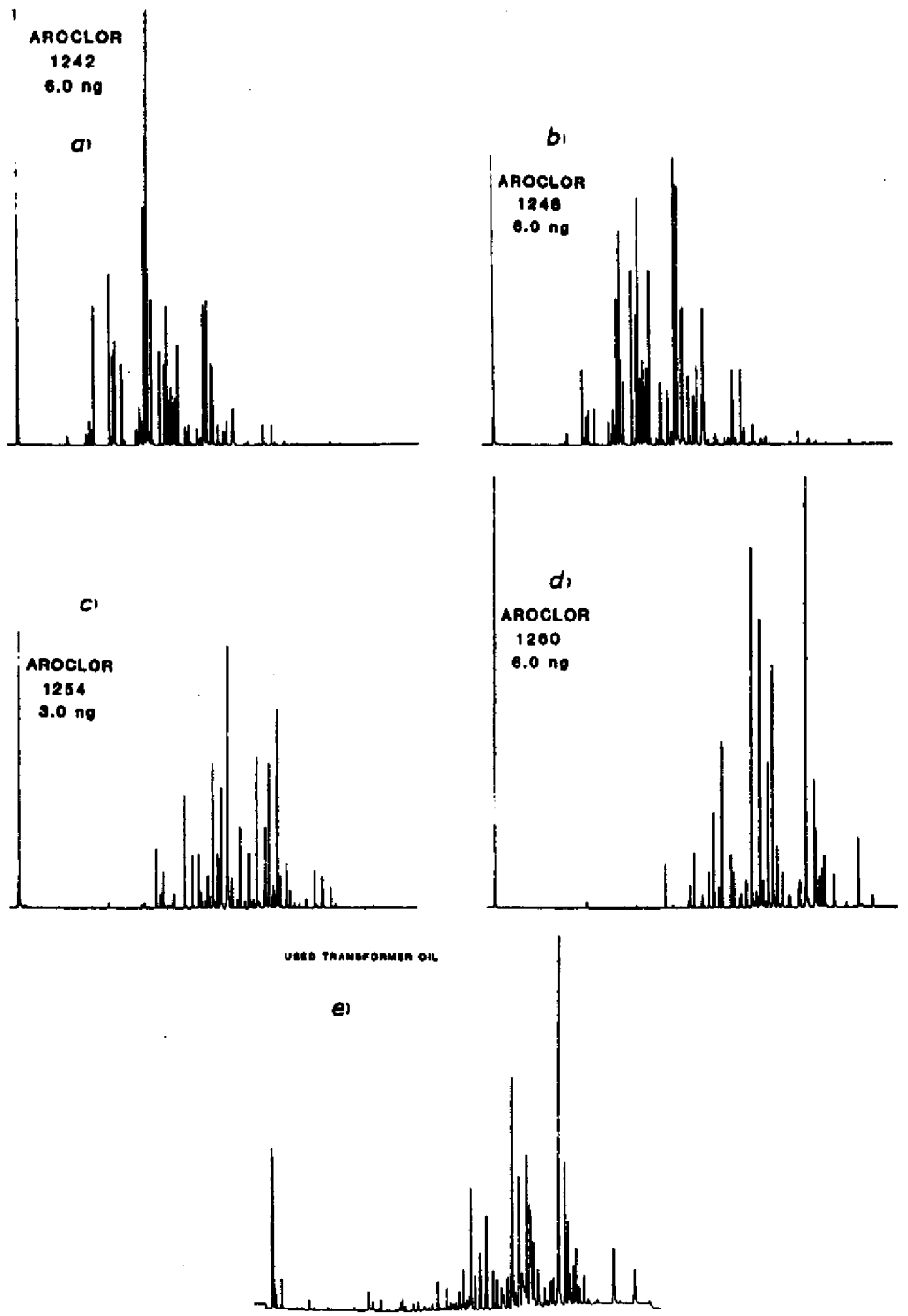

Figure 1. Gas chromatograms of the Aroclors (a) 1242, (b) 1248, (c) 1254, (d) 1260, and (e) used transformer oil.

residues in samples. This is especially true in laboratory studies designed to evaluate the kinetics of uptake and elimination kinetics associated with their bioconcentration. In studies concerning PCB environmental distribution or transport it is necessary to be able to characterize these substances in the various environmental compartments. Capillary gas chromatographic methods have been developed for this purpose (*8*, *9*, *12*, *13*) and provide not only the total PCB concentration in a sample but also the isomer distribution (*14*).

A gas chromatogram can be considered a "fingerprint" or characteristic profile of a sample containing the residue of a complex mixture. Such profiles of commercial samples of the Aroclors are given in Figure 1. Also shown is a chromatogram of used transformer oil obtained from an industrial waste dump where numerous Aroclors were alleged to have been dumped.

A visual examination of the PCB isomer profiles in the chromatograms shows that a large difference exists between the isomer distribution when Aroclor 1242 is compared to

Chart I. Data Matrix for Classification Problem

sample no.	peak number 1	2	·	i	·	M
1				·		
2				·		Aroclor 1242
·				·		Aroclor 1248
k	·	·	·	x_{ki}		
·						
N						

Chart II. Data for a High Level Classification Problem

sample no.	Aroclor content 1242 1248 · j ·	PCB isomer concn 1 2 · · i · · M
1		
·		
k	y_{kj}	x_{ki}
·		
N		

Aroclor 1260. Less marked, but discernible, differences are observed when Aroclor 1248 is compared to Aroclor 1254. Alteration of constituents in an individual Aroclor mixture such as might result from use or from electrical equipment failure is not as readily detected by visual inspection of chromatographic data. Also strong similarities exist between Aroclor 1260 and the used transformer oil.

In this and similar evaluations of complex mixtures, it is also of interest to make an assignment of a substance of unknown source, such as the used transformer oil, to a class. Here, the class assignment is to one of the Aroclors. In this case the unknown "appears" to be similar to Aroclor 1260 with a small amount of lower percent chlorinated biphenyl isomers. Thus, a quantification of the relative amount of each Aroclor present is of further interest. This constitutes a generalized classification problem and requires a method of level 3 pattern recognition for solution as described below.

THEORY

Classification problems can be formulated at different levels (*15*) depending on the information required from the analysis. At the first or lowest level, the objective is only to classify a sample as beloning to one of a set of predefined classes. At the second level the objective of the analysis is to classify an unknown into one of a number of defined classes, but with the additional possibility that it is not a member of any of them. At the third or highest level of classification, the probability of a class assignment is first calculated. Then quantitative information beyond classification, such as the concentration of constituents in a sample, is obtained.

Pattern recognition studies are carried out in stages. In the first stage a calibration set containing objects or samples of known class (in this case different Aroclors) are characterized numerically. The numerical characterization (model) of each sample is done by converting its gas chromatographic peak heights or areas to a row vector, the elements of which are the concentrations of the constituents. The result is a data matrix (training set) as shown in Chart I where i is the peak index and k the sample index. Hence, x_{ki} is the concentration of constituent i in sample k. The samples of unknown class assignment are numerically characterized in the same way to form a *test set*.

In the second stage of the analysis, a mathematical model is derived from each of the training sets which allows the samples of the test set to be classified. The unknowns in this problem are replicate analyses of a transformer oil sample.

For the highest level of classification problem, the samples of the training set can have, associated with them, external variables that may be related to isomer composition. This is shown in Chart II where X is matrix of GC data and Y is a matrix of external variables. Mathematical rules are then developed which will (1) classify samples and (2) predict the external variable values for the samples. For the case in which an unknown sample is a mixture of more than one Aroclor, the external variables would reflect the relative Aroclor content. If the same variables are avilable for the training set samples, models can be developed to predict the test set (unknown) samples. This is encouraging since a majority of the PCBs enering the environment are of technical Aroclor origin. Once an Aroclor enters the environment it can be altered in terms of isomer distribution. This is a calibration problem formulated as a pattern recognition analysis at the highest level. Methods of pattern recognition operate at different levels. Hence, it is necessary to establish in advance the desired level.

Current quantitative methods assume that alteration of isomer distribution can be approximated by using linear combinations of Aroclors (*16*). This assumption excludes the effects of isomer distribution changes in environmental samples. The history of the transformer oil in this study is not known and no assumptions were made in the selection of samples for the training set that were based on information about the sample.

To illustrate and compare the different levels of information which can be obtained in classification studies, two methods of pattern recognition have been used. These were the K-nearest neighbor (KNN) (*17*) method, which can give classification results (level 1) and SIMCA (*SIM*ple Classification by *A*nalogy, e.g.) pattern recognition method (*18*) which operates at the highest level (level 3).

KNN Pattern Recognition. The KNN method of pattern recognition has been applied in a number of cases to chemical data characterizing samples (*17*). It has a number of properties which make it applicable to the present problem. With this procedure, the classification of a sample is based on its distance to its K-nearest neighbors. The distances of each sample to the other members of the data set are calculated and classification of an unknown is based on its distance to its four nearest neighbors. The value for K was selected to be 4 since each training set contains four members (samples).

SIMCA Pattern Recognition. The SIMCA method of pattern recognition has been described (*18*) and used in classification problems involving the analysis of complex gas chromatography data (*19*). In classification analyses the training set for a q-class problem (here $q = 4$, the number of Aroclor formulations) is modeled by a set of principal components models (eq 1), one for each class. In eq 1, x_{ki} represents the elements of the concentration data matrix in Figure 1, $\bar{x}_i$ is the class mean of the peak i, and A is the number of component terms in the model. Each principal component term ($t_{ka}b_{ai}$ in eq 1) is composed of the loading, b_{ai}, and component score, t_{ka}

$$x_{ki} = \bar{x}_i + \sum_{a=1}^{A} t_{ka}b_{ai} + e_{ki} \qquad (1)$$

Usually the analysis is done so that the component terms in the model account for the systematic variation about the mean in the class. The e_{ki} values are the residuals representing the combination of measurement error and model error. The residuals can be made small by including as many product terms in the model as justified by cross validation (*20*).

For the classification aspects of this work, the number of component terms (A in eq 1) was arbitrarily set as two for each class model. This was done so that the data could be visually inspected in the initial stages of the analysis and was possible due to the high reproducibility and low error of measurement in the data. While most of the structure or variance in the data set was in the class means, this approach allowed for the detection of the variation in the class data.

Classification of an unknown sample is made by fitting its data to the Q class models. From the residuals, a standard deviation for each sample and each class is calculated. The class assigment is based on the standard deviation, which should be sufficiently small to place it inside or near the modeled class.

If a definite class assignment cannot be made, i.e., if a sample is not similar to one class, it is possible that the unknown is a mixture of Aroclors. It is then possible to deconvolute the sample into the relative amounts of the Aroclor components.

This can be done with a variety of techniques depending on the nature of the deconvolution data. The traditional method is ordinary least squares using multiple regression. This requires that the mixture be precisely a linear combination of the known Aroclors. In addition, it is required that there be no systematic variation between samples of the same Aroclor class. Moreover, PCBs accumulated in environmental samples could undergo changes in isomer distributions or change as a consequence of isomer degradation. The linear model is then no longer adequate. The SIMCA method does not require that these conditions be met to evaluate the similarity of samples.

Since data analytic methods exist that take the possibility of nonlinearity into account, but give the linear results if these indeed are adequate, we have preferred to use these more general methods. Hence, we have applied the partial least squares (PLS) with latent variables method (*18*). We have two blocks of data, Y and X (see Chart II). It is assumed that the elements of Y can be predicted from X through some latent variables, u and t. This is stated as

$$\mathbf{Y} \leftarrow u \leftarrow t \leftarrow \mathbf{X} \tag{2}$$

The X block is modeled by eq 3. This model allows the classification of test samples in the same way as the principal components (PC) class models.

$$x_{hi} = \bar{x}_i + \sum_{a=1}^{A} t_{ha} b_{ai} + e_{hi} \tag{3}$$

Analogously, the Y block is modeled by eq 4.

$$y_{hj} = \bar{y}_j + \sum_{a=1}^{A} u_{ha} c_{aj} + f_{hj} \tag{4}$$

Equations 3 and 4 are principal-components-like models but differ in that they are derived so as to *simultaneously* minimize the residuals e_{hi} and f_{hj} while yielding the latent variables t_{ha} and u_{ha} that give optimal prediction of Y and modeling of X. A predictive relationship (eq 5)

$$u_{ha} = d_a t_{ha} + k \tag{5}$$

can be written which allows an estimation of the y's from the x's for the samples of unknown composition. This is given in eq 6, where d_a is the regression coefficient from eq 5.

$$y_{hj} = \bar{y}_j + \sum_{a=1}^{A} t_{ha} d_a c_{aj} \tag{6}$$

The determination of the model complexity (number of component terms) in calibration models is dictated by the constraints that the model (eq 3 and 4) must be derived to optimaly predict the y data while best modeling the x data. Therefore, the procedure of cross validation (*20*) was employed to determine A, which gives the latent variables t and u, consistent with the above constraints. In the PLS algorithm this is done by systematicaly deleting the data for samples (x's and y's). The sum of the squared residuals are determined for the deleted objects and this model is used to estimate the deleted y's. The sum of the squared residuals is determined for the deleted objects and the process repeated until all objects have been deleted once and only once. This sum of squared residuals (CVSS) is corrected for degrees of freedom between the new model and the reference, less complex model. If the ratio of CVSS is less than one, the new model is retained. If the ratio is greater than one, there is no improvement in adding a new component to the model and the analysis is stopped.

The PLS method has been applied to problems related to the present one in which the number of variables was much greater than the number of samples (*20*).

EXPERIMENTAL SECTION

Gas Chromatographic Analysis. A Varian Model 3700 gas chromatograph (Varian Instruments Co., Palo Alto, CA) equipped with a ^{63}Ni electron capture detector (ECD) was used for sample analysis. The data sampling and GC program operations were controlled by a Varian Autosampler Model 8000 which also delivered a calibrated amount of samle to the GC injection port. Chromatographic conditions were similar for the analysis of all samples: temperature program rate, 120 °C initial temperature, programmed at 2 °C/min to a final temperature of 255 °C; detector temperature, 320 °C; injector temperature, 220 °C. The separation of PCB congeners was accomplished with a glass capillary chromatographic column (30 m × 0.25 mm i.d.) coated with a C_{87} stationary phase (Quadrex Corp., New Haven, CT). Hydrogen, linear velocity of 32 cm/min, wa used as the carrier gas, and nitrogen delivered at 15 mL/min was used as the detector makeup gas (*12*).

Integration and Laboratory Data Base. Output from the electrometer was interfaced to a PDP 11/34 computer through a Laboratory Peripheral System interface obtained from Digital Equipment Corp. Their software system, PEAK-11, was used to acquire and integrate the gas chromatographic signal representing the chromatogram. The data from the analyses were transferred to a second PDP 11/34 computer and processed with a laboratory data base system based on the Digital Standard MUMPs-11 computer language (*14*). The processed sample data were archived on magnetic tape before a copy of the data from the analysis of Aroclor standards (Aroclors 1242, 1248, 1254, and 1260) was selected for further analysis.

In addition, data representing mixtures of these Aroclors in the relative proportions (by weight) of (a) 1:1:1:1, (b) 1:1:1:0, (c) 1:1:0:1, (d) 0:1:1:1, (e) 1:0:1:1, (f) 1:1:0:0, (g) 1:0:0:1, (h) 0:0:1:1, (i) 0:1:0:1, (j) 1:0:1:0, and (k) 0:1:1:0 were also included in the data file for PLS analysis. Four replicate analyses of each Aroclor were obtained as were four replicates of a 1:1:1:1 mixture of the Aroclors. The unknown transformer oil was analyzed in triplicate. These data were transferred via an RS-232 link to a Cromemco System III (Cromemco, Inc., Mountain View, CA) Z-80 based microcomputer where the data were stored in a format suitable for analysis by the SIMCA programs. Data from the analysis of these samples were transferred to the Z-80 microcomputer in a similar manner.

The pattern recognition data analyses were accomplished with a Zenith Z-100 microcomputer running under CP/M. The software used was SIMCA 3B BASIC, which contains the KNN procedure in addition to SIMCA and PLS, and is available from Principal Data Components, 2505 Shepard Blvd., Columbia, MO, or Sepanov AB, Ostrand 14, Enskede, Sweden. The PLS algorithm has been published (*21*).

Data Analysis. Two types of pattern recognition analyses were carried out in the study: (1) classification of an unknown sample(s) as a specific Aroclor and (2) a calibration in which the relative amounts of specific Aroclors were estimated in a classified sample. GC data were obtained from 34 chromatograms in which 69 specific isomers were quantified in appropriate concentration units.

For the calibration study, 11 standards were prepared in which the relative Aroclor concentration varied as described above. These were used with the training sets (individual Aroclors) in the classification study. The data are available from the author on request.

RESULTS

Since class assignment is based on internal isomer distribution of the samples, the data for each sample were nor-

Table I. Standard Deviation of Fit to Aroclor Class Model

transformer oil sample	Aroclor 1242	1248	1254	1260
21	1.26	1.12	0.87	0.27
22	1.28	1.13	0.87	0.26
23	1.27	1.12	0.87	0.27
std dev Aroclor class	0.014	0.014	0.012	0.012

malized to a mean of zero. This makes each sample equivalent in terms of total PCB concentration.

In the classification analysis a preliminary graphic display of the training sets and unknowns was done by constructing a principal components plot of 23 samples. The result is given in Figure 2 which shows that the transformer oil samples are similar to Aroclor 1260. A principal components model with two components was derived for each class and the residual standard deviation of fit of each unknown to the respective class models was calculated. This result is given in Table I. It is seen that the transformer is indeed closest to Aroclor 1260 but still far outside the "normal class distance" of 0.012 units for Aroclor 1260.

The KNN results are given in Table II. They give a consistent picture and it is seen that the Aroclors are nearest the other members of their training sets with the next nearest neighbors being on the 1:1:1:1 samples. The transformer oil samples are nearest to the 1260 samples.

For the composition analysis the PLS method was applied to the calibration data set. A three-dimensional ($A = 3$) PLS model was derived for the calibration set and the relative

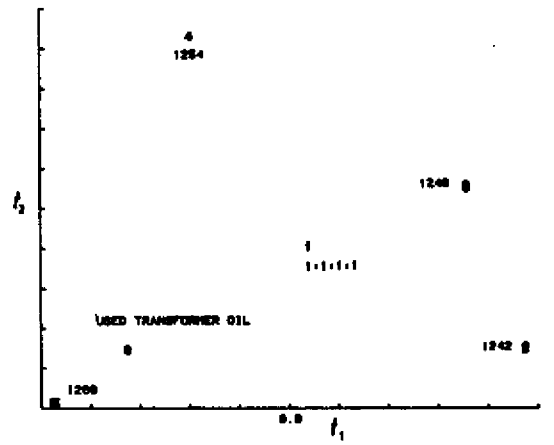

Figure 2. Principal components plot of the data for the Aroclors, transformer oil, and 1:1:1:1 mixture of the Aroclors showing that the transformer oil is similar to Aroclor 1260.

Aroclor concentration calculated for transformer oil sample. These results are given in Table III.

For comparison, PLS estimates of the calibration samples 20 and 24 are included (Table III). For the analysis of standards the numbers in parentheses are the composition of the mixture as prepared by mixing the standards. It is seen that the predictions are accurate within 0.10 or less. These results thus indicate that the transformer oil is predominantly Aroclor 1260 plus a small amount (8%) of 1254. These results are not subject to the influence of an analyst's opinion of

Table II. KNN for Aroclor Series and Unknown Transformer Oil

sample no.	dist[a]	sample no.	dist	sample no.	dist
1 (1242)	0.02 (2)[b]	9 (1254)	0.03 (11)	17 (1:1:1:1)	0.02 (18)
	0.02 (3)		0.03 (10)		0.06 (19)
	0.03 (4)		0.03 (12)		0.06 (20)
	0.73 (8)		0.73 (19)		0.59 (8)
2 (1242)	0.02 (1)	10 (1254)	0.02 (11)	18 (1:1:1:1)	0.02 (17)
	0.02 (3)		0.02 (12)		0.06 (19)
	0.03 (4)		0.03 (9)		0.06 (20)
	0.72 (8)		0.73 (19)		0.58 (8)
3 (1242)	0.02 (1)	11 (1254)	0.02 (10)	19 (1:1:1:1)	0.03 (20)
	0.02 (2)		0.02 (12)		0.06 (17)
	0.03 (4)		0.03 (9)		0.06 (18)
	0.73 (8)		0.72 (19)		0.58 (8)
4 (1242)	0.03 (1)	12 (1254)	0.02 (11)	20 (1:1:1:1)	0.03 (19)
	0.03 (2)		0.02 (10)		0.06 (17)
	0.03 (3)		0.03 (9)		0.06 (18)
	0.72 (8)		0.73 (19)		0.58 (8)
5 (1248)	0.02 (6)	13 (1260)	0.04 (14)	21 (tr. oil)	0.02 (22)
	0.03 (7)		0.04 (15)		0.03 (23)
	0.03 (8)		0.05 (16)		0.29 (13)
	0.59 (20)		0.28 (22)		0.30 (15)
6 (1248)	0.02 (5)	14 (1260)	0.02 (15)	22 (tr. oil)	0.02 (21)
	0.03 (7)		0.03 (16)		0.03 (23)
	0.03 (8)		0.04 (13)		0.28 (13)
	0.59 (20)		0.29 (22)		0.29 (15)
7 (1248)	0.01 (8)	15 (1260)	0.01 (16)	23 (tr. oil)	0.03 (22)
	0.03 (6)		0.02 (14)		0.03 (21)
	0.03 (5)		0.04 (13)		0.29 (13)
	0.58 (20)		0.29 (22)		0.29 (15)
8 (1248)	0.01 (7)	16 (1260)	0.01 (15)		
	0.03 (6)		0.03 (14)		
	0.03 (5)		0.05 (13)		
	0.58 (20)		0.29 (22)		

[a] Distance to four nearest neighbors (samples 1-20) is calculated; for unknowns (samples 21-24) the distances to the five nearest neighbors is calculated. [b] Sample number in parentheses.

Table III. Aroclor Composition Estimated by PLS Method (A = 3)

sample no.	Aroclor 1242	1248	1254	1260
Standard Mixtures				
20 (1:1:1:1)[a]	0.17 (0.25)	0.24 (0.25)	0.29 (0.25)	0.28 (0.25)
24 (1:1:1:0)	0.37 (0.33)	0.33 (0.33)	0.24 (0.33)	0.04 (0.00)
Transformer Oil				
21	0.03	0.04	0.08	0.84
22	0.03	0.03	0.08	0.84
23	0.03	0.03	0.08	0.84

[a] Actual Composition.

similarity of sample to Aroclor standard.

DISCUSSION

The results obtained here illustrate the utility of the SIMCA method of pattern recognition in the analysis of standards and environmental samples characterized by complex gas chromatography data. These results from the two pattern recognition methods SIMCA and KNN are consistent. It is assuring that the same results are obtained with two different well-understood methods. In this example, it was possible to classify a sample of PCB containing transformer oil as being similar to the isomer distribution present in Aroclor 1260 and to estimate its relative Aroclor composition.

This is significant since the majority of the PCBs enter the enviroment as one of the four Aroclers and no rugged numerical method to date is available to classify an environmental sample based upon isomer composition or according to its source type. Once an Aroclor enters the environment, it can be altered in terms of internal composition. Alteration of composition can occur by admixture with other Aroclors such that residues present in a sample represent a complex mixture of Aroclors. However, changes in the Aroclor can also result from differential partitioning of the PCB isomers between various environmental compartments (i.e., water, sediment, biota).

The history of the transformer oil in this study is not known, except for the fact that it was from an industrial waste dump in New Jersey where numerous Aroclor formulations were alleged to have been dumped. It could have undergone weathering and/or dilution. The method of analysis described here indicates that this substance is a mixture of the various Aroclors and the calculations discussed herein support this point.

Alternatively, PCB residues from biological samples could involve Aroclor alterations resulting from bioaccumulation that result in an altered chromatographic pattern. Such data from isomer specific accumulation and/or degradation are complex and such alterations are frequently encountered in environmentally derived samples. The calibration model for the PCBs derived here can be used with PLS to determine the extent to which this alteration occurs. By subtracting the three PLS components from a gas chromatogram of an environmental sample containing PCBs, the residuals would represent the isomer-specific variation that had occurred in such a sample. We are presently collecting data to illustrate this application of the method.

The SIMCA method of pattern recognition has a number of advantages when compared to other classification methods. These advantages are more easily understood when classification problems are viewed in terms of the levels of information which can be extracted and utilized from the data in such a study as the one described here. The combination of classification and calibration represents a high level of information utilization. Additionally, the PLS method is very fast and easily programmed for running on microprocessor-based systems. Hence, it can be integrated into data acquisition and management systems for any analytical technique in which the output characterizing a sample(s) is complex and multivariate. The method operates with any ratio between the number of variables and number of objects (samples, cases). This is in contrast to traditional methods such as multiple regression and linear discriminant analysis. We are presently exploring the utility of the PLS method in the analysis of other complex chromatography data.

Registry No. Aroclor 1242, 53469-21-9; Aroclor 1248, 12672-29-6; Aroclor 1252, 89577-78-6; Aroclor 1260, 11096-82-5.

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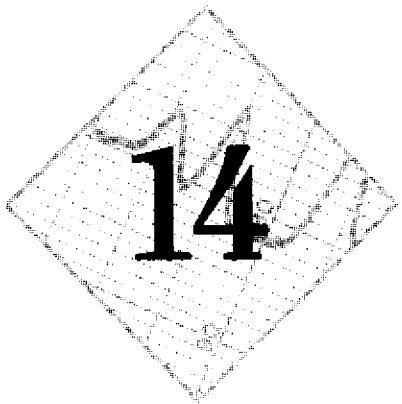

Patterns of PCDD, PCDF and PCB Contamination in Great Lakes Fish and Birds and Their Characterization by Principal Components Analysis

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PATTERNS OF PCDD, PCDF, AND PCB CONTAMINATION IN GREAT LAKES FISH AND BIRDS AND THEIR CHARACTERIZATION BY PRINCIPAL COMPONENTS ANALYSIS

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ABSTRACT

Contamination of the Great Lakes with polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) has created concern because of the adverse effects of these chemicals on fish and wildlife. In addition, the use of fishery resources for recreation and food can be limited if harmful concentrations of these contaminants occur. Concerns about these residues are based on the observations that PCDDs and PCDFs in fish and birds are composed primarily of the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachloro-dibenzofuran (TCDF) and other 2,3,7,8-chlorine substituted penta- and hexa-chlorodibenzo-p-dioxins and -furans. The concentrations of 2,3,7,8-TCDD in fish and birds are greatest in regions where chlorinated organic chemicals are manufactured or near hazardous waste sites.

The application of SIMCA, a principal components chemometric method, demonstrated that residue profiles differed between regions sampled. Ratios of PCDFs to PCDDs in the bird eggs examined were smaller than those observed in fish -- perhaps indicating that in birds the rate of metabolism of PCDFs is higher than that of PCDDs.

INTRODUCTION

Two points of focus are presented here. First, we briefly discuss a principal components method of pattern recognition. Second, we report results of the analysis of Great Lakes fish and bird samples and in part, use them to illustrate the relevance of the chemometrics approach in the study of environmental residues.

Because polychlorinated dibenzo-p-dioxins (PCDDs), -dibenzofurans (PCDFs), and -biphenyls (PCBs) consist of complex mixtures of hundreds of individual isomers, a powerful approach to data interpretation is needed to enable investigators to comprehend and characterize environmental profiles and distribution. The use of chemometric methods can improve data quality by

identifying the samples in a group that are most similar, or by providing a statistical classification method for identifying the group or class to which a sample belongs (1).

Chemometrics, as defined by Kowalski (2), includes the application of multivariate statistical methods to the study of chemical problems. The most advanced level of pattern recognition offers the possibility of predicting effects by using latent variables derived from the dependent (measured) and independent variables (3). This approach is often used in quantitative structure-activity relations.

SIMCA is a multivariate chemometric method based on principal components that has been applied to a variety of chemical problems. Dunn et al. (4) used this method in their examination of the composition of PCBs mixtures, and Stalling et al. (5) used it to examine a limited number of samples containing PCDF. The latter study was based on data reported by Rappe et al. (6). These two reports described application of an advanced chemometric tool in residue studies and illustrated the use of pattern recognition to extract quantitative information about sample similarity.

The discovery of PCDF and PCDD residues in aquatic and terrestrial ecosystems has provided an impetus for increased environmental and toxicological studies. Determination of sources, transport mechanisms, fate and biochemical effects are key elements in assessing their environmental significance.

The state of knowledge about TCDD, PCDDs, and PCDFs in the environment was recently reviewed by experts assembled at a "Planning Workshop on Bioavailability of Dioxins" convened by the U.S. Environmental Protection Agency. An excerpt from a draft report of the meeting (7) states: "Data on the impact of dioxin [on ecosystems] are essentially nonexistent, and relatively few data are available describing the effects of these chemicals on single species ... Toxic effects of [dioxins and related compounds] in actual aquatic ecosystems have not been studied."

The contamination of the Great Lakes with polychlorinated biphenyls poses the potential for co-contamination from PCDFs as a consequence of their pyrolytic conversion from PCBs (8). In addition, PCBs as manufactured are known to contain mg/kg concentrations of PCDFs. Few environmental data exist on the occurrence of PCDFs in relation to PCBs or TCDD. No extensive investigations have been reported in which PCDF and PCDD residue levels in fish and fish-eating birds were compared.

Concerns about these residues are based on the observations that PCDDs in fish and birds are composed primarily of highly toxic 2,3,7,8-substituted chlorodibenzo-p-dioxins of which 2,3,7,8--TCDD predominates. Data on the occurrence and concentrations of other 2,3,7,8-chlorine substituted congeners are presented here. It is therefore important that additional techniques be explored to extract information from the present research concerning the environmental behavior and effects of these chemicals.

Residues of PCBs and organochlorine pesticides have been studied intensively in fish-eating birds in the Great Lakes (9). We undertook analyses of fish-eating birds for PCDDs, PCDFs, and PCBs to gain some insight into the relative bioaccumulation of these chemicals. These data, when compared with the concentration of residues in fish, can provide some insight into important bioaccumulation factors.

METHODS

Sampling

Fish were collected at a number of locations in the Great Lakes from 1979 to 1982 as part of a survey of PCDFs, PCDDs, and PCBs in the United States, performed at the Columbia National Fisheries Research Laboratory. Details were reported by Stalling et al. (10). In general, samples analyzed represented pooled samples from three or four whole individual fish. Species studied included the common carp (Cyprinus carpio), coho salmon (Oncorhynchus kisutch), lake trout (Salvelinus namaycush), bloater (Coregonus hoyi), brown trout (Salmo trutta), and walleye (Stizostedion vitreum vitreum).

Eggs of Forster's tern (Sterna forsteri) were collected in 1983 from nests in two colonies in Wisconsin--one on Lake Poygan and the other at Oconto Marsh, Green Bay--as part of a study on impaired reproduction. This study was carried out cooporatively with the of the University of Wisconsin Sea Grant Program (H.J. Harris) and the U.S. Fish and Wildlife Service (T.J. Kubiak), with additional support from the Wisconsin Department of Natural Resources and the Green Bay Metropolian Sewer District.

Lake Poygan is a relatively clean lake whereas Green Bay is heavily contaminated from the Fox River with many industrial chemicals--particuarly PCBs and chlorophenols, which are known sources of PCDFs and PCDDs. A decline in reproductive success and increased incidence of deformed young have been observed in the Green Bay Colony (11, 12). Samples of a black-crowned night heron (Nycticorax nycticorax) and double-crested cormorants (Phalacrocorax auritus) collected in the Green Bay vicinity were also analyzed.

Eggs of herring gull (Larus argentatus) were obtained in 1983 from 8 of the 13 colonies on the Great Lakes regularly monitored for organochlorine compounds by the Canadian Wildlife Service as part of the Great Lakes Water Quality Program (9). Ten randomly collected fresh eggs were homogenized and subsamples of equal weight were pooled for analysis.

Analysis of PCBs

Residues of PCB in extracts of tissue and egg samples were enriched by using a combination of gel permeation chromatography on BioBeads S-X3 and 1:1 (v/v) cyclohexane:methylene chloride. Adsorption column chromatography on silicic acid was used to separate PCBs from other co-extractives and contaminants (13). Isomer specific analyses were made by temperature-programmed electron capture detection with a C_{87}-hydrocarbon glass capillary

column. We calibrated 105 individual isomers by using an equal weight mixture of Aroclor 1242:1248:1254:1260. (The last two digits of the Aroclor number designate the percentage chlorine in the macerial.) We determined molar response factors from a flame ionization detector using the computer-based calculation methods described by Schwartz et al. (14).

After determining the concentrations of individual isomer, we retrieved the data from the MUMPs based laboratory data base, and transferred them to an IBM-XT (IBM Corporation, Boca Raton, Florida 33432) by way of an RS-232 link, using the program Cyber (Department of Linguistics, University of Illinois at Champaign-Urbana, Urbana, IL). For principal components analysis we used SIMCA-3B for MS-DOS based microcomputers (Principal Data Components, 2505 Shepard Blvd., Columbia, MO 65201).

Analysis of PCDFs and PCDDs

The method for determination of PCDDs and PCDFs in biological samples described by Smith et al. (15) was used for the analysis of samples of fish, tern eggs and whole terns. Samples were spiked before extraction with 50 ng/kg each of ^{13}C-2,3,7,8-TCDD, ^{37}Cl-2,3,7,8-TCDF, and ^{13}C-octachlorodibenzo-p-dioxin (OCDD). In routine high resolution-gas chromatography/low resolution-mass spectrometry, we used a 30-m DB-5 capillary column. We conducted isomer specific analyses of PCDDs by using the method of Buser (16), using either a 60-m Silar 10C or an SP2330 capillary column.

In analyzing herring gull eggs, we used the procedure developed by Norstrom and Simon (17) with modifications to improve recovery of higher chlorinated dioxins. Following extraction, the acetonitrile partitioning step was replaced with gel permeation chromatography on Biobeads SX-3 using 1:1 (v/v) hexane:methylene chloride and an Autoprep 1002A GPC instrument (Analytical Biochemistry Laboratories, Columbia, MO 65201). Following the preliminary alumina column cleanup, the HPLC-GPC and sodium methoxide treatment were discarded. After Florisil column chromatography, the combined 25:75 hexane:methylene chloride and methylene chloride fractions were evaporated near dryness, taken up in 0.5-1 ml of hexane and chromatographed on 1 g of acid alumina (Fisher A948, 80-200 mesh activated at 190 C] packed into a Pasteur pipet. This step ensured that traces of PCBs were removed. The first 8 ml eluate was discarded [100:1 (v/v) hexane:methylene chloride] and PCDDs were eluted with 8 ml of 1:1 hexane: methylene chloride which was evaporated near dryness and transferred to a 100 microliter Reacti-vial with toluene. The final volume was adjusted to 25 microliters.

TCDD-OCDD were determined by HRGC/LRMS using a 30 m DB-5 fused silica column (J&W Scientific) in a Hewlett-Packard 5987 instrument equipped with a a splitless injector. Isomer specific identification of peaks was accompolished using a 60 m SP-2330 fused silica column (Supelco). Precision of the method was established by triplicate analysis of a Herring gull egg homogenate from Lake Ontario. These results are summarized in Table 1.

Table 1. Reproducibility of Three Analyses of a Composite Sample of Herring Gull Eggs Collected in 1983 from Snake Island, Lake Ontario. Values for TCDD and OCDD results were corrected for recovery by using an internal standard.

Time of analysis and statistic	2,3,7,8-TCDD ng/kg	2,3,7,8-TCDD % Recovery ^{13}C-TCDD	1,2,3,7,8-Penta-CDD ng/kg	1,2,3,6,7,8-Hexa-CDD ng/kg	OCDD ng/kg	OCDD ^{13}C-OCDD %Recovery
Jan. 84	81	81.2	8	11	14	52.3
May 84	98	100.5	12	27	28	101.2
May 84	91	70.8	9	13	31	74.0
Mean	90.0	84.2	9.7	17.0	24.3	75.8
SD	8.5	15.0	2.0	8.7	9.1	24.5
%SD	9.5	17.9	21.5	51.3	37.3	32.3

Principal Components Analysis

We transferred data from the analysis of the samples to an IBM-XT computer in the form of a linear array, using the program FINP, or transferred them from the laboratory data base (14) and examined them by calculating principal components sample scores (Thetas) and variable loading terms (Betas), using the program CPRIN from the SIMCA-3B programs. Sample similarity was determined by calculating sample scores (θ-theta values; see Equation [1]). The likeness of samples can be assessed by the relation of samples in plots derived from principal components models. The statistical technique of cross-validation (18) was used to determine the number of components that were statistically significant.

The calculations involved in principal components are summarized in Equation [1]. The objective was to derive a model of a data set having *k*

$$X_i = \overline{X}_i + \sum_{a=1}^{A} B \cdot \theta + E_i \qquad [1]$$

samples and *i* variables in which the concentration or value of any measured value, X_{ik}, could be calculated. The principal component term is the product of θ_{ka} and B_{ai}, where θ_{ka} (Theta) is designated the a^{ht} component "score" for sample k, and B_{ai} (Beta) is designated as the "loading" for variable *i* in principal component *a*. The term $\overline{X}_i$ is the mean of variable *i* in all samples. The residual term (or unexplained part of the measurement not modeled) is designated E_i, and "A" describes the number of principal components extracted from the data. A more detailed discussion of this approach was presented by Dunn *et al.* (4).

RESULTS and DISCUSSION

Analyses of PCDDs, PCDFs, and PCBs can create large data sets that are difficult to interpret because there are 75 PCDDs, 135 PCDFs, and 209 PCBs. Isomer compositions may vary widely due to differential partitioning or metabolism of compounds. In addition, residue profiles in the biota may differ locally because of wide variations in effluents, combustion, or other source inputs. Chemometric methods can greatly improve the analyst's ability to describe and model residues in these diverse samples.

The power of principal components modeling of multivariate data such as those encountered in these complex mixtures, originated from graphical presentations of sample similarity, as well as from statistical results calculated by the SIMCA-3B programs (19). Sample data are treated as points in higher dimensional space, and projections of these data are made in two- or three-dimensional space in a way that preserves most of the existing relations among samples and variables (19). This feature is especially helpful in visualizing data of more than three dimensions.

An example of the use of principal components calculations for describing PCB data (Figure 1) should serve to illustrates this approach. Four replicate samples of Aroclors 1242, 1248, 1254, and 1260, and an equal weight mixture of each Aroclor were analyzed by gas chromatography for 105 individual PCB isomers. Three replicate analyses of a used transformer oil were also made.

The concentration data obtained from each analyses were expressed as fractional parts and normalized to sum to 100. The normalized data were statistically analyzed and three principal components (A=3) were calculated. The sample scores (Theta-1, Theta-2, and Theta-3) in each component were used to plot the samples in a 3-D graph (Figure 1). The inset plot in the upper right quadrant of the 3-D figure, which represents the 3-D data as viewed parallel with the Z-axis, shows that much of the sample information may be discerned from the 2-D plot (X-axis = Theta-1, Y-axis = Theta-2). However, it is not possible to determine from the 2-D plot whether the used-oil sample was composed of more than three Aroclors.

The 3-D figure preserves more than 95% of the sample variance of the entire data set. From this plot one can learn that PCB mixtures of two Aroclors form a straight line, that three Aroclor mixtures form a plane, and that possible mixtures of the four Aroclors are bounded by the intersection of the four planes. Samples not bounded by the four planes, or located in the space formed by their intersection, may be derived from mixtures of Aroclors, but are not identical with them. The locus of the points representing used oil samples showed that the sample was composed of about 80% Aroclor 1260, 10% 1254, and 5% each of Aroclors 1242 and 1248 (4).

Quality control aspects of the analysis are reflected in the nearly identical proximity of each of the replicate analysis. Broader use of this statistical technique to examine sample residue profiles from different locations could lead to improved understanding of complex mixtures of contaminants and related problems.

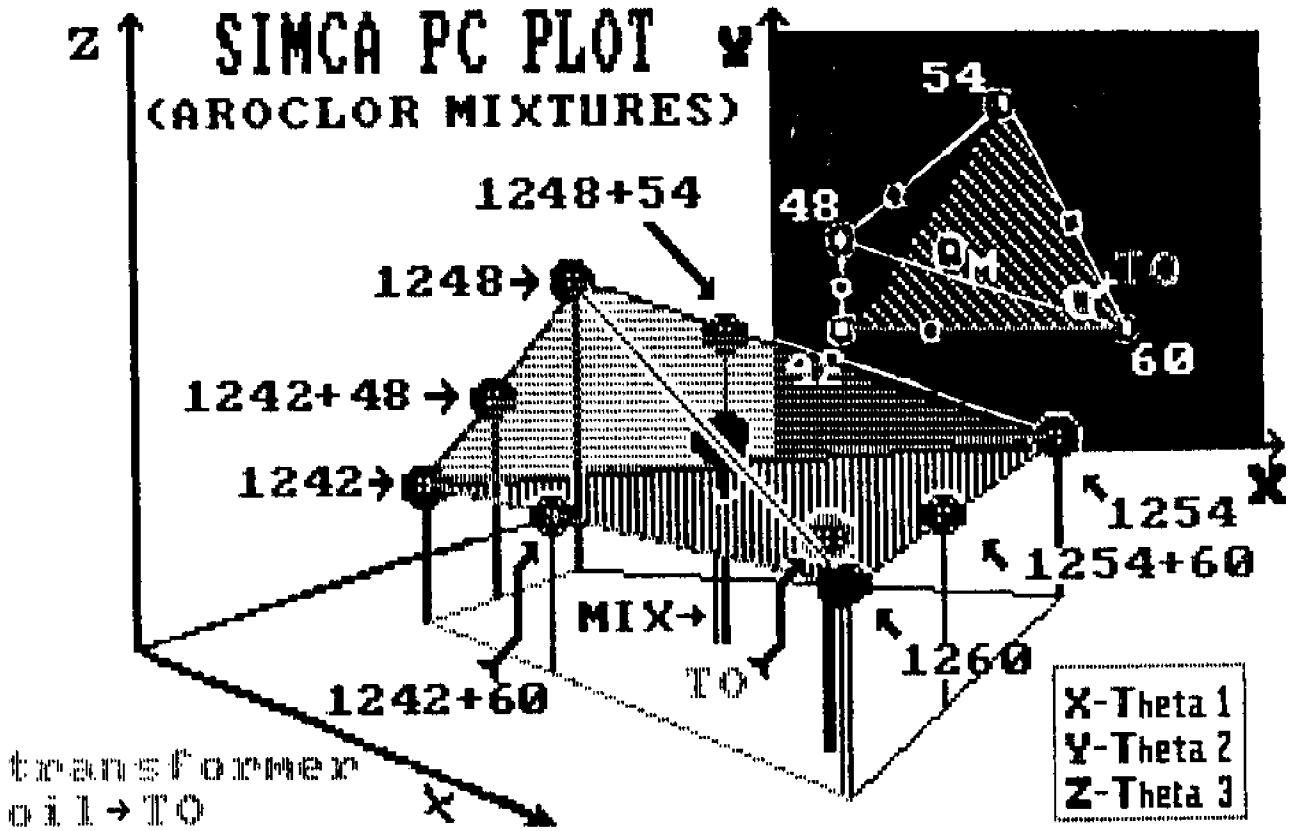

Figure 1. Three Dimensional Plot of Principal Components Scores (Theta-1,-2,-3) Representing Normalized Isomer Composition Data for Aroclors 1242, 1248, 1254, 1260, a 1:1:1:1 Mixture (MIX) of Each Aroclor, and Used Transformer Oil Containing PCBs (TO). The points represent four analyses for each Aroclor and three analyses for the transformer oil. The plot in the upper right quadrant is the view parallel to the Z-axis.

Residues in Eggs of Forster's Tern and Birds of Green Bay

Recently, in and around Green Bay, a decline in reproductive success has been observed in colonies of Forster's terns and common terns and crossed-bill syndrome has been observed in cormorants and herons have been found dead or moribund (11,12). U.S. Fish and Wildlife specialists, Green Bay, Wisconsin, requested that the Columbia National Fisheries Research Laboratory conduct PCB, PCDD, and PCDF analyses on birds and eggs. Because 2,3,7,8-TCDD is teratogenic, its presence in the birds was of special concern.

The samples from the two Wisconsin locations were also analyzed for

individual PCB isomers by the method described by Schwartz et al. (14). Residue levels for the total PCB content (Table 2) represented the sum of the individual PCBs present in the sample.

For the SIMCA analyses, the individual PCB isomer concentrations were normalized to sum to 100. Then the data were examined by using the SIMCA-3B program to calculate principal components and plot sample scores, in a manner identical to that discussed for the Aroclor mixtures. The plot of sample data illustrated that residue profiles differed in the geographic locations examined (Figure 2).

The PCBs in eggs of Green Bay birds were similar to Aroclor 1254 and the total PCB concentrations were about 6 times greater than those in eggs from Lake Poygan. Residue compositions in Lake Poygan eggs were less consistent and tended to lie farther from a line between Aroclors 1254 and 1260 (Figure 2). We found that the geographical origin of the samples (Lake Poygan or Green Bay) could be determined from the normalized residue data with a certainty of 0.85. In classifying the samples we used the SIMCA-3B program "CLASSI."

Two Aroclor 1260 standards (A_1 and A_2) were included in these analyses. One standard was from the Columbia National Fisheries Research Laboratory, and the other from the Patuxent Wildlife Research Center (U.S. Fish and Wildlife Service, Laurel, MD). A difference of about 30% in the concentration of one constituent was responsible for the small difference observed between the two Aroclor 1260 standards (Figure 2.)

The analysis of 13 samples of tern eggs revealed that concentrations of PCDDs and total PCBs in samples were elevated in samples from the Green Bay colony where reproductive problems had been observed (Table 2). Analysis of moribund herons and cormorants collected from Green Bay and Lake Michigan revealed similar patterns of PCDDs and PCDFs (Table 3). Morbidity of these birds had reportedly increased.

PCBs of particular concern were isomers without chlorine atoms in the ortho- ortho'-positions of the biphenyl molecule--e.g., 3,3',4,4',5-pentachloro-biphenyl. These PCBs induce aryl hydrocarbon hydroxylase liver enzyme activity at concentrations approaching those observed to induce such activity following exposure of laboratory rats and mice to PCDD and PCDF isomers having chlorine atoms in the 2,3,7,8-positions of the dibenzo-p-dioxin and dibenzofuran molecules (20).

Although concentrations of total PCDFs were greater in eggs from Green Bay than in those from Lake Poygan, concentrations of individual PCDFs were consistently near the limit of detection (1-5 ng/kg); consequently determinations of specific PCDF isomers were not made. The concentrations of both PCDDs and non-ortho, ortho'-chlorine substituted PCBs were significantly higher than those of PCDFs in Green Bay.

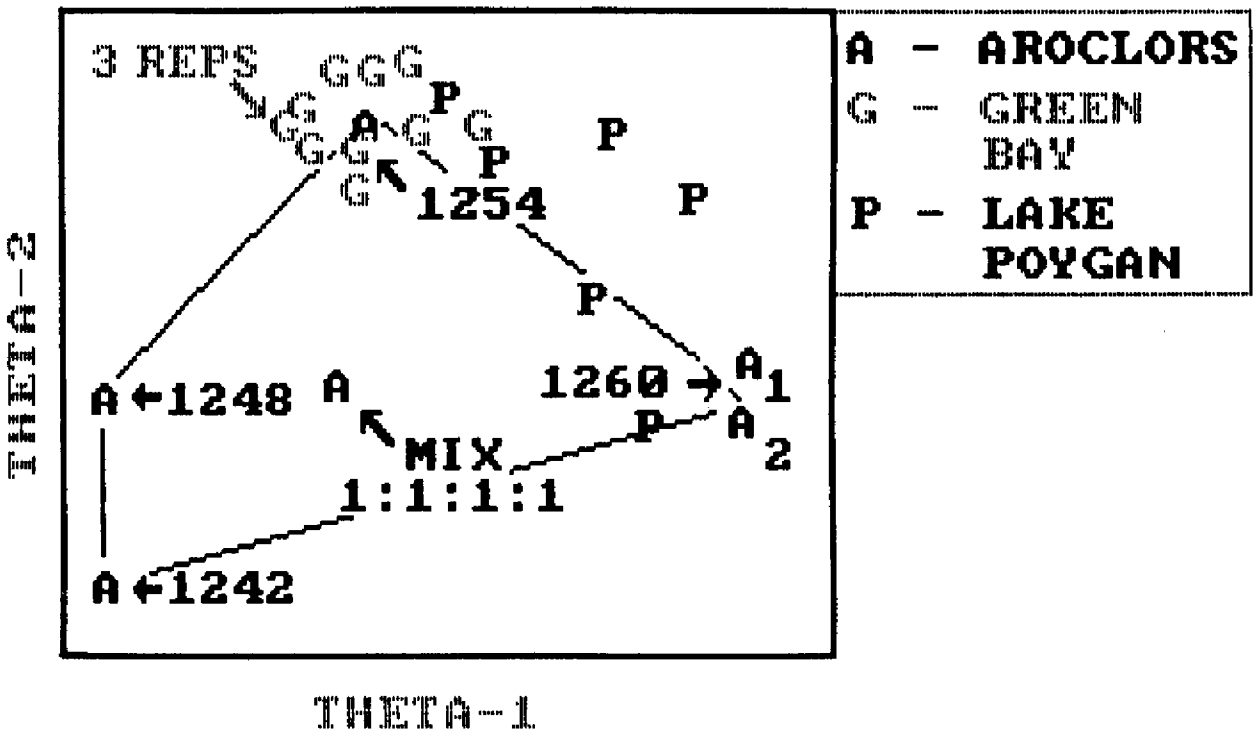

Figure 2. Principal Components Plot Derived from Analysis of Aroclor Standards, Their Equal Mixture, and Forster's Tern Eggs.

Table 2. Mean Residues (SD in parentheses) PCDDs, PCDFs, and PCBs in Eggs of Forster's Terns from Two Colonies in Wisconsin.

	PCDDs (ng/Kg)		Total	PCBs	
	2,3,7,8-	Total	PCDFs	Total	non-o,o'
Location	TCDD	PCDDs	(ng/kg)	(mg/kg)	(ug/kg)
Green Bay	47 (35)	114 (46)	19[1] (6)	39 (17)	11 (15)
Lake Poygan	9 (7)	21 (13)	9[2]	7 (3)	4 (8)

[1]Analysis of 6 eggs
[2]Analysis of 2 eggs

Residues of PCDDs in these tern eggs and in one bird sample subjected to isomer-specific analyis were composed almost entirely of the following compounds: 2,3,7,8-TCDD; 1,2,3,7,8-pentachloro-isomers; 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachloro-isomers; both heptachloro-isomers; and octachloro-dibenzo-p-dioxin.

Table 3. Residue Levels of PCDDs, PCDFs, and PCBs in Fish-Eating Birds from Green Bay and Lake Michigan.

Bird Species Year[1]	PCDDs (ng/Kg) 2,3,7,8-TCDD	PCDDs (ng/Kg) Total PCDDs	PCDFs (ng/Kg) 2,3,7,8-TCDF	PCDFs (ng/Kg) Total PCDFs	PCBs Total (mg/kg)	PCBs non-o,o' (ug/kg)
Night Heron (1978)	59	188	8	8	115	0.4
Night Heron (1978)	12	88	nd[2][2]	nd[20][2]	19	1.2
Night Heron (1982)	21	214	4	53	NA[4]	1.2
Cormorant[4] (1983)	4	25	2	10	NA	1.0

[1]Black crowned night heron and couble-crested cormorant.
[2]Detection limit shown in brackets.
[3]NA=analysis not performed.
[4]Average of three samples of nestlings from Spider Island and Gravelly Island, Lake Michigan.

In a sample of sediment from the Fox River, tetra- through hexachloro-dibenzofurans were present in higher concentrations than the corresponding PCDDs. The questions of bioavailability of PCDFs and PCDDs from these sediments and the composition of these residues in fish consumed by the birds have yet to be addressed.

These data suggest that the rate of metabolism of PCDFs was higher than that of PCDDs in birds, or that the relative proportions of PCDFs in the diet was much lower in these birds than in any Great Lakes fishes or sediments yet analyzed. Histopathology studies of in cormorant nestlings are being made at the request of Fish and Wildlife personnel, Green Bay Field Office, to determine if the reproductive problems can be related to the presence of these residues.

Residues of PCDDs in Herring Gull Eggs

Data for the eight colonies of herring gulls monitored in 1983 are presented in Figure 3 as 2,3,7,8-TCDD and the sum of 1,2,3,7,8-pentachloro- and 1,2,3,6,7,8-hexachloro-dibenzo-p-dioxin, the only 2,3,7,8-substituted isomers found in each congener group. Nearly all concentrations of hepta-chloro- and octachloro-dibenzo-p-dioxin were below the 10 ng/kg limit of quantitation and were therefore not included.

In general, the sum of higher chlorinated PCDD residue levels was similar to that of 2,3,7,8-TCDD, except in Saginaw Bay and Lake Ontario. Concentrations of 2,3,7,8-TCDD ranged from 9 to 26 ng/kg in eggs from colonies of Lakes Superior, Michigan, Huron, and Erie, and 90 and 141 ng/kg respectively in eggs from colonies of Lake Ontario and Saginaw Bay. Higher chlorinated PCDDs were also higher in eggs from Saginaw Bay than in those from other areas.

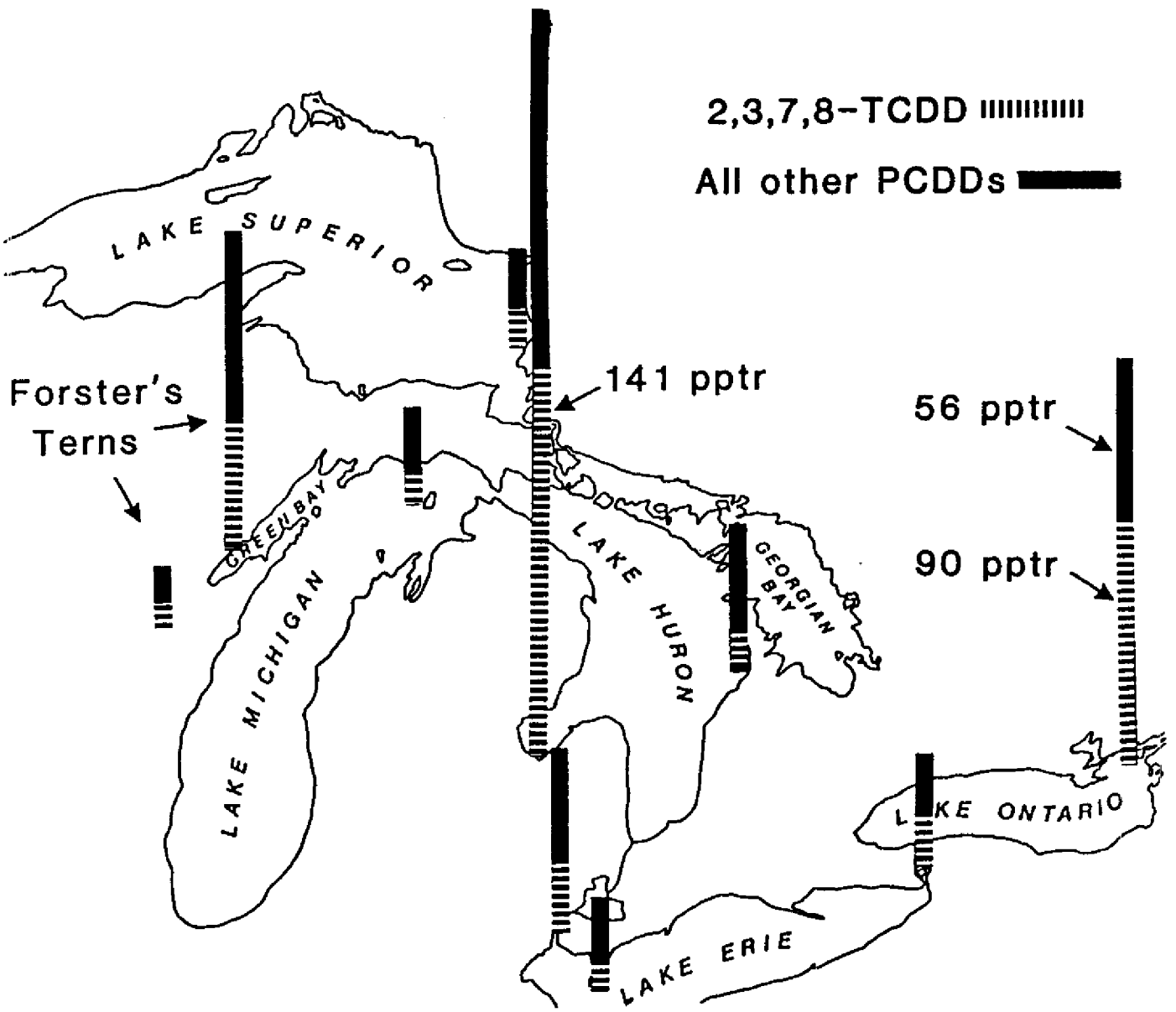

Figure 3. Residues of Chlorinated Dibenzo-p-dioxins in Herring Gull and Forster's Tern Eggs Collected from the Great Lakes Region. (pptr = ng/Kg).

The methodology used for the determination of PCDDs in herring gull samples (17) was unsuitable for ultra-trace PCDF determination because low-level interferences remained after cleanup. Preliminary comparison of this method with that reported by Smith et al. (15) indicated that these interferences were removed by the carbon enrichment technique, allowing trace determination of all PCDDs and PCDFs in the same sample by electron impact-GC/MS. An additional benefit was the removal of interferences from methoxy-polychlorodiphenyl ethers (PCDPEs) and methoxy-PCBs in the determination of PCDDs. A carbon enrichment column step consequently should be introduced immediately after the gel permeation step. Future analyses of Herring gull samples will include PCDFs as well as PCDDs.

Principal component analysis was used to determine if residue profiles were similar at different geographical locations or if temporal trends could be detected in pattern changes. We examined the concentrations of TCDD, penta-CDD, and hexa-CDD for the period 1981-1983, using the SIMCA-3B program, after these variables were summed and expressed as fractional parts. Two principal components were extracted from the data. A letter designating the origin of the sample was plotted at the point corresponding to the Theta-1 and Theta-2 coordinates (Figure 4). A plot of the variable loadings (Beta values) revealed that high relative concentrations of 2,3,7,8-TCDD were responsible for the clustering of Lake Ontario and Niagara River samples. Most of the other samples clustered together, with the exception of the 1983 Niagara River and 1982 and 1983 Detroit River samples. These samples clustered separately because of similar levels of tetra- and hexa PCDDs, and low relative levels of penta-CDD and these samples might be considered for reanalysis because their loci in the plot differ markedly from the main cluster of samples from the Detroit and Niagara rivers. Lake Ontario samples designated "Q" are replicates.

We have observed striking similarities in the occurrence of PCDD isomers in samples of herring gull eggs, tern eggs, and adult herons from all five Great Lakes. The same 2,3,7,8-chlorine substituted isomers are present: 2,3,7,8-tetrachloro-; 1,2,3,7,8-penta-chloro-; 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin. The ratios or composition of these isomers are somewhat similar, with the largest variation being associated TCDD.

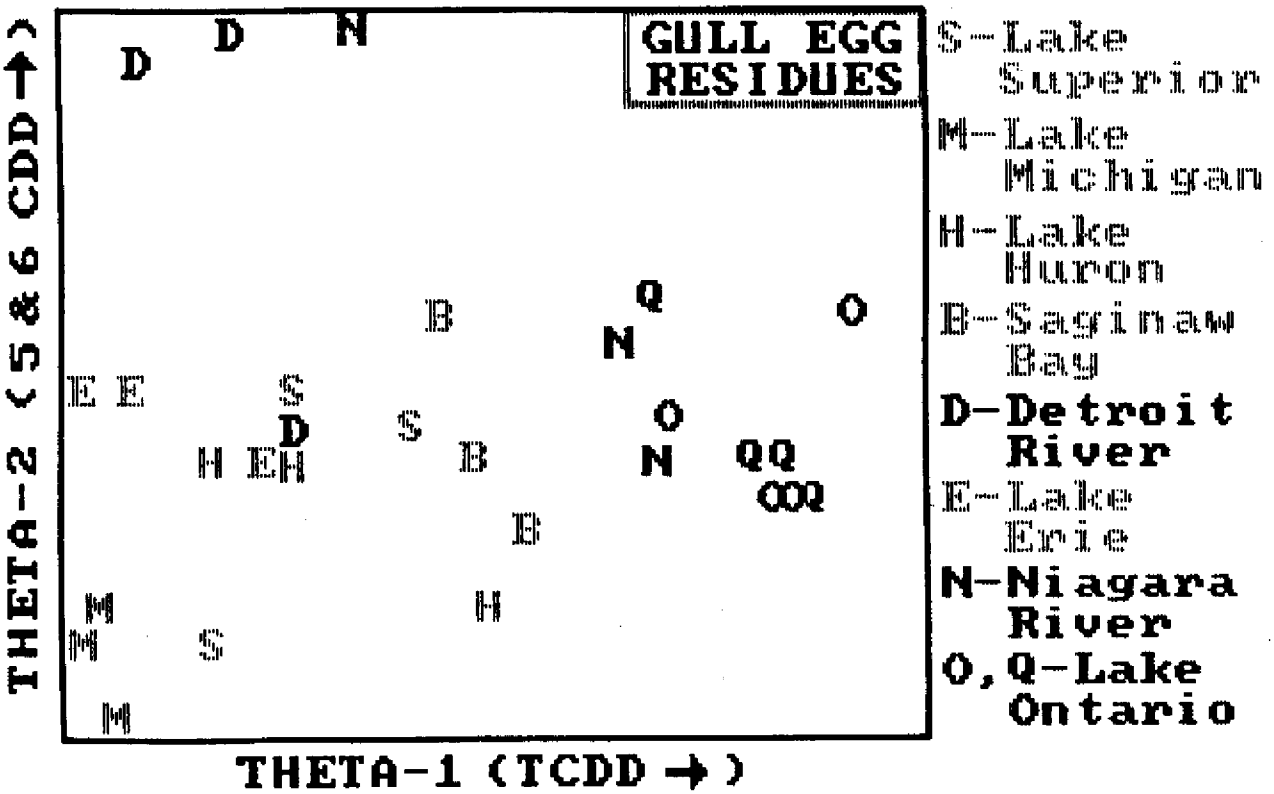

Figure 4. Principal Components Plot Derived from Data Representing the Normalized Dioxin Residue Levels for TCDD, Total Penta-, and Hexachlorodibenzo-p-dioxins in Herring Gull Eggs Collected from the Great Lakes Region.

PCDD and PCDF Residues in Great Lakes Fish

The distribution of PCDFs and PCDDs in fish from the Great Lakes (Figure 5) was determined as a first step in evaluating the impacts that these toxic contaminants might have on the various Great Lakes fish stocks when toxicity data for the various compounds are available. A more detailed treatment of statistics related to this analysis is beyond the scope of the present discussion.

Concentrations of PCDFs and TCDD were seemingly greater in samples from regions having large-scale chemical production or hazardous waste disposal facilities. Fish samples analyzed from Lake Siskiwit--an island lake in Isle Royal National Park, Lake Superior that does not receive significant input of contaminants other than by rainfall or atmospheric deposition (22)--contain no detectable residues of TCDD and had the lowest concentrations of PCDFs in all samples measured.

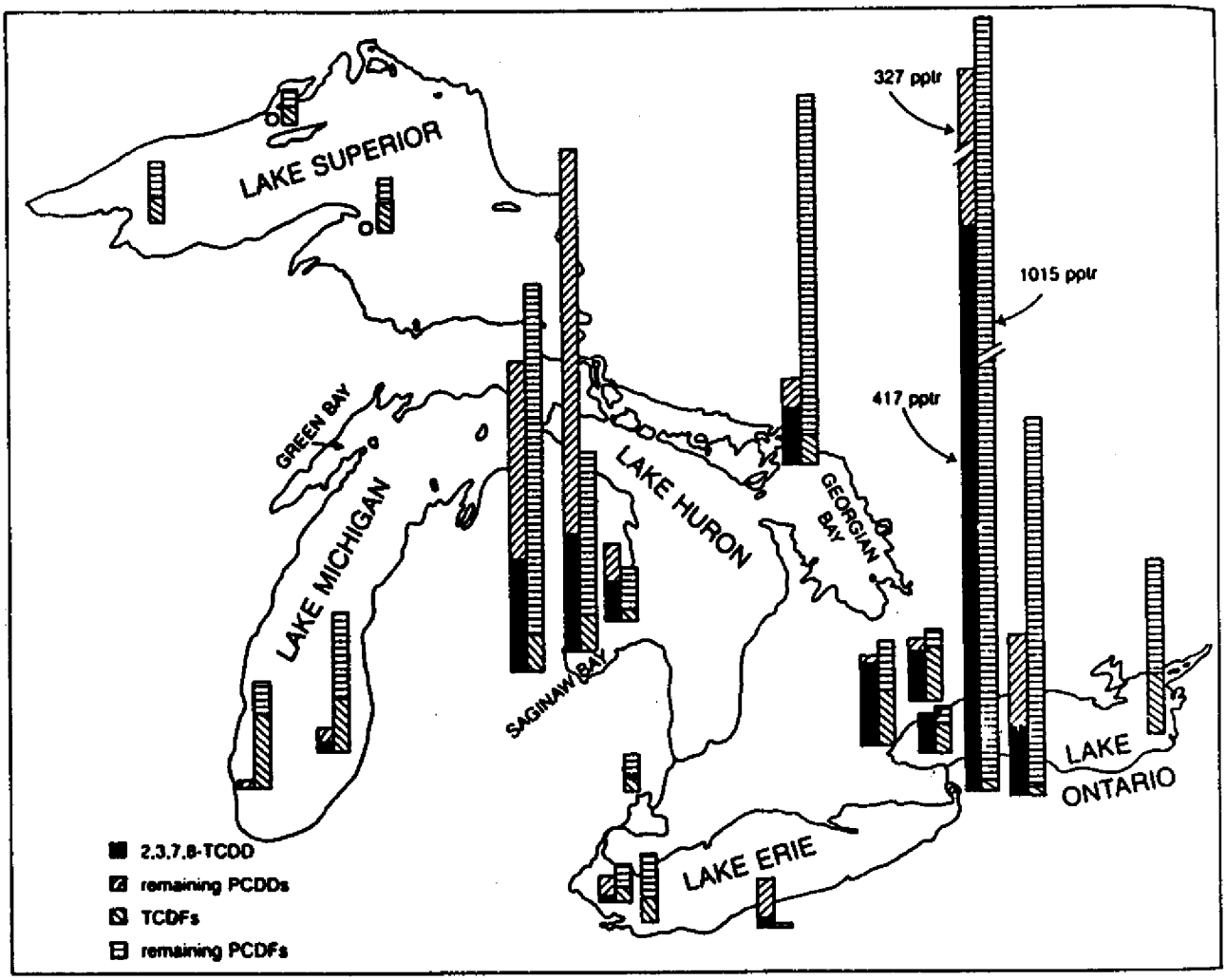

Figure 5. Distribution and Concentration of PCDDs and PCDFs in Fish from the Great Lakes.

These analyses identified the major PCDD and PCDF isomers in fish as those having 2,3,7,8-chlorine substitution. In all samples, 2,3,7,8-TCDD was the major isomer observed and the PCDFs detected in samples resembled the isomer distribution present in human liver samples from Yusho patients (21). PCDF residues in the limited number of fish samples subjected to isomer-specific analysis were composed primarily of 2,3,7,8-TCDF and 2,3,4,7,8-pentachlorodibenzofuran (10).

SUMMARY

Concerns with chlorinated dibenzo-p-dioxins and dibenzofurans in the biota are based on the observations that PCDDs and PCDFs in fish and birds are composed primarily of the highly toxic 2,3,7,8-tetrachloro-dibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and other 2,3,7,8-chlorine substituted penta- and hexachlorodibenzo-p-dioxins and -dibenzofurans. The residue concentrations of 2,3,7,8-TCDD in fish and birds are greatest in regions in which chlorinated organic chemical manufacturing facilities or hazardous waste sites are located. PCB isomers with no chlorine substitution in the ortho, ortho'--positions were observed in excess of ug/kg concentrations and their toxicological impact should be explored because they show structure-activity relations similar to those of 2,3,7,8-chlorine substituted PCDFs and PCDDs.

The patterns of PCDDs and PCDFs in fish and birds suggest that isomers with 2,3,7,8-chlorine substitution are preferentially bioaccumulated or biomagnified. These findings support the need for isomer specific analyses, especially for the 2,3,7,8-substituted congeners, to more adequately assess the hazard of these contaminants.

The compositions of PCDD and PCDF residues in fish and birds of the Great Lakes are simple in comparison with those of likely source materials, such as chlorophenol and PCB wastes. The PCDFs and PCDDs produced from municipal or chemical waste combustion are even more complex. More information is required on the environmental fate of these complex mixtures to enable the association of sources with residues in the environment.

It has been shown that pattern recognition techniques may be applied to the characterization of complex environmental residues. More extensive use of isomer specific analysis, when combined with chemometric techniques, should provide a better insight into sources and permit a quantitative understanding of how the composition of these chemicals change as they pass through the food chain and are distributed in the environment.

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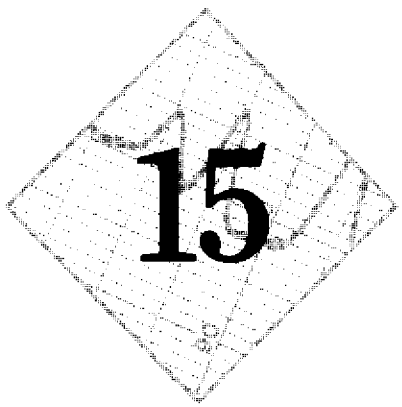

Polychlorinated Biphenyls: Congener-Specific Analysis of a Commercial Mixture and a Human Milk Extract

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Polychlorinated Biphenyls: Congener-Specific Analysis of a Commercial Mixture and a Human Milk Extract

Stephen Safe,* Lorna Safe, and Michael Mullin

On the basis of the relative retention times and response factors of all 209 synthetic polychlorinated biphenyls (PCBs), this paper reports the first congener-specific analysis of a commercial PCB preparation, Aroclor 1260, and the PCB composition of a human milk extract. The analysis indicates that Aroclor 1260 contains nearly 80 different PCB congeners with the major components identified as 2,2',3,4',5',6-, 2,2',4,4',5,5'-, 2,2',3,4,5,5'-, and 2,2',3,4,4',5'-hexachlorobiphenyl and 2,2',3,3',4,4',5-, 2,2',3,3',4,5,6'-, 2,2',3,4,4',5,5'-, and 2,2',3,4',5,5',6-heptachlorobiphenyl. In contrast, the major PCB components of the human milk fraction were the 2,4,4'-tri-, 2,4,4',5-tetra-, 2,2',4,4',5-penta-, 2,3',4,4',5-penta-, 2,2',3,4,4',5'-hexa-, 2,2',4,4',5,5'-hexa-, 2,2',3,3',4,4',5-hepta-, and 2,2',3,4,4',5,5'-heptachlorobiphenyls. The significance of congener-specific PCB analysis is discussed in terms of the structure–activity effects on PCB persistence, bioaccumulation, and toxicity.

Polychlorinated biphenyls (PCBs) are highly stable industrial chemical products that are synthesized by the direct chlorination of biphenyl. Commercial PCBs are distinguished by their stability and resistance to breakdown by acids, bases, oxidation, and reduction, their miscibility with numerous organic solvents, their nonflammability, and their excellent electrical insulation properties. Because of these highly desirable physical properties PCBs have enjoyed widespread use as industrial fluids, flame retardents, diluents, hydraulic fluids, and dielectric fluids for capacitors and transformers. Due to their widespread use, careless disposal practices, and environmental stability, PCBs have been widely identified in diverse environmental matrices including fish, wildlife, and domestic animals, rivers, lakes, and oceans and their underlying sediments, aquatic and marine flora, air, rain, and snow (Risebrough et al., 1968; Fishbein, 1972; Buckley, 1982; Ballschmiter et al., 1981; Wasserman et al., 1979). It was also apparent from several analytical studies that PCBs preferentially bioaccumulate in the food chain and residues are routinely detected in human adipose tissue, blood and human milk (Wasserman et al., 1979; Cordle et al., 1978; Holdrinet et al., 1977; Safe, 1982). Thus, the chemical stability of PCBs is paralleled by their environmental stability and potential for environmental transport, and it is evident from analytical surveys that PCBs are the

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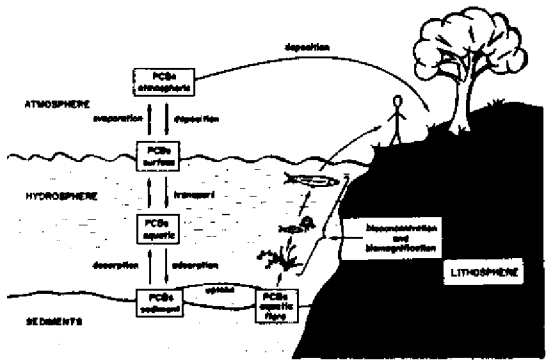

Figure 1. Environmental fate of PCBs.

most ubiquitous industrial chemical pollutant in the global ecosystem.

Numerous gas chromatographic studies using packed or capillary columns have confirmed the complexity of all commercial PCB formulations (Sawyer, 1978; Webb and McCall, 1976; Burse et al., 1983; Newton and Laski, 1983; Kerkhoff et al., 1982; Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Ballschmiter and Zell, 1980; Mullin et al., 1981; Albro et al., 1981; Albro and Parker, 1979). Some of the difficulties inherent in PCB analytical protocols are similar to those encountered in the analysis of related organochlorine pesticides and pollutants such as lindane, DDT, DDE, hexachlorobenzene, dieldrin, and related hexachlorocyclopentadiene-derived insecticides. Diverse extraction and cleanup procedures have been devised to preferentially remove coextractives that are present in different matrices and interfere with routine quantitative gas chromatographic (GC) and GC–mass spectrometric (MS) analysis. In contrast to the organochlorine pesticides, the qualitative analysis for PCBs presents several unique problems. Most analytical schemes for PCBs use the various commercial PCB preparations as quantitative reference standards. The PCB concentrations are estimated by comparing the relative intensities of several diagnostic peaks observed in the commercial reference standards and in the analyte. The accuracy in determining PCB levels is highly variable and matrix dependent; for example, the PCBs that are present in many waste industrial fluids or in (retro-filled) transformers usually resemble a specific commercial mixture and comparative GC analysis can yield accurate quantitative results. In contrast, gas chromatographic analysis of PCBs in extracts from diverse environmental matrices clearly indicates that these mixtures can be strikingly different from the commercial PCB analytical reference standards. These differences in composition reflect the major differences in the physical properties (e.g., water solubility and volatility) and biodegradability of the individual PCBs present in the commercial mixtures. Figure 1 illustrates some of the effects that would alter the composition of a commercial PCB preparation introduced as a pollutant into an aquatic or marine environment; these include physical partitioning between the water–sediment and water–air interfaces, sediment desorption processes, and biomagnification and bioconcentration with aquatic forms of life and the food chain. Thus, it is not surprising that the composition of PCB extracts from these environmental matrices can vary widely and often do not resemble any commercial mixture (Kerkhoff et al., 1982; Jenson and Sundstrom, 1974; Mullin et al., 1981; Hansen, 1979; Harvey and Steinhauer, 1974; Wolff et al., 1982). Quantitative analysis on these mixtures is usually determined by pattern or peak matching methods using artificially reconstituted mixtures of different commercial PCB formations. At best, these results are only semiquantitative estimates of the total PCB levels in these environmental samples.

High-resolution glass capillary GC analysis can provide a solution to some of these analytical problems. The high resolving power of coated silica or quartz capillary columns offers a method that can separate the PCBs present in most samples; the identities of the individual peaks must then be determined by using synthetic standards and by retention index addition methods (Ballschmiter and Zell, 1980). This latter technique predicts the relative retention times (RRTs) of specific PCBs and has been used to assign the structures of the individual PCB congeners present in diverse analytes (Kerkhoff et al., 1982; Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Ballschmiter and Zell, 1980; Mullin et al., 1981, 1984; Albro et al., 1981). This method relies on the RRT values that have been determined for the limited number of available synthetic PCB standards. However, accurate quantitation of the individual PCB components in a mixture can only be accomplished by comparing the observed RRT and peak height (or area) data for a PCB–containing extract and the RRT and molar (or weight) response factors for synthetic PCB standards.

It was apparent to us that high-resolution PCB analysis must not only incorporate a high-resolution separation method but must also provide results which will confirm identities and concentrations of each individual PCB present in any mixture (Mullin et al., 1981). Moreover, since several reports clearly indicate that the toxicity of PCBs are structure dependent (Poland and Knutson, 1982; Parkinson and Safe, 1981; Safe et al., 1982; Poland et al., 1979; Yoshimura et al., 1979; Goldstein et al., 1977), the capability for isomer-specific PCB analysis will be an important method for assessing the potential environmental and human health impact of PCBs. This analytical approach requires the synthesis and characterization of all 209 PCB congeners and determination of their GC RRTs and molar response factors. We have recently completed the synthesis of the 209 PCB congeners (Mullin et al., 1984), and these standards can now be used for congener-specific PCB analysis. This paper compares the composition of a human milk PCB extract with commercial Aroclor 1260, which is used as a low-resolution quantitative standard for the GLC quantitation of many PCB mixtures derived from environmental matrices.

EXPERIMENTAL SECTION

The individual PCB analytical standards were synthesized as described and the retention times and response factors were determined relative to the standard, octachloronaphthalene (Mullin et al., 1984, 1981). The high-resolution capillary gas chromatography was performed by using a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector. A 50-m fused silica capillary column (0.2-mm i.d.) coated with SE-54 (Hewlett-Packard) was used to separate the PCB isomers and congeners. The oven temperature was programmed at a rate of 1.0 °C min^{-1} from 100 to 240 °C. The injector and detector temperatures were 270 and 330 °C, respectively. Sample volume, 6.0 μL, was injected by using an automatic sampler with splitting in the injector (10:1 split ratio, vented from 0.75 to 1.75 min). The hydrogen carrier gas was held at a constant pressure of 2.25 kg cm^{-2} to give the optimized linear velocity ($\bar{u}$) at 100 °C of 45 cm s^{-1}. The retention times (RT) of the PCBs were expressed relative to that of the octachloronaphthalene (OCN, RT = 124.9 min) standard; the relative response factors (by weight)

Table I. Quantitative and Qualitative Analysis of PCBs in Aroclor 1260 and a Human Breast Milk Extract

congener name[a]	% in Aroclor 1260	% in Human Milk[b]	congener name[a]	% in Aroclor 1260	% in Human Milk[b]
PCB-018	0.12		PCB-118	0.49	6.5
PCB-017	0.05		PCB-134	0.35	
PCB-024	0.01		PCB-114		0.33
PCB-016	0.04		PCB-131	0.07	
PCB-029	0.02		PCB-122	0.12	0.53
PCB-026	0.02		PCB-146	1.3	1.9
PCB-028	0.04	8.8	PCB-153	9.6	12.
PCB-021	0.01		PCB-141	2.5	0.29
PCB-033	0.09	2.2	PCB-176	0.33	
PCB-053	0.04		PCB-137	0.22	0.87
PCB-022	0.01	0.65	PCB-130		0.59
PCB-045	0.07		PCB-138	6.5	10.
PCB-046	0.02	0.25	PCB-158	0.70	0.55
PCB-052	0.25	1.9	PCB-129	0.20	
PCB-043	0.02		PCB-178	1.2	
PCB-049	0.06	0.66	PCB-175	0.49	
PCB-048	0.29	0.37	PCB-187	4.5	1.5
PCB-044	0.11	0.78	PCB-183	2.3	1.4
PCB-037	0.04	2.9	PCB-128	0.47	0.33
PCB-042	0.04		PCB-167	0.16	0.85
PCB-041	0.25	1.3	PCB-185	4.1	0.11
PCB-040	0.03		PCB-174	5.5	0.39
PCB-100	0.02		PCB-177	1.9	0.61
PCB-074	0.03	11.	PCB-171+202	1.2	0.37
PCB-070+-076	0.15	0.61	PCB-156	0.45	4.87
PCB-095	2.7		PCB-173	0.06	
PCB-091	0.07		PCB-200	0.78	
PCB-056+-060	0.14	0.71	PCB-157		0.47
PCB-084	0.65		PCB-172	0.78	0.31
PCB-101	2.5	0.97	PCB-180	9.1	5.3
PCB-099	0.13	4.8	PCB-193	0.47	0.19
PCB-119		0.08	PCB-191	0.10	0.90
PCB-083	0.04		PCB-199	0.33	
PCB-097	0.45		PCB-170	6.8	5.3
PCB-087	0.45	0.82	PCB-201	2.9	0.85
PCB-085	0.13		PCB-203	3.1	0.79
PCB-136	1.4		PCB-196	2.5	0.18
PCB-110	1.7	1.0	PCB-189	0.15	2.4
PCB-154	0.02		PCB-195	3.1	0.31
PCB-082	0.11		PCB-207	0.08	
PCB-151	2.5	0.59	PCB-194	1.7	0.48
PCB-144+-135	1.5	0.51	PCB-205	0.11	0.06
PCB-107	0.03	0.31	PCB-206	0.85	0.24
PCB-149	7.4		PCB-209	0.06	0.09

[a] Congener names adapted from Ballschmiter and Zell (1980). [b] Human milk sample collected and extracted by the Michigan Department of Public Health under Cooperative Agreement CR807192 with the Large Lakes Research Station, U.S. Environmental Protection Agency.

for the PCBs were expressed relative to that of OCN (RRF = 1.0 for 1 ng of OCN) by using integrated peak areas. (note: RRF = (height of congener peak/concentration of congener peak)/(height of internal standard peak/concentration of internal standard peak). Table I summarizes the percent composition of a human milk PCB extract and Aroclor 1260. The analysis of Aroclor 1260 and the human milk PCB extract has previously been reported (Mullin et al., 1981) prior to the availability of all the PCB congeners as analytical standards. To assure the accuracy of the data, the total PCB concentration in the Aroclor 1260 was calculated and compared to the expected value (32 ng/mL found and 30 ng/mL expected), yielding 94% accuracy.

RESULTS AND DISCUSSION

Analytical Data. In a previous study 195 of the 209 PCB congeners have been separated by using a 50-m fused silica capillary column coated with SE-54 (Mullin et al., 1984). Five pairs of isomers, namely, 31/28, 56/60, 70/76, 203/196, and 135/144, and two pairs of nonisomeric congeners, 202/171 and 81/145, were not completely resolved by using this column, and several other separation methods are currently being investigated [note: the numbering scheme of Ballschmiter and Zell (1980) is used for PCB congener identification]. Preliminary results in our laboratory suggest that only isomers 31/28, 70/76, and 203/196 are components of the commercial PCBs and only the latter pair of octachlorobiphenyls are potentially persistent in the environment. In addition, isomers 31/28 and 203/196 can be separated on the capillary column although quantitation of the isomer pairs is not possible if both compounds are present.

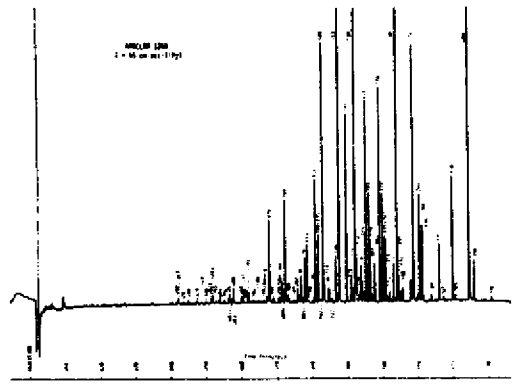

Figure 2. Congener-specific GC analysis of the commercial PCB, Aroclor 1260.

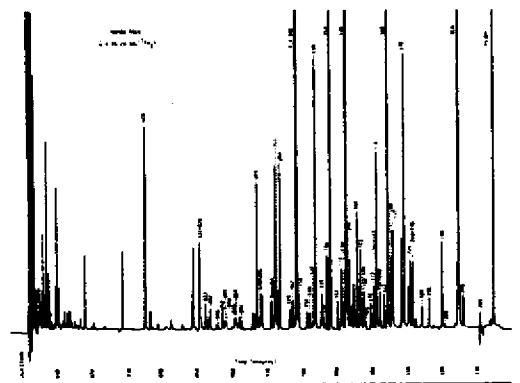

Figure 3. Congener-specific GC analysis of a human milk extract from the State of Michigan (note the occurrence of 2,2',4,4',5,5'-hexabromobiphenyl, which is present due to the PBB contamination in Michigan).

The isomer specific GC analysis of commercial Aroclor 1260 is illustrated in Figure 2 (note: the resolution of isomers 196 and 203) and represents the first such report for any PCB mixture. Figure 3 illustrates the high-resolution isomer-specific analysis of the PCBs extracted from a human milk sample obtained in the State of Michigan. This gas chromatogram does not resemble the pattern of any commercial PCB, and pattern matching methods would not yield meaningful quantitative results. However, the high-resolution isomer-specific GC approach permits quantitation of all the individual PCB components present in this mixture. Several PCB congeners, including 2,2',4,4',5,5'-hexa- (no. 153), 2,2',3,4,4',5'-hexa- (no. 138), 2,2',3,3',4,4',5-hepta- (no. 170), and 2,2',3,4,4',5,5'-hepta- (no. 180), are major components of both Aroclor 1260 and

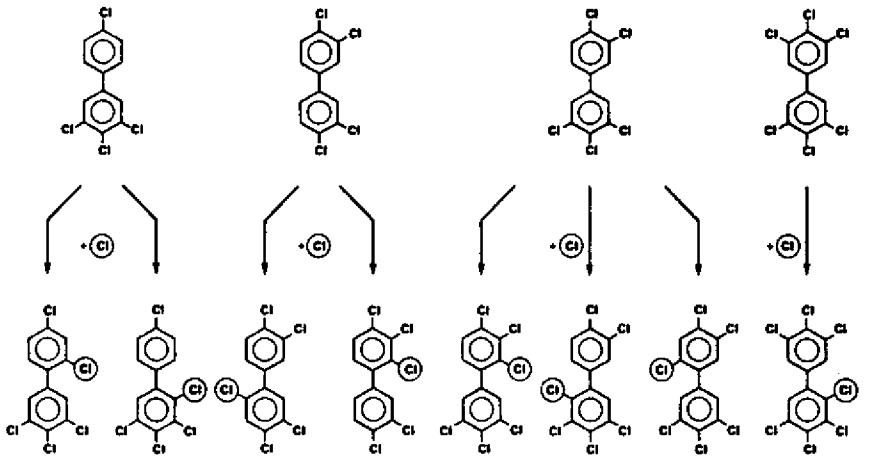

Figure 4. Structures of the most active coplanar PCBs and their mono-*o*-chloro-substituted analogues.

the human milk extract. These four PCB congeners possess several common structural features including (a) six or more chlorine atoms per biphenyl moiety and (b) the presence of only three different substitution patterns (i.e., 2,4,5-, 2,3,4,5-, and 2,3,4-) on both phenyl rings. PCBs no. 153 and 180 do not contain adjacent unsubstituted carbon atoms and are therefore resistant to metabolic breakdown (Matthews and Dedrick, 1984), and their persistence in human tissues is not unexpected. The results also suggest that the higher chlorinated PCBs (no. 138 and 170) that contain a 2,3,4-trichlorophenyl group are also resistant to metabolism and environmental breakdown and readily bioaccumulate in human tissues.

Another major PCB present in the human milk extract (4.87%), no. 156, is a minor component of Aroclor 1260 and other commercial PCBs (Jensen and Sundstrom, 1974; Ballschmitter and Zell, 1980) and has previously been identified as a major PCB contaminant of Japanese human milk extracts (Safe, 1982). The four remaining major PCB congeners identified in the human milk extract, no. 28, 74, 99, and 118, are minor components of Aroclor 1260 (<0.49% for all four isomers). It is likely that these pentatrichlorinated PCB congeners are derived from the lower chlorinated PCB formulations; however, it is noteworthy that with the exception of congener no. 28, all of these compounds also contain 2,4,5-trichloro substitution on one of the phenyl rings and a *p*-chloro group on the second phenyl ring. This high-resolution analytical study has also identified 2,4,4'-trichlorobiphenyl as a major PCB component and confirms a previous report that identified this compound in a Japanese human milk extract (Yakushiji et al., 1979). The reasons for the persistence of this congener are not apparent. It was also of interest to note that several other compounds including no. 95 (2.7%), no. 149 (7.4%), no. 185 (4.1%), no. 174 (5.5%), and no. 195 (3.1%) comprise 22.8% of the PCBs present in Aroclor 1260 but are minor components (0.81%) of the human milk PCB extract. With the exception of no. 195, all of these compounds possess a 2,3,6-trichloro- or 2,5-dichloro-substitution pattern on at least one of their phenyl rings, and because of the two adjacent unsubstituted carbon atoms rapid metabolic degradation of these congeners would be expected (Matthews and Dedrick, 1984). The lack of persistence of no. 195 is not clear; however, it has been reported for a series of hexachlorobiphenyl isomers that in some animals there is a decrease in tissue persistence of PCBs with increasing *o*-chloro substituents (Sparling and Safe, 1980). The effects of structure on the fate of PCBs in the environment, in biological samples, and in human tissues are currently under investigation in our laboratory, and this high-resolution analytical approach will play a critical role in delineating the environmental fate of individual PCB congeners.

High-Resolution PCB Analysis: Toxicologic Implications. The proposed mechanism of action of the toxic halogenated aryl hydrocarbons such as the PCBs and polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), naphthalenes (PCNs), and azobenzenes (PABs) has been derived from studies on the activities of the most toxic member of this group, namely, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related PCDDs (Poland and Knutson, 1982; Poland et al., 1979; Poland and Glover, 1980). Results obtained from several studies are consistent with a mechanism of action for the toxic halogenated aryl hydrocarbons in which the first step is their reversible binding to the cytosolic receptor protein. It was also apparent that three PCB congeners that are the most toxic, namely, 3,3',4,4'-tetra-, 3,3',4,4',5-penta-, and 3,3',4,4',5,5'-hexachlorobiphenyl (Kohli et al., 1980; Ozawa et al., 1979; Biocca et al., 1981; Marks et al., 1981; McKinney et al., 1976; Yoshihara et al., 1979; Silkworth and Grabstein, 1982) are minor to trace components of the commercial PCBs and are unlikely to be the sole contributors to the toxicity of these mixtures (Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Kamops et al., 1979).

Several previous studies have reported the correlation between the rank order of the AHH induction potencies and receptor binding avidities for the toxic halogenated aryl hydrocarbons and their toxicities (Parkinson and Safe, 1981; Poland et al., 1979; Safe et al., 1982), and this suggests that the two biologic assays can be used as indicators of toxicity. Our research initially focused on the activity of PCBs as inducers of AHH in immature male Wistar rats, and rat hepatoma H-4-II-E cells in culture and their binding affinities to rat hepatic cytosolic receptor protein (Safe et al., 1982; Bandiera et al., 1982; Sawyer and Safe, 1982).

A comprehensive study of all 209 PCBs was not feasible; however, several reports indicated that the most potent AHH inducers are substituted at both para and two or more meta positions. It was concluded that the introduction of *o*-chloro substituents would reduce biphenyl ring coplanarity and the AHH-inducing activity of the PCB congeners. These structural considerations suggest that the 4,4'-dichloro-substituted biphenyls, namely,

4,4'-di-, 3,4,4'-tri-, 3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta-, and 3,3',4,4',5,5'-hexachlorobiphenyl are the most likely PCB congeners that induce AHH. Initial studies confirmed that only four of these compounds (3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta-, and 3,3',4,4',5,5'-hexachlorobiphenyl) induce AHH and bind to the cytosolic receptor protein (Sawyer and Safe, 1982; Bandiera et al., 1982). The predictive potential of these two assays is confirmed by frequent reports that confirm the toxic potencies of the coplanar PCBs (Safe et al., 1982; Poland et al., 1979; Yoshimura et al., 1979). It is known that ortho-substituted biphenyls exhibit less coplanar conformational character due to steric interactions; thus it was assumed (Poland et al., 1979; Goldstein et al., 1977) that o-chloro substituted PCBs would not bind to the receptor protein and not elicit toxic and biologic effects mediated through this protein. We synthesized and tested all the mono-ortho derivatives of the four most active coplanar PCBs (see Figure 4), and the results confirm their AHH induction and receptor binding activities and clarifies the identities of those PCBs that are present in commercial Aroclors and exhibit the mixed-type enzyme induction properties (i.e., induction of PB plus MC type activity) comparable to those observed for Aroclors 1254 and 1260. The mono-ortho coplanar PCBs that have previously been identified in commercial PCBs include 2,3,3',4,4'-penta-, 2,3',4,4',5-penta-, 2',3,4,4',5-penta-, 2,3,3',4,4',5-hexa-, and 2,3,3',4,4',5,5'-heptachlorobiphenyl (Ballschmiter and Zell, 1980; Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Mullin et al., 1981; Albro et al., 1981) and two of these compounds, 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl, have been identified as major components of the human milk PCB extract. Although the toxicities of the mono-ortho coplanar PCBs have not been thoroughly investigated, it is apparent that the effects of many of these compounds resemble those reported for 2,3,7,8-TCDD. For example, 2,3',4,4',5-penta-, 2,3,3',4,4'-penta-, 2,3,3',4,4',5-hexa-, and 2,3,3',4,4',5'-hexachlorobiphenyls cause thymic atrophy in rats (Parkinson et al., 1983). 2,3,3',4,4'-Pentachlorobilphenyl administered to mice and rats results in a wasting syndrome (weight loss), edema, liver lipid accumulation, extensive hepatic damage, and splenic atrophy (Yamamoto et al., 1976); 2,3',4,4',5-pentachlorobiphenyl and Aroclor 1254 cause 100% embryo mortality in eggs from pullets receiving the PCB in their diet at a level of 20 ppm (Ax and Hansen, 1975), whereas several PCB congeners that do not induce AHH were inactive at the same dose level; administration of 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl to rats caused increased liver weights, increased liver lipids, and thymic atrophy (Yoshihara et al., 1979). Most of the mono-ortho coplanar PCBs induce AHH and cause thymic atrophy in responsive C57BL/6J mice but do not elicit these effects in the nonresponsive DBA/2J mice (Parkinson et al., 1982; Robertson et al., 1984).

These data indicate that most of the mono-ortho analogues of the coplanar PCBs elicit toxic effects that resemble (qualitatively) those caused by 2,3,7,8-TCDD, and some of these congeners (2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl) have been identified in Aroclor 1260 and the human milk PCB extract examined in this study. Future research should establish the quantitative contributions of this group of compounds to the toxic and biologic effects of commercial PCBs and the PCB residues that persist in human tissues.

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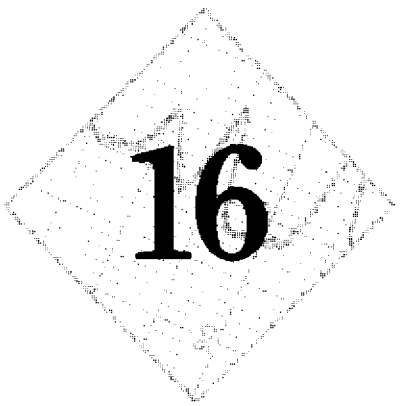

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Precision and Accuracy in the Determination of Organics in Water by Fused Silica Capillary Column Gas Chromatography/Mass Spectrometry and Packed Column Gas Chromatography/Mass Spectrometry

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Two general methods for the identification and measurement of organic compounds in water are compared. One method employs packed column chromatography and the other fused silica capillary column chromatography. The two gas chromatography/mass spectrometry (GC/MS) methods use different pH conditions for the liquid–liquid extractions with methylene chloride. Single laboratory total method accuracy and precision data are presented for over 80 analytes in water at the low parts per billion level. With the packed column method, 54% of the analytes have a mean recovery of 70% or more. With the capillary column method, 74% of the analytes have a mean recovery of 70% or greater. Both methods give mean relative standard deviations for concentration measurements of about 20%.

Concentration measurements with gas chromatography/mass spectrometry (GC/MS) are strongly identified with the selected ion monitoring (SIM) method of data acquisition. The scope, precision, and sensitivity of SIM have been thoroughly reviewed recently (*1*). In many applications, for example, the broad spectrum analysis of environmental samples (*2*) or the measurement of a long list of target compounds in a complex sample matrix, the preferred data acquistion method is the repetitive measurement of spectra as separated components emerge from the chromatograph. This technique requires a computer data acquisition system for the practical storage and retrieval of all the data. Storage and retrieval of complete mass spectra are required to allow their examination, which permits reliable identifications of the components separated from often complex mixtures. A number of investigators have recognized (*3–5*) the potential efficiency (cost effectiveness) of making concentration measurements with the same mass spectral data stored in the data system files.

The purpose of this report is to present data which define the scope and limitations of this type of simultaneous identification and measurement process. The scope and limitations are presented in terms of the total method precision and accuracy for a classical liquid–liquid partition, extract concentration, and repetitive scanning GC/MS analysis. The test compounds selected for study were from a group that are of environmental interest but are not sufficiently volatile for the inert gas purge and trap method (*5, 6*). The liquid–liquid extraction from water utilized methylene chloride, which was considered the general purpose solvent with the most favorable properties and fewest disadvantages (*7*). The GC/MS results were obtained with both conventional packed and wall-coated fused silica capillary columns. Two variations of the general method are designated method 625(8) and method 625.1 for convenient reference.

EXPERIMENTAL SECTION

Materials. The compounds used in this study were the highest purity materials available from commercial sources and were used without purification. Typically 100.0 mg of each compound was dissolved in a separate 100-mL portion of acetone to form a series of stock solutions. For benzidine and 3,3′-dichlorobenzidine the solvent was methanol; for most polycyclic aromatic hydrocarbons with a molecular weight greater than 260 the solvent was benzene. Mixtures of analytes were prepared by combining aliquots of the individual stock solutions and diluting the mixture with acetone. Generally aliquots in the range of 0.3–3.0 mL were diluted to 10 mL or 100 mL to give analyte concentrations in the range of 10–30 ng/μL. Some analytes which were mixtures of many compounds and several compounds which displayed poor chromatographic behavior were used at higher concentrations. Benzidine and 3,3′-dichlorobenzidine in methanol were added to the mixture immediately before use to preclude losses by reactions of these analytes with acetone. The standard solutions of mixtures of analytes were used for standard additions to the water matrix and to calibrate the GC/MS systems. All solvents were distilled in an all-glass apparatus within 24 h of their use. Reagent water was prepared by passing distilled water through a column containing about 1 lb of granular activated carbon.

Instrumentation for Method 625. Analyses were carried out with a Varian Model 1400 gas chromatograph interfaced to a Finnigan Model 3200 mass spectrometer with an all-glass transfer line and an all-glass jet-type enrichment device. All compounds except those of the phenolic group were chromatographed with a 1.8 m × 2 mm i.d. glass tube packed with 3% SP-2250 on 100/120 mesh Supelcoport. Compounds of the phenolic group were chromatographed with a 1.8 m × 2 mm i.d. glass tube packed with 1% SP-1240DA on 100/120 mesh Supelcoport. The columns were placed in the chromatograph oven so that the injection end was in contact with the septum to facilitate on-column injection which was used throughout the study. The injector temperature was maintained at 260 °C and the transfer line at 240 °C. The carrier gas was helium at a flow of 30 mL/min. The spectrometer sweep was controlled by an interface to a Digital Equipment Corp. Model PDP-8/E computer operating under revision E0 of the System 150 operating system. Data acquired were stored in disk files under the disk operating system and displayed via a cathode ray rube (CRT), a printer, or an incremental plotter. All data were collected at a nominal 70 eV in the electron impact ionization mode. The temperature of the ion source was unknown but probably exceeded 70 °C.

Instrumentation for Method 625.1. Analyses were carried out with a Finnigan Model 9610 gas chromatograph interfaced to a Finnigan Model 4000 mass spectrometer. All compounds were chromatographed with a 28 m × 0.25 mm i.d. fused silica capillary column coated with SE 54. The column was extended through the interface oven and into the spectrometer ion source slightly without intercepting the electron beam. The chroma-

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Table I. Accuracy and Precision for Measurements of Substituted Benzenes with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), μg/L	R	mean recovery, %	S, %	C(M), μg/L	R	mean recovery, %	S, %
1,2-dichlorobenzene	146	3.8	5	105	122	10	7	61	10
1,3-dichlorobenzene	146	3.8	5	50	31	10	7	58	6.1
1,4-dichlorobenzene	146	13.3	7	37	4.6	10	7	68	11
1,2,4-trichlorobenzene	180	3.8	5	86	23	10	7	76	11
1,2,3-trichlorobenzene	180	4	8	58	9.9	not measured			
1,3,5-trichlorobenzene	180	4	8	40	5.8	not measured			
1,2,3,4-tetrachlorobenzene	214	4	8	59	12	not measured			
1,2,3,5-tetrachlorobenzene	214	4	8	50	9.1	not measured			
pentachlorobenzene	248	4	8	55	9.8	not measured			
hexachlorobenzene	284	3.8	5	73	18	10	7	98	24
nitrobenzene	123	3.8	5	91	40	10	7	6	6.5
2,4-dinitrotoluene	165	13.3	7	35	3.1	10	7	79	11
2,6-dinitrotoluene	165	3.8	5	70	21	10	7	116	19

tograph was equipped with a Grob-type splitless injector which was used throughout the study. The injector and interface oven temperatures were maintained at 260 °C. The carrier gas was helium at a linear velocity of 28 cm/s. The spectrometer sweep was controlled by an interface to a Data General Model Nova 3 computer operating under revision 3.1C of the Finnigan-Incos operating system. Data acquired were stored in disk files under the disk operating system and displayed via a CRT or a printer-plotter. All data were collected at a nominal 70 eV in the electron impact ionization mode. The temperature of the ion source was 220 °C.

Procedure for Method 625. Eight 1-L aliquots of reagent water were placed in a series of 2-L separatory funnels. To each of seven aliquots was added 0.2 mL of the mixed standard solution of analytes in acetone. The eighth reagent water aliquot was carried through all subsequent operations as a reagent blank. Alternatively, for some analytes, 2.0 mL of the mixed standard solution of analytes in acetone was added to 10 L of reagent water in a 10-L separatory funnel. After being mixed, 1-L aliquots of the solution were placed in a series of seven 2-L separatory funnels. A separate portion of reagent water was used for the reagent blank. The detailed procedure for extraction and analysis of the water samples has been published (8). Briefly, the analytes were twice partitioned between pH adjusted water and methylene chloride by mixing the two liquid phases in the separatory funnel. The first partition was at pH 11, the second at pH 2, and the methylene chloride extracts were analyzed separately. Both extracts were dried by passing them through a column of purified anhydrous sodium sulfate, concentrated to a low volume with a Kuderna–Danish apparatus, and analyzed by on-column injection into the packed chromatographic columns described under Instrumentation for Method 625. Before analysis, a known quantity of the internal standard anthracene-d_{10} was added to each extract to give a concentration approximately the same as the expected concentrations of the analytes. An aliquot of the extract obtained at pH 11 was injected into the SP-2250 column at 50 °C. This temperature was held for 3 min, allowing the solvent to elute, and then programmed at 8 °C/min to 265 °C. Mass spectrometer data acquisition began with the initiation of temperature programming. The spectrometer was scanned repetitively from 45 to 460 amu at 8 ms/amu, and with a settling time of 2.3 ms/amu, the scan time was 4.3 s/spectrum. The 265 °C temperature was held for approximately 25 min or until the last analyte eluted. An aliquot of the extract obtained at pH 2, which contained the phenols, was injected into the SP-1240DA column at 60 °C. This temperature was held for 3 min, programmed at 6 °C/min to 190 °C, and then held until the last analyte eluted. Again data acquisition began with the initiation of temperature programming.

Procedure for Method 625.1. Mixtures of analytes in reagent water were prepared substantially the same as described under Procedure for Method 625. The extraction procedure was also the same with the single major exception that the first set of methylene chloride–water partitions was at pH 7, the second at pH 2, and the methylene chloride extracts were combined before concentration and analyzed together. Three internal standards were added to the dried concentrated extract before analysis. The internal standards were naphthalene-d_8, anthracene-d_{10}, and chrysene-d_{12}, and they were added to give concentrations approximately the same as the expected concentrations of the analytes. An aliquot of the extract was injected into the fused silica capillary column at 40 °C. This temperature was held for 4 min, and then programmed at 10 °C/min to 275 °C. Mass spectrometer data acquisition began with the initiation of temperature programming. The spectrometer was scanned repetitively from 45 to 460 amu at 0.95 s/spectrum and with a 0.05 s pause at 45 amu. The 275 °C temperature was held for 30 min or until the last analyte eluted.

Calibration and Quantitation. Methods 625 and 625.1 employed very similar single-point calibration procedures. To the mixed standard solutions in acetone, which were used to prepare the water solutions, were added quantities of the internal standards so that the concentrations of analytes and standards were the same or very similar. These calibration standards were analyzed by using the GC/MS conditions described in each procedure. Response factors were computed from the quantitation ion peak areas and the quantities of analytes and internal standards injected. The quantitation ions used are shown in Tables I–VII. The response factors were used, along with the measured extract component peak areas and the known quantities of internal standards added to the extracts, to compute the concentrations of analytes in the water solutions. The response factors were always measured with 4 h of the extract analyses, and the concentrations in the calibration standards were always within a factor of 2 of the concentrations in the extracts. With this procedure the calibration error was minimized. The only difference in the calibration procedures for the two methods was that Method 625 used a single internal standard for all analytes, and Method 625.1 used three internal standards. The analytes in Method 625.1 were divided into three groups by chromatographic retention time, and one internal standard was used for each group. In all cases it was established that the ratio of analyte area to internal standard area was linear with respect to the amount of analyte injected at constant internal standard amount over the short concentration range of concern. With Method 625 quantitation ion peak areas, response factors, and water concentrations were determined by using a System 150 program called Superquan. Details of this program are available from the authors as a separate report. With Method 625.1 quantitation ion peak areas, response factors, and extract concentrations were determined by using the standard Finnigan-Incos program Quan.

Quality Control. A reagent water blank was extracted with each batch of samples that was processed. Generally, no samples were analyzed unless this reagent water was free of all background signals from the measured compounds. In one batch of samples background signals at the quantitation ions for dibutyl phthalate, benzidine, and 3,3'-dichlorobenzidine were detected in the blank, and corrections were made in the measured concentrations of these compounds. The 70-eV mass spectrum of bis(perfluorophenyl)phenylphosphine (DFTPP) was measured at least once each working day by using the gas chromatographic inlet system.

Table II. Accuracy and Precision for Measurements of Substituted Phenols with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), μg/L	R	mean recovery, %	S, %	C(M), μg/L	R	mean recovery, %	S, %
phenol	94	8.3	7	25	7.4	20	7	42	2.3
2-fluorophenol	112	8.3	7	36	11	20	7	55	11
2-chlorophenol	128	8.3	7	51	17	20	7	72	11
2-nitrophenol	139	8.3	7	46	16	20	7	73	10
4-nitrophenol	65[a]	16.3	7	32	3.8	20	7	29	12
2,4-dichlorophenol	162	8.3	7	60	13	20	7	74	12
2,4-dimethylphenol	122	8.3	7	27	7.8	20	7	70	6.2
2,4-dinitrophenol	184	41.6	7	57	18	20	7	67	15
4-chloro-3-methylphenol	142	8.3	7	64	11	20	7	76	14
2,3,6-trichlorophenol	196	8.3	7	66	9.5	not measured			
2,4,6-trichlorophenol	196	not measured				20	7	78	16
3,4,5-trichlorophenol	196	8.3	7	76	4.9	not measured			
2,4,6-trimethylphenol	121	not measured				20	7	66	3.8
2-methyl-4,6-dinitrophenol	198	41.6	7	56	22	20	7	121	23
pentachlorophenol	266	16.6	7	76	8.4	30	7	95	24

[a] The quantitation ion for Method 625.1 was 139.

Table III. Accuracy and Precision for Measurements of Polycyclic Aromatic Hydrocarbons with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), μg/L	R	mean recovery, %	S, %	C(M), μg/L	R	mean recovery, %	S, %
naphthalene	128	3.8	5	106	35	10	7	73	8
acenaphthylene	152	3.8	5	84	50	10	7	76	13
acenaphthene	154	3.8	5	76	20	10	7	91	21
fluorene	166	3.8	5	73	21	10	7	93	19
anthracene	178	3.8	5	75	16	10	7	94	5.9
phenanthrene	178	13.3	8	42	4.7	10	7	91	12
fluoranthene	202	3.8	5	84	15	10	7	99	21
pyrene	202	3.8	5	81	13	10	6	104	25
benz[a]anthracene	228	not measured				10	7	91	24
chrysene	228	3.8	5	89	13	10	7	102	15
benzo[a]pyrene	252	3.8	5	71	13	10	7	66	19
benzo[b]fluoranthene	252	13.3	8	35	7.3	10	7	114	29
benzo[k]fluoranthene	252	3.8	5	82	15	10	7	87	24
benzo[ghi]perylene	276	3.8	5	79	13	20	7	78	32
indeno[1,2,3-cd]pyrene	276	3.8	5	73	13	20	7	82	31
dibenzo[a,h]anthracene	278	3.8	5	79	12	10	6	121	41

The measured spectrum was compared with published criteria (9), and if the criteria were not met, the ion source and other mass spectrometer operating variables were adjusted until an acceptable spectrum was obtained. Chromatographic performance was evaluated with test mixtures of analytes on a daily basis, and no measurements were made unless satisfactory performance was attained.

RESULTS AND DISCUSSION

Tables I–VII show the test analytes used with Methods 625 and 625.1. The methods are defined in the Experimental Section, and the analytes are grouped in the tables according to their structures or other common features. Each table gives for each method and analyte the following information: (a) the quantitation ion; (b) the concentration made, C(M), in water; (c) the number of complete method replicates, R; (d) the mean recovery for the R replicates in terms of the percentage of the C(M); and, (e) the estimated standard deviation (S) of the percentage recovery.

Figure 1 shows the distribution of mean recoveries among Method 625 analytes. Of the 82 analytes tested with Method 625, 44 (54%) had mean recoveries of 70% or greater. Five analytes have zero recovery which is attributed to chemical reactions in water at pH 11. These analytes are all found in Table IV and are chlorinated hydrocarbon pesticides. With Method 625.1, 57 analytes (74%) had mean recoveries of 70% or greater, and the distribution of recoveries is shown in Figure 2. The five analytes which gave zero recovery with Method 625 were all recovered with Method 625.1. A few analytes that were measured at several concentrations were included in the above totals at each level. For several reasons not all analytes were measured with both methods, but 66 different analytes were tested with both methods.

Figure 3 shows the distribution of estimated standard deviations of the mean recoveries among Method 625 analytes. Of the 77 measurable method 625 analytes, 70 (91%) had an estimated standard deviation of 25% or less. Two dichlorobenzenes had particularly variable recoveries (Table I) and the third a low mean recovery. This is partly attributed to their volatility, which leads to losses during extract concentration, but mainly to imprecise peak area measurements caused by poor resolution from the solvent on the packed chromatography column. With Method 625.1, 66 (86%) of the analytes had an estimated standard deviation of the mean recovery of 25% or less (Figure 4). The dichlorobenzenes are well resolved from the solvent on the fused silica capillary column and demonstrated acceptable method precision. Some of the compounds with the most variable recoveries were the higher molecular weight polycyclic aromatic hydrocarbons (Table III), which may not be transferred efficiently and consistently to the column in the splitless injector system.

It is important to recognize that Method 625 was designed for use with two separate packed chromatography columns.

Table IV. Accuracy and Precision for Measurements of Chlorinated Hydrocarbon Pesticides with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (*m/z*)	*C*(M), μg/L	*R*	mean recovery, %	*S*, %	*C*(M), μg/L	*R*	mean recovery, %	*S*, %
hexachlorocyclohexane (α isomer)	109[a]	5	6	0		10	7	90	18
hexachlorocyclohexane (β isomer)	109[a]	6	10	91	23	10	7	95	7.3
hexachlorocyclohexane (γ isomer)	109[a]	7	6	0		10	7	87	12
hexachlorocyclohexane (δ isomer)	109[a]	6	10	72	27	10	7	92	4.9
1,1-bis(4-chlorophenyl)-2,2-dichloroethane (DDD)	235	10	6	97	6.8	not measured			
1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT)	235	10	6	82	9.7	10	7	85	8.6
1,1-bis(4-chlorophenyl)-2,2-dichloroethene (DDE)	246	10	10	87	18	10	6	104	22
aldrin	66[b]	10	6	86	3.9	10	7	90	5.6
endrin	81	10	10	0		10	7	66	48
dieldrin	79	10	6	96	5.2	10	7	108	20
heptachlor	100[c]	10	6	84	7.5	10	7	110	14
heptachlor epoxide	81[d]	10	6	93	5.9	10	7	119	22
chlordane (γ isomer)	373	not measured				20	7	93	13
endosulfan I	75[e]	6	10	0		10	6	87	51
endosulfan II	195	6	10	0		10	7	107	80
endosulfan sulfate	272	7	10	80	29	10	7	71	11
toxaphene	*f*	not measured				300	6	72	9.1

[a] The quantitation ion for Method 625.1 was 219. [b] The quantitation ion for Method 625.1 was 263. [c] The quantitation ion for Method 625.1 was 272. [d] The quantitation ion for Method 625.1 was 353. [e] The quantitation ion for Method 625.1 was 195. [f] The sum of ion abundance at masses 159, 161, 195, and 197 was used for this measurement (*10*).

Table V. Accuracy and Precision for Measurements of Phthalic Acid Esters, *N*-Nitroso Compounds, and Isophorone with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (*m/z*)	*C*(M), μg/L	*R*	mean recovery, %	*S*, %	*C*(M), μg/L	*R*	mean recovery, %	*S*, %
dimethyl phthalate	163	3.8	5	40	7.3	10	7	80	10
diethyl phthalate	149	3.8	5	73	15	10	7	118	13
di-*n*-butyl phthalate	149	3.8	5	89	16	10	7	70	14
n-butylbenzyl phthalate	149	3.8	5	71	17	10	7	88	10
di-*n*-octyl phthalate	149	3.8	5	81	15	10	7	70	30
di-2-ethylhexyl phthalate	149	3.8	5	89	8.3	10	7	85	6.4
N-nitrosodimethylamine	74	not measured				100	7	42	14
N-nitrosodi-*n*-propylamine	130	not measured				20	7	67	6.0
N-nitrosodiphenylamine	169[a]	3.8	5	68	14	10	7	89	13
isophorone	82	3.8	5	87	25	10	7	64	14

[a] Measured as diphenylamine because compound decomposes in the injection port.

Table VI. Accuracy and Precision for Measurements of Miscellaneous Halogenated Compounds with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (*m/z*)	*C*(M), μg/L	*R*	mean recovery, %	*S*, %	*C*(M), μg/L	*R*	mean recovery, %	*S*, %
hexachloroethane	117	3.8	5	76	23	10	7	55	6.2
bis(2-chloroethyl) ether	93	13.3	7	36	4.8	10	7	66	10
bis(2-chloroisopropyl) ether	45	not measured				10	7	67	23
bis(2-chloroethoxy)methane	93	13.3	7	39	5.3	10	7	67	15
hexachlorobutadiene	225	3.8	5	74	18	10	7	64	15
hexachlorocyclopentadiene	237	not measured				10	7	38	7.1
4-chlorophenyl phenyl ether	204	13.3	8	37	7.1	10	7	96	21
4-bromophenyl phenyl ether	248	3.8	5	71	16	10	7	89	21
1-fluoronaphthalene	146	4.0	8	53	5.6	not measured			
1-fluoronaphthalene	146	4.8	5	88	25	not measured			
1-fluoronaphthalene	146	13.3	7	41	4.3	10	7	76	7.9
2-chloronaphthalene	162	3.8	5	78	23	10	7	75	9.1

Table VII. Accuracy and Precision for Measurements of Substituted Biphenyls with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), µg/L	R	mean recovery, %	S, %	C(M), µg/L	R	mean recovery, %	S, %
4,4'-dibromobiphenyl	312	10	16	91	16	not measured			
4,4'-dibromobiphenyl	312	13.3	8	39	4.4	not measured			
4,4'-dibromobiphenyl	312	36	8	37	4.5	not measured			
4,4'-dibromooctafluorobiphenyl	296	4	8	47	4.2	not measured			
4,4'-dibromooctafluorobiphenyl	296	10	16	88	14	10	7	112	48
Aroclor 1221	188	91	8	61	10	not measured			
Aroclor 1248	294	not measured				50	7	88	5.8
Aroclor 1254	326	91	8	70	10	not measured			
4,4'-diaminobiphenyl(benzidine)	184	not measured				50	7	128	94
4,4'-diamino-3,3'-dichlorobiphenyl	252	33	8	43	14	50	7	154	67

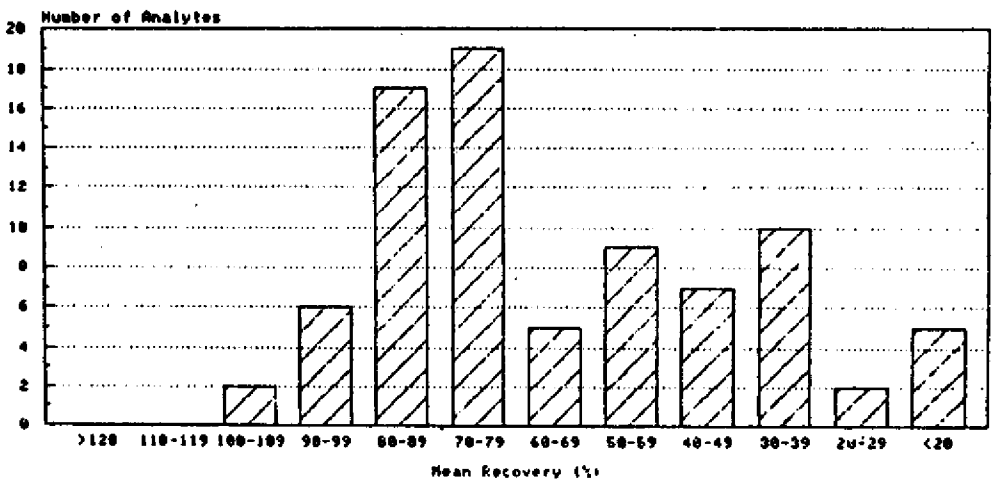

Figure 1. Distribution of mean recoveries with Method 625.

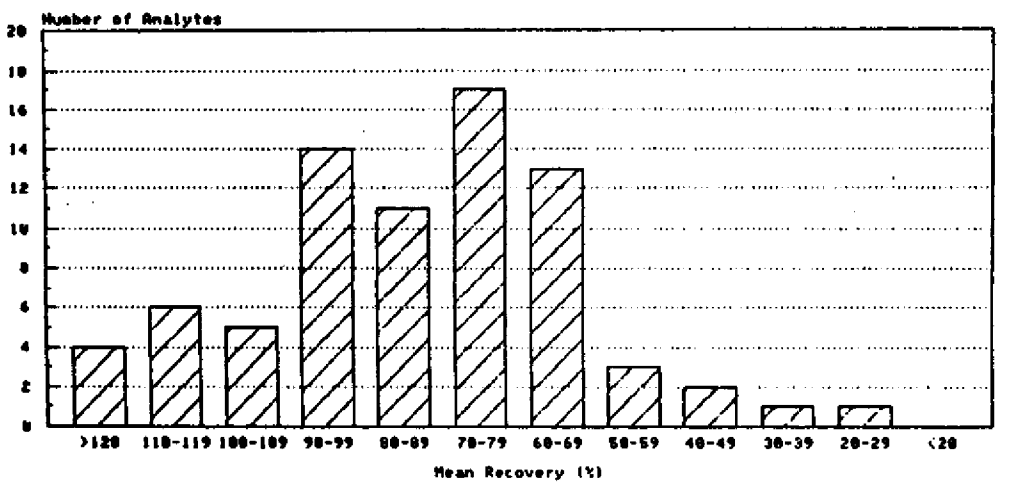

Figure 2. Distribution of mean recoveries with Method 625.1.

One column was selected for optimum separation of a group of phenols (Table II) and the other was selected for optimum separation of a variety of halogenated organics and aromatic compounds (Tables I, III–VII). Therefore, it was necessary to provide a chemical separation of these two groups before chromatography, and liquid–liquid extractions at pH 11 and pH 2 were selected to implement this broad class separation. With the development of capillary columns and particularly fused silica capillary columns capable of superb separations of the compounds in Tables I–VII, the broad class chemical separation became unnecessary. It was desirable to eliminate the pH 11 extraction because it was no longer necessary, and many organic compounds are sensitive to strongly basic conditions (*11*). Therefore, a pH 7 extraction followed by a pH 2 extraction was selected for Method 625.1. The pH 7 extraction minimizes the risk of acid and base catalyzed reactions of analytes. The pH 2 extraction ensures extraction of acidic phenols that may not be extracted efficiently at pH 7. An optional third extraction at pH 12 may be used for very basic compounds that are not extracted efficiently at pH 7.

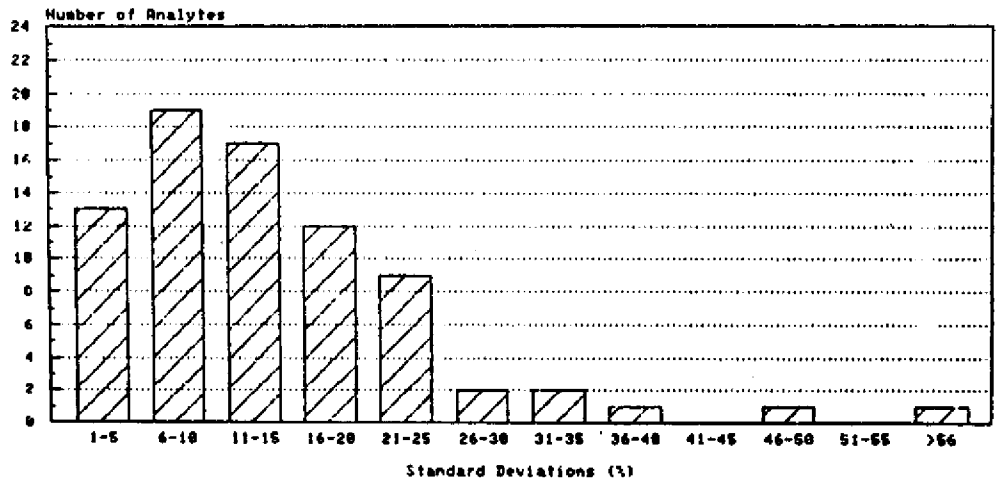

Figure 3. Distribution of standard deviations of mean recoveries with Method 625.

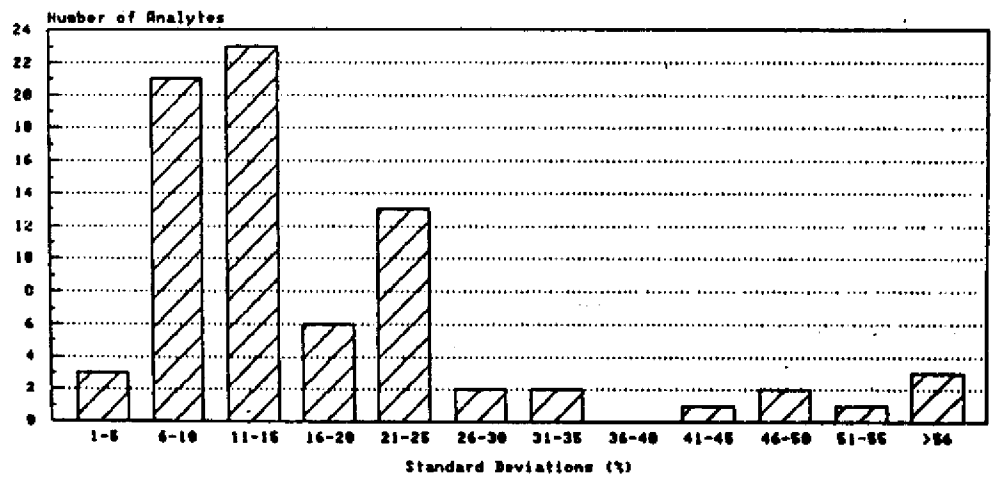

Figure 4. Distribution of standard deviations of mean recoveries with Method 625.1.

This option was not included in Method 625.1 but has been recommended (7). The combined extracts are then chromatographed with the fused silica capillary column.

The fundamental differences in the two methods led to several differences in the way the methods were implemented in this study. The test analytes were studied in batches, and more batches were used with Method 625 because of lower chromatographic resolution with the packed columns. Nearly all analytes were measured in two large batches with Method 625.1 Other significant additional differences were the use of on-column injection and a GC/MS interface enrichment device with Method 625, and the use of Grob splitless injection and no interface device with Method 625.1.

Method Bias. Methods 625 and 625.1 were designed to reveal the extraction and extract processing efficiencies and to allow an evaluation of method bias. With both methods the internal standard is added to the concentrated methylene chloride extract immediately before chromatography. It is assumed there is no bias during injection, chromatography, and mass spectral analysis because any losses of analytes should be compensated with the internal standard calibration technique. Therefore, the measured concentrations may be compared with the amounts added to the water samples to determine the mean percentage recovery or method bias. These values are in Tables I–VII as previously discussed. A major source of low recovery (and variability) is loss of relatively volatile analytes during extract concentration. This is most apparent in the recoveries of the dichlorobenzenes and trichlorobenzenes in Table I, the more volatile phenols in Table II, and isolated relatively volatile compounds in the other tables. Qualitatively there appears to be some improvement in the recoveries of these more volatile compounds with Method 625.1. This may be partly the result of more accurate peak area measurements which are made possible by far better resolution of the sample components from the solvent peak. It was not possible to measure *N*-nitrosodimethylamine (Table V) with Method 625 because its signal was obscured by the solvent peak; however, with Method 625.1 resolution was adequate for peak measurement, but losses during extract concentration are still apparent.

Five chlorinated hydrocarbon pesticides (Table IV) gave zero recovery with Method 625, but excellent recoveries with Method 625.1. This is attributed to the base sensitivity of the compounds since each chromatographs well on the packed column. The α and γ isomers of hexachlorocyclohexane each have two chlorines in a trans axial configuration with hydrogen which is a favorable arrangement for base-catalyzed dehydrochlorination. The δ isomer has similar relationships, but apparently reacts slower with base. Clearly Method 625.1 is the preferred method for the analysis of this group, especially for the γ isomer, which is the active pesticide component in mixtures of these isomers. Similar base sensitivity was ob-

served with the two endosulfan isomers, which are sulfurous acid esters with the sulfur and two oxygens part of a seven-membered ring. Endosulfan sulfate, which has the same general structure but sulfur in the higher oxidation state, was recovered in good yield from the basic solution of Method 625. Perhaps sulfur in the lower oxidation state is more susceptible to attack by hydroxide ion. Endrin, a stereoisomer of dieldrin, was not recovered from the basic solution of Method 625, but dieldrin was recovered in excellent yield.

The compound 4,4'-diaminobiphenyl (benzidine, Table VII) was not measured with Method 625 because its standard solutions in acetone were found to be unstable. Benzidine slowly reacts with acetone to form a monoimine that is readily recognized by its mass spectrum, which contains an intense molecular ion at mass 224 and an M − 15 ion at mass 209. In time, a second compound appeared which was identified as the bis(imine) by its similarly characteristic mass spectrum. Solutions of benzidine in methanol are stable, and these were used in the preparation of water samples for Method 625.1. Nevertheless, the recovery of benzidine and its 3,3'-dichloro derivative were quite erratic as shown in Table VII. It has been reported (*12*) that benzidine is sorbed on sodium sulfate during the drying of organic extracts and is subject to thermal decomposition during Kuderna-Danish (KD) extract concentration. This was confirmed by processing three solutions containing 100 μg of benzidine in methylene chloride through the KD step only. Recoveries were 19, 36, and 69%, and when the drying plus KD step was tested, recoveries dropped to 7, 13, and 26%. It is concluded that benzidine and some of its derivatives are not viable analytes with these methods.

Qualitatively, Table II shows generally higher mean recoveries of phenols with Method 625.1. In addition to the improved resolution of the more volatile phenols from the solvent, a factor discussed previously, the use of a combined extract with Method 625.1 probably contributes to somewhat better recoveries. Some phenols are partitioned into methylene chloride at pH 11, and this reduces their recovery in the pH 2 extract with Method 625. In an independent study, approximately 12% of the 2,4-dimethylphenol and smaller amounts of 4-chloro-3-methylphenol were found in the pH 11 extract. These diversions are often neglected in routine applications of Method 625 and may account for some small part of the lower recoveries.

The compound *N*-nitrosodiphenylamine (Table V) decomposes in a hot gas chromatograph injection port and is measured as diphenylamine. Therefore, this compound cannot be unambiguously measured with the two methods unless they are modified to separate diphenylamine prior to gas chromatography. A similar problem exists with another potential analyte, the benzidine precursor 1,2-diphenylhydrazine, which decomposes to azobenzene in the injection port. Hexachlorocyclopentadiene (Table VI), an important intermediate in the synthesis of many chlorinated bicyclohydrocarbon pesticides, was found susceptible to decomposition at low concentrations in solvents unprotected from light. No data were obtained with Method 625, but when solutions were protected from light, the compound was measurable with Method 625.1. The low recovery of hexachlorocyclopentadiene may be partly accounted for by slow degradation during storage or processing.

In general, Methods 625 and 625.1 are least biased with the stable, higher molecular weight substituted benzenes, phenols, polycyclic aromatic hydrocarbons, chlorinated pesticides, phthalic acid esters, and other halogenated compounds. Two low recoveries of aromatic hydrocarbons reported in Table III with method 625 are assumed due to systematic errors because there are no obvious reasons why these two compounds should behave differently from the others of the group.

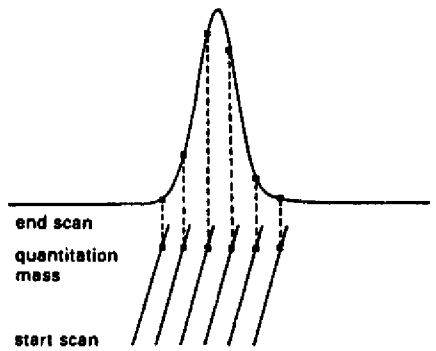

Figure 5. Schematic representation of peak area measurements with data acquisition by repetitive measurement of spectra.

Given this generality, the methods may be extended to numerous classes of compounds with similar properties. Overall Method 625.1 is qualitatively superior to Method 625 and provides generally improved recoveries, especially for phenols, base-sensitive compounds, and the more volatile compounds that are not well resolved from the solvent on a packed chromatography column. The fused silica capillary column used with Method 625.1 provides superior chromatographic resolution of components compared to a packed column, and this undoubtedly contributes to greater accuracy with complex environmental samples. The advantages of superior chromatographic resolution have been discussed by many authors (*1*).

Method Precision. The total method precision, as expressed in the estimated standard deviations of the percentage recoveries, is a function of the precision of many individuals method steps. Variabilities in the solvent extraction, extract concentration, and internal standard addition to the concentrated extract all contribute to the overall precision. One variable present in the GC/MS analysis using the repetitive measurement of spectra method of data acquisition is illustrated in Figure 5. The peak profile shown represents the real concentration of any component as it enters and leaves the ion source of the mass spectrometer. The series of diagonal line segments below the profile represents the repetitive sweep of the mass spectrometer through the 45–450 amu mass range. The points on the line segments represent the mass of the single ion used for the peak area measurement. The corresponding points on the real concentration profile represent the concentration at which the quantitation ion's abundance is measured. Since the mass spectrometer sweep is at regular intervals, but the concentration maximum may occur at any random time, there is no assurance that a quantitation ion's abundance will be measured when the maximum concentration of the substance is in the mass spectrometer. Therefore, peak areas, rather than peak heights, are used as a more consistent measure of compound concentration.

The precision of the area measurement depends on the number of points per peak, and the number of points depends on the spectrometer sweep time, the width of the peak, and the number of ions used to measure each compound. One quantitation mass was selected for each compound except toxaphene to minimize contributions from coeluting or background materials that generate ions with masses common to those in the spectrum of the analyte. With Method 625, the spectrometer sweep time of 4.3 s allowed at least six measurements of the abundance of the quantitation mass 99% of the time. Frequently seven or more points were obtained per peak since many peaks were 30 s wide at the base line. With Method 625.1, the spectrometer sweep time of 1 s allowed at least five measurements of the abundance of the quantitation ion.

It is very difficult to determine the extent that variations in area measurements affect total method precision, because area variabilities are not easily separated from variabilities in making standard solutions, syringe filling, injection, and chromatography. However, the sum of these variabilities can be compared to the total method precision, which includes the variations in extraction efficiency and extract processing. With packed columns and on-column injection, careful, experienced, and skilled operators achieve relative standard deviations (RSD) in the range of 2–7% for the measurement of response factors (*13*). With fused silica capillary columns, 1 s sweep times, and splitless injection RSDs in the range of 4–12% have been reported (*14*) for the measurement of response factors for most analytes. In the latter study the mean RSD was 11.4%, and this included a number of the same problem analytes such as *N*-nitrosodimethylamine, benzidine, etc. discussed in this paper.

For Method 625 the mean RSD for concentration measurements computed from the data in Tables I–VII is 21%. For Method 625.1 this estimate of mean total method precision is 20%. In computing these means the measurement s of four compounds with standard deviations greater than 67% were excluded. It appears that, on average, at least half of the total method variability results from the sample extraction and processing steps, and for some compounds, sample preparation may account for as much as 75% of the total method variability.

CONCLUSION

The accuracy and precision data presented in this paper serve as base lines for comparison of alternative analytical methods and the estimation of matrix effects. The reagent water data obtained with a single calibration point at nearly the same concentration as the analytes probably represents the maximum performance of the methods. With real environmental samples a multipoint calibration is used, and no significant loss in accuracy or precision is expected as long as the samples have matrices similar to the reagent water, that is, surface water, groundwater, and drinking water. Significantly different method performance is expected with samples that are highly contaminated with water-soluble organic solvents or other materials that dramatically change the sample matrix. In one reported study (*11*) Method 625 was used to measure some of the same analytes in reagent water and in a diverse group of wastewaters. Although many compounds showed depressed mean recoveries in wastewater, a surprising number did not, including some substituted phenols. The pH conditions of Method 625.1 are expected to reduce the emulsion problems associated with Method 625 and wastewater (*11*), and this should reduce the observed matrix effects for some compounds. The application of the method of standard additions or adoption of an isotope dilution calibration procedure (*15*) are approaches to improved accuracy and precision which could be implemented with these methods to overcome sample matrix effects.

The application of the selected ion monitoring (SIM) method of data acquisition could provide improved peak area measurement accuracy and precision. Also improved sensitivity is available with SIM, but the loss of qualitative information is significant and usually unacceptable. With repetitive measurements of complete mass spectra, more than one ion could be used for quantitative analysis, but this is not expected to improve sensitivity by more than 50% since one of the two ions is usually the base peak. Finally, method detection limits may be estimated by using the data in Tables I–VII and a published procedure (*16*). These methods detection limits generally fall in the range of 1–10 μg/L.

Overall, Methods 625 and 625.1 allow well-supported identifications of large numbers of compounds in water samples. However, the precision and accuracy of the concentration measurements are generally less than those which may be achieved with the selected ion monitoring method of data acquisition and isotope dilution calibration (*1*).

ACKNOWLEDGMENT

The authors express their appreciation to William Middleton, Jr., who provided excellent technical assistance in preparing standard solutions and conducting most of the sample processing prior to analysis.

Registry No. Water, 7732-18-5; 1,2-dichlorobenzene, 95-50-1; 1,3-dichlorobenzene, 541-73-1; 1,4-dichlorobenzene, 106-46-7; 1,2,4-trichlorobenzene, 120-82-1; 1,2,3-trichlorobenzene, 87-61-6; 1,3,5-trichlorobenzene, 108-70-3; 1,2,3,4-tetrachlorobenzene, 634-66-2; 1,2,3,5-tetrachlorobenzene, 634-90-2; pentachlorobenzene, 608-93-5; hexachlorobenzene, 118-74-1; nitrobenzene, 98-95-3; 2,4-dinitrotoluene, 121-14-2; 2,6-dinitrotoluene, 606-20-2; phenol, 108-95-2; 2-fluorophenol, 367-12-4; 2-chlorophenol, 95-57-8; 2-nitrophenol, 88-75-5; 4-nitrophenol, 100-02-7; 2,4-dichlorophenol, 120-83-2; 2,4-dimethylphenol, 105-67-9; 2,4-dinitrophenol, 51-28-5; 4-chloro-3-methylphenol, 59-50-7; 2,3,6-trichlorophenol, 933-75-5; 2,4,6-trichlorophenol, 88-06-2; 3,4,5-trichlorophenol, 609-19-8; 2,4,6-trimethylphenol, 527-60-6; 2-methyl-4,6-dinitrophenol, 534-52-1; pentachlorophenol, 87-86-5; naphthalene, 91-20-3; acenaphthylene, 208-96-8; acenaphthene, 83-32-9; fluorene, 86-73-7; anthracene, 120-12-7; phenanthrene, 85-01-8; fluoranthene, 206-44-0; pyrene, 129-00-0; benz[*a*]anthracene, 56-55-3; chrysene, 218-01-9; benzo[*a*]pyrene, 50-32-8; benzo[*b*]fluoranthene, 205-99-2; benzo[*k*]fluoranthene, 207-08-9; benzo[*ghi*]perylene, 191-24-2; indeno[1,2,3-*cd*]pyrene, 193-39-5; dibenzo[*a,h*]anthracene, 53-70-3; hexachlorocyclohexane (α-isomer), 319-84-6; hexachlorocyclohexane (β-isomer), 319-85-7; hexachlorocyclohexane (γ-isomer), 58-89-9; hexachlorocyclohexane (δ-isomer), 319-86-8; aldrin, 309-00-2; endrin, 72-20-8; dieldrin, 60-57-1; heptachlor, 76-44-8; heptachlor epoxide, 1024-57-3; chlordane (γ-isomer), 5566-34-7; endosulfan I, 959-98-8; endosulfan II, 33213-65-9; endosulfan sulfate, 1031-07-8; toxaphene, 8001-35-2; dimethyl phthalate, 131-11-3; diethyl phthalate, 84-66-2; di(*n*-butyl) phthalate, 84-74-2; *n*-butyl benzyl phthalate, 85-68-7; di(*n*-octyl) phthalate, 117-84-0; di(2-ethylhexyl) phthalate, 117-81-7; *N*-nitrosodimethylamine, 62-75-9; *N*-nitrosodi(*n*-propyl)amine, 621-64-7; *N*-nitrosodiphenylamine, 86-30-6; isophorone, 78-59-1; hexachloroethane, 67-72-1; bis(2-chloroethyl) ether, 111-44-4; bis(2-chloroisopropyl) ether, 39638-32-9; bis(2-chloroethoxy)methane, 111-91-1; hexachlorobutadiene, 87-68-3; hexachlorocyclopentadiene, 77-47-4; 4-chlorophenyl phenyl ether, 7005-72-3; 4-bromophenyl phenyl ether, 101-55-3; 1-fluoronaphthalene, 321-38-0; 2-chloronaphthalene, 91-58-7; 4,4′-dibromobiphenyl, 92-86-4; 4,4′-dibromooctafluorobiphenyl, 10386-84-2; Arochlor 1221, 11104-28-2; Arochlor 1248, 12672-29-6; Arochlor 1254, 11097-69-1; benzidene, 92-87-5; 4,4′-diamino-3,3′-dichlorobiphenyl, 91-94-1; DDD, 72-54-8; DDT, 50-29-3; DDE, 72-55-9.

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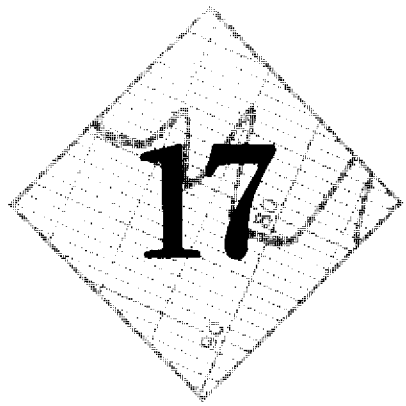

Preparation of *n*-Alkyl Trichloroacetates and Their Use as Retention Index Standards in Gas Chromatography

Ted R. Schwartz, Jimmie D. Petty and Edwin M. Kaiser

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Preparation of *n*-Alkyl Trichloroacetates and Their Use as Retention Index Standards in Gas Chromatography

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High-resolution gas chromatography has proved to be an effective means of quantifying single components in the presence of complex mixtures (*1–3*). When this analytical technique is used in conjunction with the Kovats or other retention index systems (*4, 5*), data generated in any laboratory will have a common reference and can be directly compared. An integral part of the Kovats retention index system is a series of homologous reference compounds employed as the retention index standards, the most common being normal alkanes used with flame ionization detection. Although the normal alkanes are the most widely used, their practical application to most environmental analyses cannot be realized due to the limited response of the electron capture detector (ECD) to the normal alkanes. Other homologous series have been proposed as substitutes for the *n*-alkanes in a retention index system that would be compatible with the ECD. Among these are the *n*-bromoalkanes (*6*) and *n*-alkyl trichloroacetates (*7*).

In a study of the distribution of polychlorinated biphenyl (PCB) isomers in environmental samples, the *n*-alkyl trichloroacetates were selected as the retention index standards, because of both their thermal stability and their adequate response in an ECD at picogram concentrations. The utility of *n*-alkyl trichloroacetate for retention indexing was first recognized by Neu and co-workers (*7*) and they have been used for such purposes in the gas chromatographic analysis of PCBs (*8, 9*). Unfortunately, the synthetic route for obtaining these compounds has not previously been described in the chemical literature. Thus the synthesis and purification of such *n*-alkyl trichloroacetates are described below. The procedure involves adding trichloroacetyl chloride to the alcohol in the presence of pyridine and warming the mixture overnight to produce the corresponding esters (45–85%). Attempts were not made to maximize yields. This procedure appears to be generally applicable to the synthesis of these types of esters.

$$Cl_3CCOCl + ROH \xrightarrow{C_5H_5N} Cl_3CCO_2R + HCl$$

EXPERIMENTAL SECTION

A slight excess of trichloroacetyl chloride was slowly added through a dropping funnel to the desired alcohol (all chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI) and 0.05 mL of pyridine in a round-bottom flask fitted with a reflux condenser. The reaction mixture was gently warmed (90 °C) overnight in a fume hood allowing HCl gas to escape. The amber-yellow crude product was transferred to a separatory funnel and washed several times with distilled water until the washings had a pH of 7. The crude product (5 mL) was transferred to the top of a 13 mm i.d. × 600 mm silica gel column (EM-60 70-230 mesh ASTM from E. Merck, Darmstadt, Germany) that had been activated at 130 °C overnight. The sample was allowed to drain into a 2 cm bed of Na_2SO_4 above the silica gel. Elution with 550 mL of 2% methyl *tert*-butyl ether in hexane was begun. The first 50 mL was discarded and the remainder was collected in a round-bottom flask. Solvent was removed by rotary evaporation. Compound purity was determined on a gas chromatograph.

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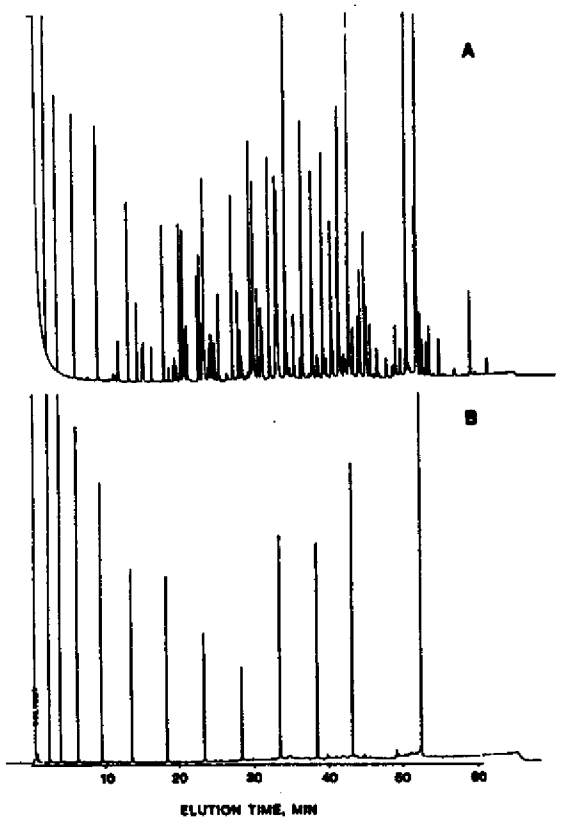

Figure 1. *n*-Alkyl trichloroacetates added to a mixture of Aroclor standards (A). *n*-Alkyl trichloroacetates chromatographed as a standard mixture (B). The concentrations of the *n*-alkyl trichloroacetates (ng/μL) are as follows: nonyl, 0.15; decyl, 0.13; undecyl, 0.15; dodecyl, 0.15; tridecyl, 0.15; tetradecyl, 0.34; pentadecyl, 0.51; hexadecyl, 1.14; octadecyl, 4.4. the gas chromatograph (Varian Model 3700, Varian Instrument, Sunnyvale, CA) was equipped with a ^{63}Ni ECD and a 0.25 mm i.d. × 30 m glass capillary column coated with Apolane-87 (C-87) (Quadrex Crop., New Haven, CT). Hydrogen, linear velocity of 32 cm/min, was used as the carrier gas, and nitrogen delivered at 15 mL/min was used as the detector makeup gas. The injector and detector temperatures were 220 °C and 300 °C, respectively. After injection the oven temperature was programmed from 120 °C to 250 °C at 2 °C/min.

Confirmation of structural identity was accomplished by infrared analysis and mass spectrometry.

RESULTS AND DISCUSSION

A high degree of purity was demonstrated by the presence of only one major peak when the esters were individually chromatographed. The mass spectra of these compounds in either electron impact or negative chemical ionization did not exhibit a molecular ion but rather a fragmentation indicative of the loss of Cl. All 12 esters exhibited the same major IR peaks. IR (neat): ν_{max} cm^{-1} 2938 (CH); 1743 (C—O ester); 1242 (C–O–C); 828 and 678 (C–Cl).

The utilitity of these compounds as retention index standards is demonstrated in Figure 1, where the *n*-alkyl trichloroacetates were first chromatographed as a mixture and subsequently with an Aroclor (polychlorinated biphenyl) mixture. In summary, although the alkyl trichloroacetates have been employed as retention index compounds, their synthesis has not previously been described in the chemical literature. The procedure described here is simple and materials are relatively inexpensive and commercially available. The response of the ECD to these compounds makes them an ideal retention index standard.

ACKNOWLEDGMENT

The authors thank James L. Johnson for his assistance in GC/MS analyses.

Registry No. Cl_3CCOCl, 76-02-8; Cl_3CCO_2R (R = nonyl), 65611-32-7; Cl_3CCO_2R (R = decyl), 65611-33-8; Cl_3CCO_2R (R = undecyl), 74339-49-4; Cl_3CCO_2R (R = dodecyl), 74339-50-7; Cl_3CCO_2R (R = tridecyl), 74339-51-8; Cl_3CCO_2R (R = tetradecyl), 74339-52-9; Cl_3CCO_2R (R = pentadecyl), 74339-53-0; Cl_3CCO_2R (R = hexadecyl), 74339-54-1; Cl_3CCO_2R (R = octadecyl), 35425-17-3; nonanol, 143-08-8; decanol, 112-30-1; undecanol, 112-42-5; dodecanol, 112-53-8; tridecanol, 112-70-9; tetradecanol, 112-72-1; pentadecanol, 629-76-5; hexadecanol, 36653-82-4; octadecanol, 112-92-5.

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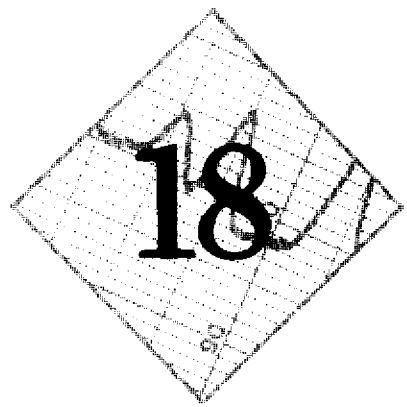

The Principle of Control Charting

Donald E. King

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The Principle of Control Charting

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Abstract

The purpose and objectives of control charting are reviewed. Control is defined as proper action in response to the observation of unexpected behaviour relative to defined limits about an expected value or mean. Precision and accuracy control are differentiated and discussed in terms of simple and complex statistical control. The advantages of a dual control system for verifying calibration control status between-run are presented. With the aid of two control standards proper control action can be taken based on knowledge of proper system function and experience with respect to probable cause and most appropriate solution.

The Principle of Control Charting

Introduction

Control carries with it the implication of action in response to an observation. It is something that is imposed rather than mere happenstance. Control implies the existence of an expected value and of some limitation to the amount of deviation from expected. A simple temperature measurement device connected to a digital display would not be a control device. Even when it is connected to a strip chart recorder it is still not considered a control device until mechanical limits are put in place which cause an alarm to sound whenever the pen deflection exceeds a predefined amount. And if no one is around to hear and react to the alarm there is still no control.

In the same way, an analytical process may be in a state of control, but it cannot be considered to be under control until the observations made have been plotted on a chart, and considered in the light of predefined limits. Even then, control has not been exerted until the human operator has made a decision based on the combination of observed fact, defined limits, and most importantly, other relevant factors.

The strip chart temperature recorder on an incubator is required, not because it is connected to a control device, nor yet because it records individual values, but because it provides a performance record for later review and evaluation. Trends, or chronic malfunctions can be seen at a glance. The frequency, as well as the magnitude, of unexpected values is a valid indication of control status. Obviously deviations caused by someone opening the door cannot be considered as indicative of a control problem unless it is opened too frequently or left open too long. It is the supervisor's responsibility to decide correctly whether to call the serviceman or to speak to the staff.

FIGURE 1: A RECORD OF OBSERVATIONS WILL REVEAL TRENDS AND VARIABILITY RELATIVE TO AN AVERAGE VALUE.

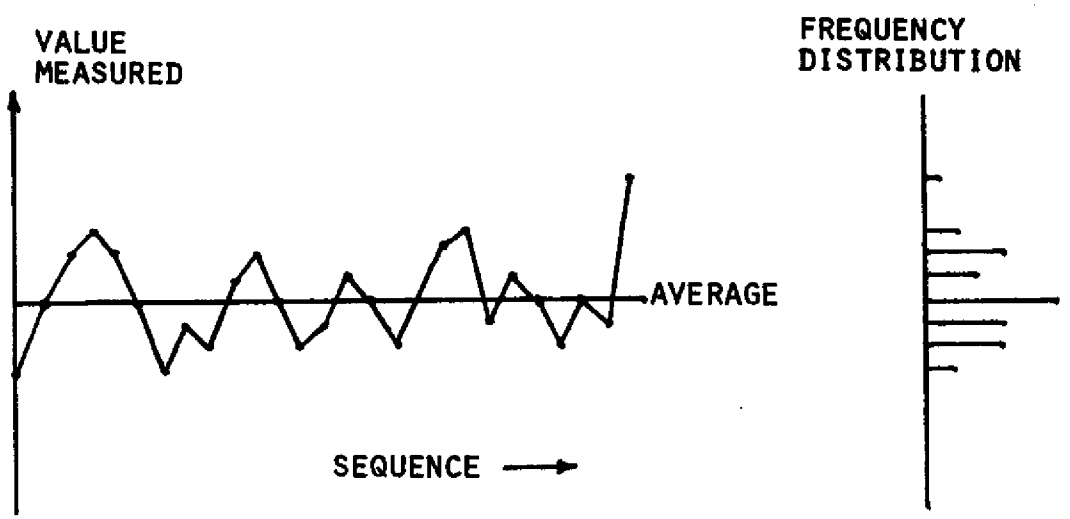


FIGURE 2: A CONTROL CHART DEFINES THE LIMITS OF ACCEPTABLE DEVIATION FROM AN EXPECTED VALUE.

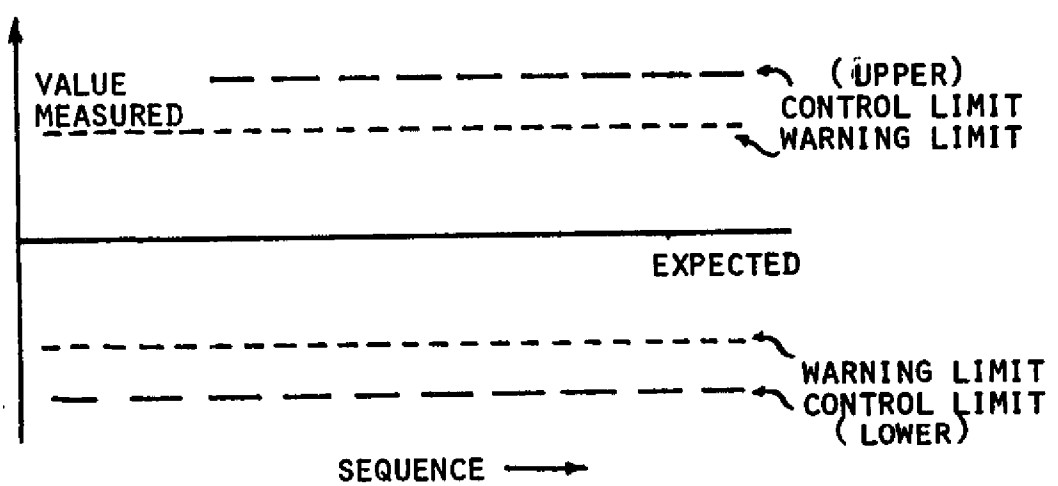
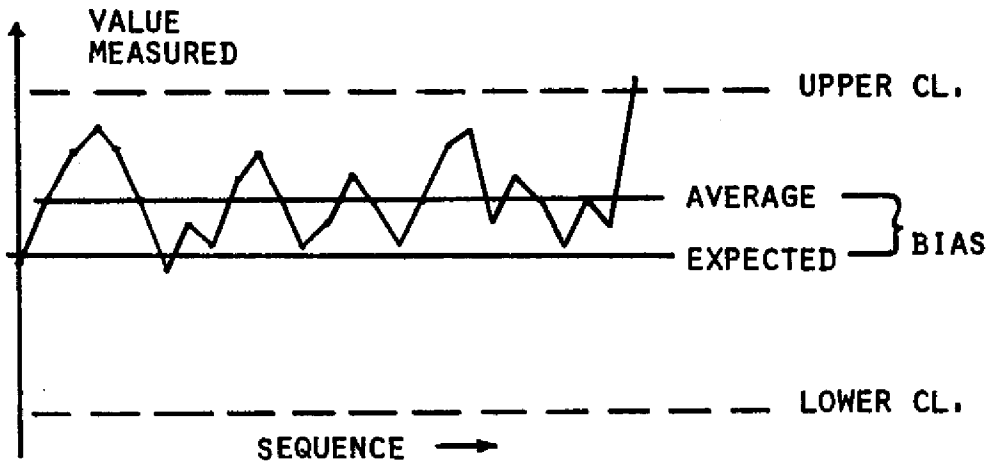


FIGURE 3: CONTROL STATUS IS ASSESSED BY COMBINING OBSERVATIONS AND CONTROL CHART LIMITS. THE VALIDITY OF THE CURRENT LIMITS SHOULD BE EVALUATED IN ADDITION TO DATA REPEATABILITY AND BIAS OF THE AVERAGE.



Clearly there is a difference between a data chart and a control chart. Figure 1 represents a series of observed results plotted in time sequence. Figure 2 is a control chart. It includes an expected value and contol limits. By imposing figure 2 on figure 1 (see figure 3) we obtain an impression of relative Control Status.

Figure 3 suggests that there is more involved to Control than simply calculating a control limit and plotting data. As shown, the limits seem far too wide compared to the current data set, and the average seems to be displaced from the expected value. One might (correctly) conclude that control is required over both the range of variation of individual values (precision), and the variation or drift of averages (accuracy). However before we discuss how to control, we should know

a) what control means,

b) what we are trying to achieve by control and control charting,

c) the areas of operation that should be considered for control, and

d) the relative priority of these areas in assuring final measurement quality.

Definition of Control

Control arises from Proper Action in Response to the Observation of Unexpected Behaviour Relative to Defined Limits about an Expected Value or Mean.

Proper action will be based on Knowledge of Proper System Function and Experience with respect to Probable Causes and the Most Appropriate Solutions.

Objectives of Control Charting

1. To make You more familiar with the Actual Operating Characteristics of Your System, as operated by You.

2. To provide a Readily Interpretable Record of the performance level achieved, for internal and external evaluation.

Control Areas

1. Equipment

   - regular inspection and maintenance
   - passive control (self-limiting)

2. Analytical Precision

   - well-defined procedures
   - properly trained staff
   - passive control (self-limiting)

3. Analytical Accuracy

   - properly prepared, traceable standards
   - well-defined calibration and standardization procedures
   - active control of standardization by means of control standards

4. Analytical Recovery

   - known sample processing efficiency
   - well characterized samples
   - active control of sample processing by means of control samples

Priorities for Control

Measurement theory suggests that results obtained within a short period of time under standardized conditions will show a central tendency. They will not deviate

excessively from this central value with more than a defined probability. It is not the point of this discussion to describe how these limits are calculated or how the probability of exceeding a limit is determined. In the end it is essentially an arbitrary decision based on statistical theories which may, or may not, be appropriate. It is more important that we discuss the nature of control as it relates to measurement and to the decision making process. In particular the difference between precision and accuracy control must be clearly appreciated.

Precision is a measure of the spread of individual values. Accuracy is a measure of the deviation of an average from an expected value. Ability to detect inaccuracy is limited by the precision available only when insufficient data is included in the average. No statement can be made about either precision or accuracy given only a single value. Recent and historical patterns are essential to evaluating control status.

As shown in figure 4a), precision and accuracy statements are always relative. Information is required as to what is acceptable, not only on an instantaneous but also on a continuous basis. Five shots closely spaced in a target may give an impression of precision. But if the marksman was only five yards from the target this may be unacceptable.

If five shots are closely spaced and the sixth is not (e.g. point marked X) one must consider the marksman somewhat erratic. The point marked y is obviously not erratic. The more precise a procedure becomes the easier it is to identify suspect values. Fewer repeat measurements are required to confirm an outlier.

It should be apparent from figure 4b) that it is not sufficient to be able to place shots precisely. One must be able to repeat this performance on successive trials.

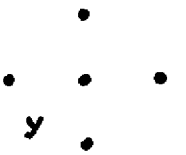
FIGURE 4: PRECISION, BIAS, AND ACCURACY CAN ONLY BE ASSESSED IN TERMS OF PAST EXPERIENCE, CONTINUING CONTROL, AND A DEFINITION OF WHAT IS CONSIDERED ACCEPTABLE.

A)

precise?

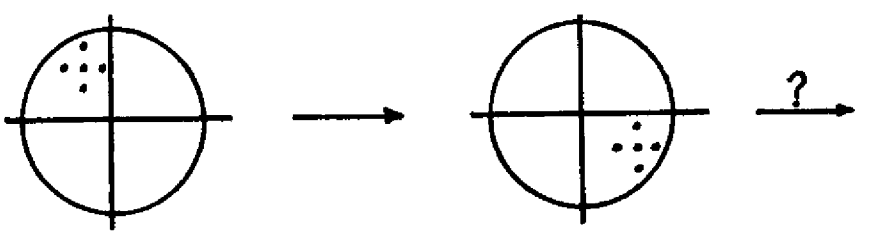
shot from... 5 yards
poor.

imprecise?

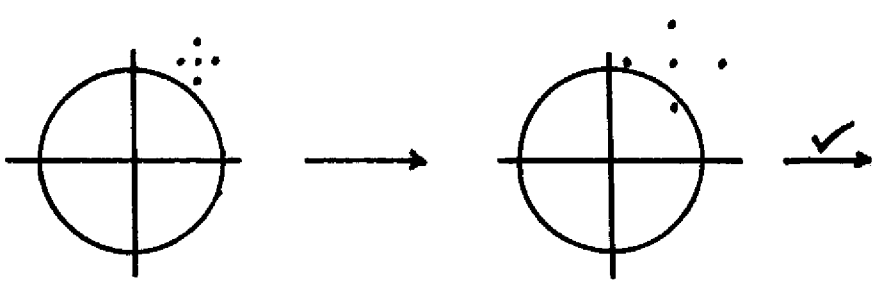


... 50 yards
good.

B) THIS MARKSMAN IS ACCURATE (IE. ON TARGET) PRECISION IS CONTROLLED, BUT BIAS IS NOT CONTROLLED. WHERE WILL HIS GROUPING GO NEXT?



C) THIS MARKSMAN IS INACCURATE (IE. OFF TARGET) PRECISION IS LESS CONTROLLED, BUT HIS BIAS IS WELL CONTROLLED. IF HIS SIGHTS ARE PROPERLY ADJUSTED HE CAN BE MADE AS ACCURATE AS NEEDED.



P R E C I S I O N

A MEASURE OF THE SPREAD OF INDIVIDUAL VALUES DUE TO
- INHERENT WITHIN-RUN VARIATION OF MEASUREMENT PROCESSES
- CONTROLLABLE BETWEEN-RUN VARIATION CAUSED BY THE STANDARDIZATION PROCESS

REPEATABILITY = WITHIN-RUN EFFECTS ONLY

REPRODUCIBILITY= BOTH WITHIN AND BETWEEN-RUN

B I A S

VARIATION OF AN AVERAGE, EITHER OVER TIME
OR BETWEEN OPERATORS
OR BETWEEN SYSTEMS / METHODS
OR RELATIVE TO AN EXPECTED VALUE

THE EXPECTED VALUE MAY BE DERIVED FROM A LONG TERM AVERAGE OR A BETWEEN-METHOD OR A BETWEEN-LAB AVERAGE.

I N A C C U R A C Y

DEVIATION OF A LONG TERM (ETC.) AVERAGE FROM A DEFINED TRUE VALUE.

THE DEFINED TRUE VALUE IS FREQUENTLY AN EXPECTED VALUE MADE OFFICIAL BY DECREE. IT'S VALIDITY WILL THEREFORE OFTEN BE METHOD DEPENDENT.

N O T E

REPEATED MEASUREMENT WILL INCREASE ONE'S CONFIDENCE IN THE AVERAGE VALUE OBTAINED. IT HAS NO EFFECT ON THE TRUTH OR ACCURACY OF THE AVERAGE. IT MAY IN FACT VERIFY ANY BIAS WHICH MAY BE PRESENT.

ACCURACY IS NOT AN ABSOLUTE. IT DEPENDS UPON WHAT ONE CONSIDERS ACCEPTABLE. THEREFORE THE PHRASE 'ON-TARGET'.

However, even if the precision can be maintained it is not good enough if the next grouping of shots is located at a different spot on the target.

Bias is usually introduced through operator error. If the gun sights are not adjusted correctly one will not expect the shots to arrive at the same location. If a different gun is used, a standard setting of the sights may not work. Calibration adjustments are always susceptible to Type I and Type II errors. The first is a failure to make an adjustment when one is required. The second involves failure to make a correct adjustment particularly when one is not required.

In figure 4b) we assume the marksman is accurate because he is on target. His ability to control bias is not being tested sufficiently. If the target were smaller, or further away, he might have problems with accuracy.

In figure 4c) we see a marksman who is inaccurate (off-target) but who is bias controlled. Readjustment of the sights would keep him on center. His precision is somewhat variable but this has little or no effect on the determination of bias or accuracy.

It should be clear at this point that precision control and bias control are independent factors. One's ability to become and remain accurate depends more on the size of the target then anything else. However if the target is small, bias control becomes critical. Precision is relatively unimportant. Its importance increases as fewer and fewer shots are taken per grouping.

Simple Statistical Control: Precision

As long as we do not interrupt a measurement process by recalibration, or otherwise change the conditions of measurement, and as long as the process has been demonstrated to be rugged (i.e. relatively unaffected by small changes in

procedure), it is safe for us to "act for the moment as if the process is in a state of statistical control". Every time we repeat the process we can expect more or less the same result. Small changes at various stages in the process will tend to have random small effects on the result. On average these will tend to cancel out.

Statistically this means that any given average calculated from a small amount of data will tend to be a good estimate of the 'true average' under existing conditions. It also implies there is a low probability that any given result will differ greatly from the average. The range of deviation likely to be observed can be estimated as some factor (often 3) times the 'standard deviation' (s). Given a series of repeated measurements ($X_1, X_2, - - - X_n$), s is calculated by the formula

$$s^2 = \frac{\Sigma X_i^2 - (\Sigma X_i)^2/n}{n - 1}$$

Given a series of duplicate results s can also be calculated by

$$s^2 = \frac{\Sigma (X_1 - X_2)_i^2}{2k} = \frac{\Sigma (D)^2}{2k}$$

where k is the number of duplicate pairs and D is the difference between the paired values.

It is important to realize that s can be calculated for any available data set. However, its suitability for predicting the likely range of deviation from an average requires that all results were determined under identical conditions and that the distribution of repeated results is approximately 'normal'. In other words the system must be in control.

Demonstrating Simple Statistical Control

The essential ingredient in Simple Statistical Control is repeatability. The actual value measured is not a concern at this point. Analysis is usually performed as a batch process. As long as the same reagents, digestion equipment, etc., are used and as long as the measurement instrumentation is stable and drift free there is no reason to believe that the average analytical response will change. Duplicate analysis of selected samples which represent the type routinely received can be used to demonstrate that Simple Statistical Control is actually occurring.

A common approach to evaluating within-run control status from duplicates is to define an acceptable degree of difference (e.g. 10% or 0.25 mg/L) irrespective of the actual performance being achieved. This represents an acceptance limit approach. The alternative is to calculate of the standard deviation under routine conditions based on recent duplicate results. The control limit is then some factor times this standard deviation. Both approaches may permit a system to operate 'out-of-control' for significant periods of time, since data obtained under uncontrolled conditions will not yield a valid control limit.

A prime requisite for establishing a proper control limit is a system that is demonstrably in-control. Demonstration is most readily achieved by graphical techniques. A day to day plot of the difference between duplicates will indicate not only the instantaneous variability of differences but also, over the long-term, will show periods of time when differences are on average smaller than previously. At such times, a higher degree of control is being achieved. This is usually related to the use of a different batch of reagents, reserviced equipment, or staff rotation. Once this has been observed, control can be exerted over the factor that has been identified and more appropriate control limits can be determined.

It should be clear that both acceptance limits and control limits are useful. The former ensures a guaranteed level of service to the laboratory client while the latter ensures that periods of poorer control status are identified so that remedial action can be undertaken. There are dangers in the use of acceptance limits related to maintaining accuracy. This will be discussed later. When used in the context of precision based on repeatability of duplicates this is not a problem.

Outliers on a day to day basis, as defined by the control limits, will occur

a) because the sample is atypical of the routine for which the performance criterion was defined.

b) the criterion is actually dependent on concentration and this factor has not been considered in defining it.

c) an unusually large deviation has occurred, as expected (e.g. 1 in 20 or 1 in 200).

d) an unusually large deviation has occurred because of an error in the analytical process, operational or instrumental problems, or individual sample processing error.

If we can "act for the moment" as if the system is capable of control, and if we know that it has been in-control, and that no changes have been introduced, a single instance of outliers must be carefully evaluated in terms of alternative explanations before one concludes that the process itself has failed. It is a characteristic of standard analytical procedures that gross total failure is highly unlikely. The judgement error of unnecessarily reanalyzing large numbers of samples because of a single instance of suspect repeatability is as serious an error as ignoring a control problem by failing to look for it. If the cause of the suspect data cannot be identified there can be no reason to believe that automatic reanalysis will provide data any more repeatable than that already available. It could provide worse data if the system is indeed on the verge of failure.

One should always be cautious when responding to control problems identified by use of duplicate (or replicate) analyses, especially when they are borderline. Acceptance limits based on actual client needs should always be incorporated into the control program action plan to avoid unnecessary or incorrect control response. Repeatability control is properly a passive activity. It documents the performance level achieved, good or bad. It does not provide solutions.

Figure 5 demonstrates a mechanism for observing the distribution and dependence of difference between duplicates on concentration. It usually reveals that a single criterion for controlling repeatability is inappropriate. It can indirectly monitor the effect of time as points are added to the diagram on a day by day basis. Figure 6 demonstrates the Range control chart approach based on time sequence. In both cases control limits are defined as a factor times the average range (difference between duplicates). However figure 5 accounts for the effect of concentration.

Complex Statistical Control: Accuracy

In order to report a result two principles are involved. There must be a measured response. But there must also be a calibration equation for converting the response to a concentration or other usable value. As already indicated, response can be assumed, for the moment, to vary in a statistically random fashion. However, it is not proper to make the same assumption with respect to calibration factors, because of the human element involved in deciding to accept or reject the data used to calibrate. Figure 7 shows how the response factor (sensitivity) might change from day to day. As shown, errors can be made in concluding that a significant change has, or has not occurred.

When the same solution is measured on several different occasions the variation between results will be larger than predicted by random chance alone, because the

FIGURE 5: THE RANGE OF DIFFERENCE BETWEEN DUPLICATES IS USUALLY SOMEWHAT DEPENDENT ON CONCENTRATION. THIS METHOD OF ACCUMULATING DATA HELPS TO SHOW HOW CONTROL LIMITS CAN BE ADJUSTED FOR THIS EFFECT. A DUPLICATE PAIR 0.017 AND 0.021 WOULD BE ADDED AS A POINT IN THE BOX MARKED *. NOTE THE EFFECT OF ROUNDING-OFF RESULTS ON THE APPARENT DIFFERENCES.

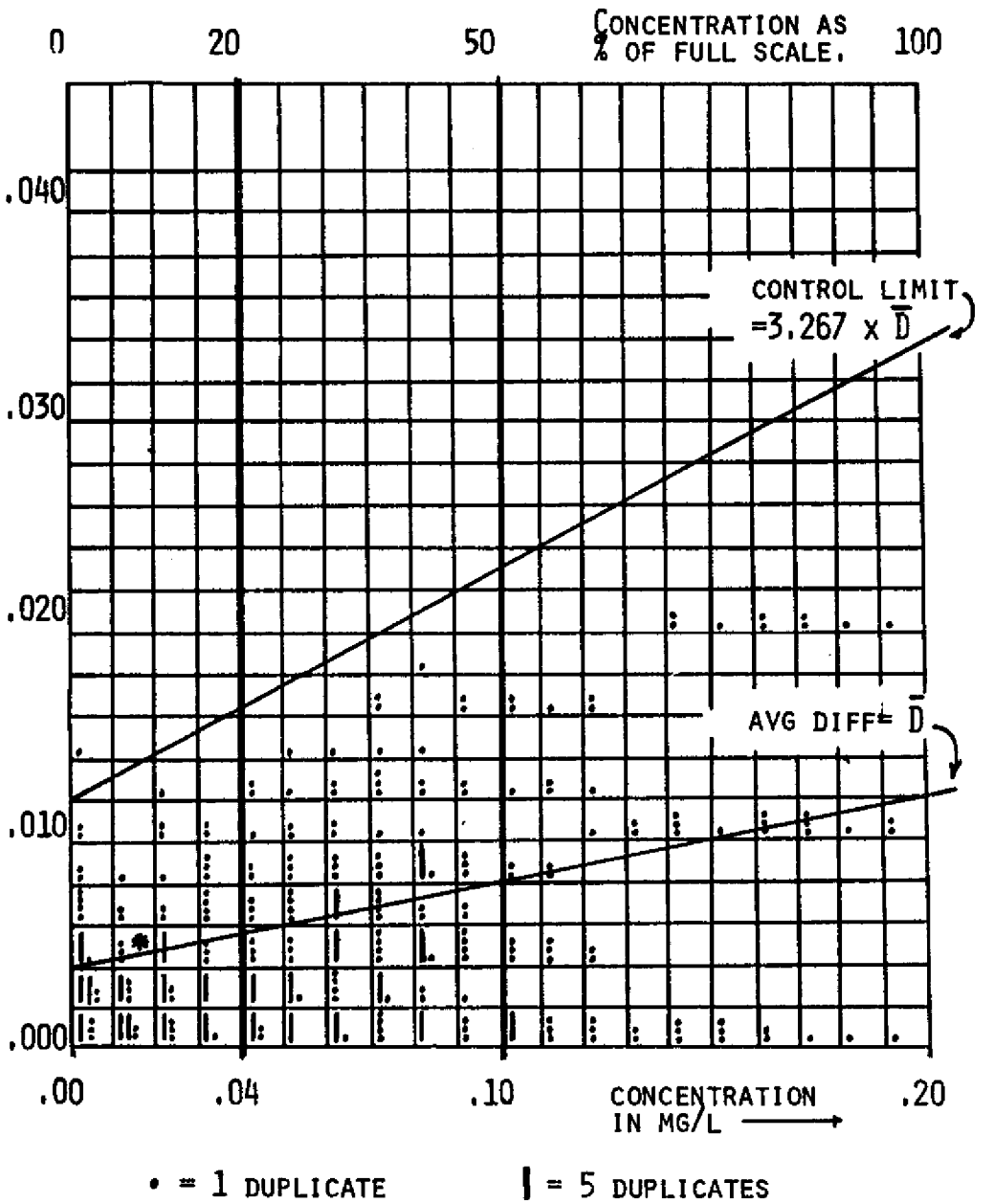
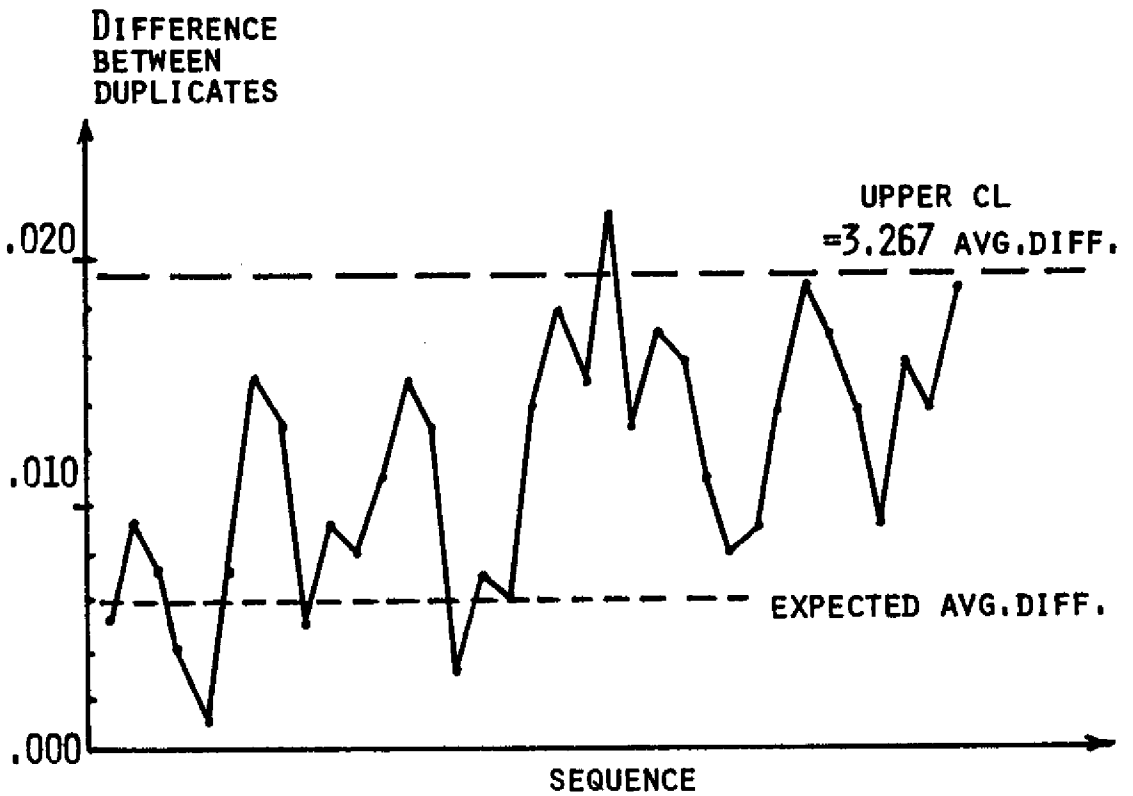


FIGURE 6: TYPICAL RANGE CONTROL CHART. THERE IS NO ALLOWANCE FOR THE EFFECT OF CONCENTRATION ON DIFFERENCE. TRUE CONTROL STATUS IS DIFFICULT TO EVALUATE. HAS THE OBSERVED AVERAGE DIFFERENCE CHANGED RECENTLY? OR HAVE WE BEEN ANALYSING A LARGER THAN USUAL NUMBER OF HIGH LEVEL SAMPLES?



PRECISION CONTROL

-ASSUMES THAT ANALYTICAL CONDITIONS INCLUDING SYSTEM CALIBRATION REMAINS UNCHANGED

-DEMONSTRATED BY ABILITY TO REPEAT ANALYSIS AND OBTAIN THE SAME RESULT WITHIN A NARROW RANGE OF VARIATION

-SELF-LIMITING CHARACTERISTIC OF ALL "STANDARD" MEASUREMENT PROCESSES

-LOW PROBABILITY THAT ANY REPLICATE RESULT WILL DIFFER GREATLY FROM THE AVERAGE

-LOW PROBABILITY THAT CONTROL ACTION WILL BE REQUIRED UNLESS THE SYSTEM FAILS COMPLETELY

-PASSIVE DATA COLLECTION AND EVALUATION IS USUALLY SUFFICIENT

WE CAN ACT FOR THE MOMENT AS IF THE PROCESS IS IN A STATE OF "SIMPLE" STATISTICAL CONTROL. THEREFORE THE USE OF A CONTROL CHART IS HELPFUL BUT NOT ESSENTIAL.

ACCURACY CONTROL

-ASSUMES THAT ANALYTICAL CONDITIONS HAVE CHANGED AND THAT RESTANDARDIZATION IS REQUIRED

-DEMONSTRATED BY A LARGER RANGE OF VARIATION BETWEEN RESULTS FROM DIFFERENT "RUNS"

-INCORPORATES THE ERRORS IN PREPARING AND PROCESSING STANDARDS

-THE RANGE OF VARIATION IS NOT LIMITED BY CHANCE

-HIGH PROBABILITY THAT ACTION WILL BE REQUIRED IN ORDER TO MAINTAIN CONTROL

-REQUIRES ACTIVE DATA COLLECTION AND EVALUATION

A CONTROL CHART IS REQUIRED TO KEEP THE PROCESS IN A STATE OF "COMPLEX" STATISTICAL CONTROL.

OBJECTIVES IN

CONTROL CHARTING ACCURACY

- TO ENSURE CONTROLLED BETWEEN-RUN VARIATION
- TO IDENTIFY UNUSUAL CALIBRATION CHANGES WHICH COULD AFFECT ACCURACY
- TO RECORD THE LEVEL AND STATUS OF CONTROL BEING MAINTAINED
- TO DETERMINE AND CHARACTERIZE THE PARTICULAR TYPES OF CALIBRATION PROBLEMS TO WHICH THIS PROCESS IS SUSCEPTIBLE

TO MAKE YOU
FAMILIAR WITH THE ACTUAL
OPERATING CHARACTERISTICS OF
YOUR SYSTEM AS
OPERATED
BY YOU

CALIBRATION PROBLEMS

- ZERO ADJUSTMENT AND CONTROL
- BLANK CORRECTION AND CONTROL
- SLOPE ADJUSTMENT AND CONTROL
- ERROR IN PREPARATION OF STANDARDS
- UNCERTAINTY IN MEASUREMENT OF STANDARDS
- CURVATURE IN USUALLY LINEAR SYSTEMS
- INSTABILITY IN CURVED SYSTEMS
- ILL-ADVISED ADJUSTMENT OF INSTRUMENT OR SYSTEM CONTROLS

calibration curve must be restandardized day by day. One cannot automatically assume that the materials used as 'standards' are correct or have been prepared without contamination or loss, or have been measured without error. Therefore once the calibration curve has been restandardized, deliberate action must be taken to ensure that today's 'true average' will be not significantly different from its previous value. This introduces the concept of Complex Statistical Control.

It should be noted that analytical recovery from individual samples is not under discussion here, although it may have some impact on the result in less 'rugged' analytical processes. Rather, we are concerned with ensuring that standards are used and interpreted properly, to avoid introducing bias between 'true averages' from day to day. It must always be kept clear that 'simple statistical control' determines only the range of variability expected. It defines the precision of the method. On the other hand, 'complex statistical control' addresses the variability of the 'true average'. It therefore maintains a degree of accuracy.

Demonstrating Complex Statistical Control

Whenever a system is restandardized there is always a significant probability that bias will be introduced. A check is required which will help to limit the range of this error. This is achieved by use of a control standard. The data in figure 7 is basically the same as figure 3) except that not only the range of variation but also the variation relative to an expected value must be examined. This difference represents the bias for that day's run. From day to day this bias will change. It may or may not be significant. It is the function of the control standard to monitor these changes.

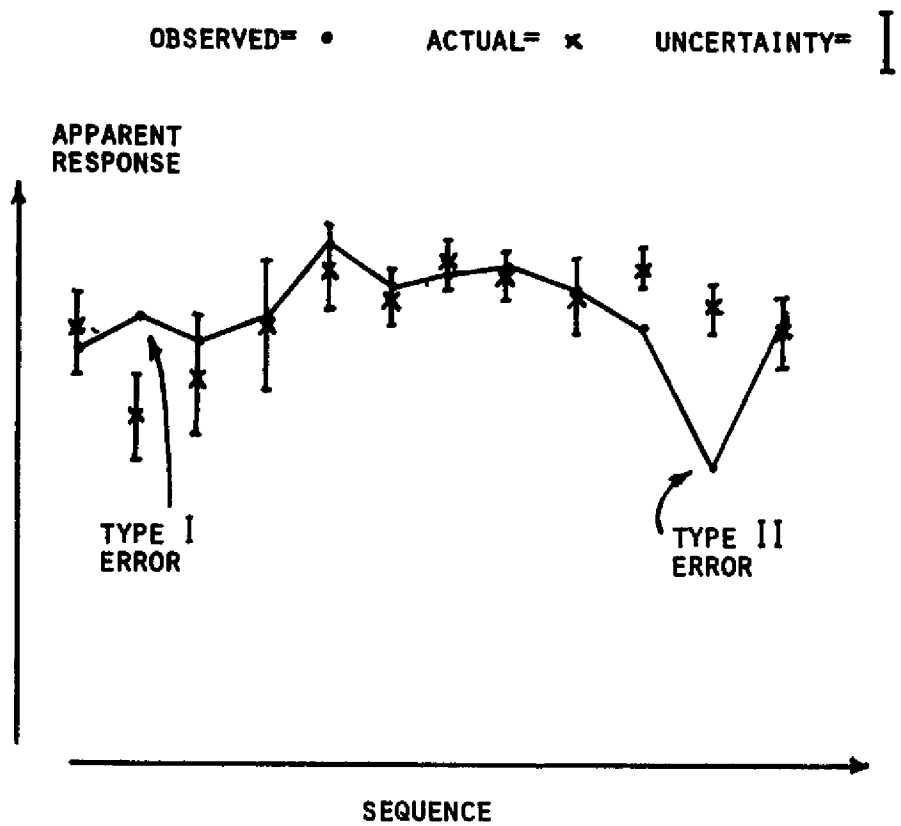
When only one analysis of a single control standard is available, it is clearly difficult to distinguish between the precision variability and the accuracy variability. However, the use of a control chart with limits based on within-run estimates of standard deviation will reveal,

a) an unexpected number of values outside the control limits but not grossly outside. These we may have to live with since they represent an acceptable level of between-run control.

b) trends in the apparent average over short periods of time. These we should eventually correct since trends are a sign of determinate error rather than chance.

c) occasional gross outliers beyond the level defined by a). These we must prevent from affecting calibration on a day-to-day basis.

FIGURE 7: INSTRUMENTAL RESPONSE FACTORS USUALLY VARY SOMEWHAT FROM DAY TO DAY. CALIBRATION ADJUSTS FOR THIS. BUT ANALYTICAL ERRORS IN THE STANDARDS CAN LEAD TO INCORRECT CALIBRATION DECISIONS WHICH WILL AFFECT ALL MEASUREMENTS IN THAT RUN.



Extraordinary deviations may occasionally reflect an error in analysis of the control standard. But more frequently they are a direct indication of calibration bias influencing today's 'true average'.

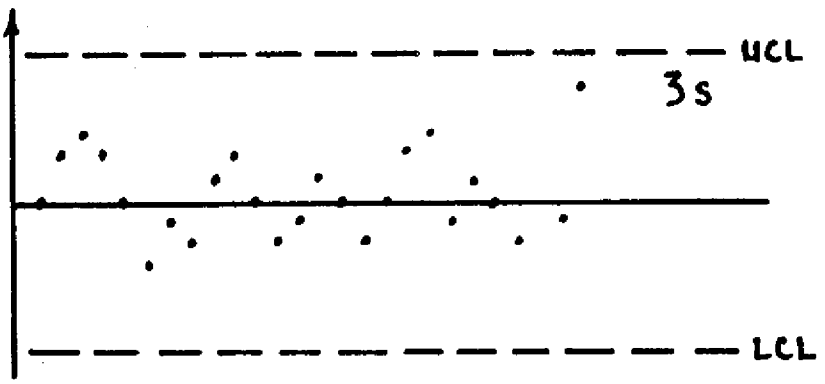
The data in figure 8a) might generate the control limit estimates shown. However this control status cannot be assumed to be good. This data may have been actually generated over several runs where the within-run standard deviation was known to be significantly better. As shown in figure 8b) the system appears to be out-of-control relative to within-run performance. But is it really? Is there a criterion for establishing an acceptable level of control over between-run variation?

Control limits are often set at 3 times the standard deviation. In setting control limits for between-run performance it should be obvious that within-run standard deviation is too tight a basis. On the other hand, between-run standard deviations will reflect the current control status. If the process is not in good control, between-run standard deviations will be too loose to exert control.

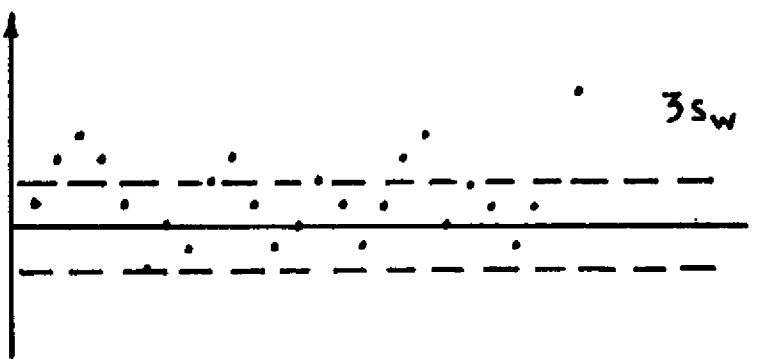
As a general rule of thumb, a ratio greater than 1.3 for between-run versus within-run standard deviation would be considered 'statistically significant' (based on the f-test for ratios of variance for about 30-60 degrees of freedom). While statistical significance is not directly indicative of practical significance, in this case it provides a basis for establishing control. Our experience indicates that the simple use of a control chart often has the effect of reducing the ratio of between-run to within-run standard deviation below this value.

FIGURE 8: IF BETWEEN-RUN EFFECTS ARE NOT CONTROLLED THEN CONTROL LIMITS BASED ON SUCH DATA CAN NOT EXERT CONTROL. A FACTOR OF 1.3 TIMES THE KNOWN WITHIN-RUN STANDARD DEVIATION WILL PROVIDE A BETTER ESTIMATE OF BETWEEN-RUN STANDARD DEVIATION FOR USE IN DEFINING MORE APPROPRIATE CONTROL.

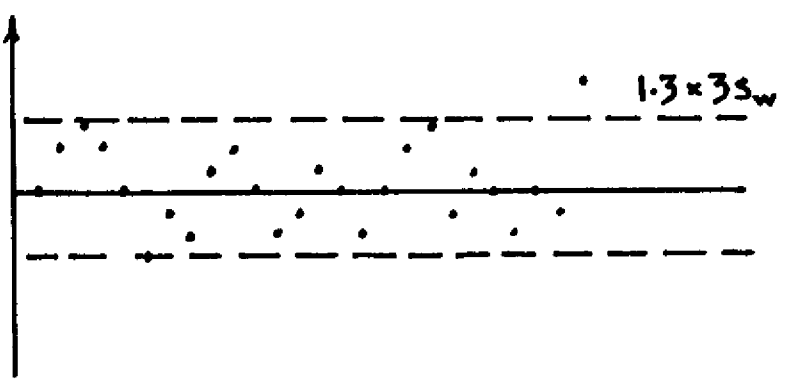
A) BETWEEN-RUN STD DEV. YIELDS LOOSE CONTROL LIMITS



B) WITHIN-RUN STD DEV. YIELDS EXCESSIVELY TIGHT LIMITS



C) 1.3 X WITHIN-RUN STD DEV. EXERTS CONTROL



Therefore, in establishing control limits for between-run performance, it is better to determine the within-run standard deviation and apply a factor of 1.3, than to use the actual between-run standard deviation. This allows room for short-term drift as well as imprecision and yet establishes active control.

It is always possible to apply an acceptance limit approach for determining when action is absolutely required. This usually implies relaxing the control limit somewhat. However, it must be kept in mind that accuracy is at stake here, not just precision. It may be permissible to allow replicate results to vary by plus or minus 10% or 20%. But, the client does not expect the 'true average' to change by this amount. When a single control standard is being used, it is impossible to determine how much of today's deviation is random and how much represents a bias. It is even less possible to determine the source of this bias.

Establishing Calibration Control

Control can only be exerted when both a cause and a solution can be determined. It is always important to realize that excessive control may not only create bias, it may also prevent the cause of the problem from being identified. Figure 9 shows a few of the different types of problems that can be revealed by a control chart. Since this chart provides no solution, proper action cannot be taken. Therefore this process must remain out-of-control.

In addition to curvature, a calibration will show variation in both slope and intercept over time. Figure 8 showed that changes were occurring but the operator had to guess the cause. More often than not this type of behaviour is not even apparent because the 'control' date is not plotted even if the control material analyzed and measured. The assumption is often made that this data reflects the capability of the process rather than lack of control over it.

Is this system under control?

1. THE CONTROL LIMITS WERE DETERMINED DURING THIS PERIOD.
2. THIS ERRATIC POINT MAY OR MAY NOT REFLECT A CALIBRATION ERROR. IT COULD BE AN ANALYTICAL OR MEASUREMENT ERROR.
3. BETWEEN-RUN PRECISION HAS IMPROVED. THE CURRENT CONTROL LIMITS ARE TOO LOOSE BUT ARE RETAINED AS ACCEPTANCE LIMITS UNTIL A REASON CAN BE FOUND FOR CHANGING THEM.
4. IS THIS DRIFT CAUSED BY BLANK OR SLOPE EFFECTS, ARE THE STANDARDS CHANGING OR IS IT THE INSTRUMENT?
5. DRIFT HAS STABILIZED. ACCEPTANCE LIMITS ARE PERMITTING A SIGNIFICANT BIAS GIVEN THE APPARENT BETWEEN-RUN PRECISION.
6. ALTHOUGH THIS RESULT LOOKS GOOD, IT IS ERRATIC RELATIVE TO RECENT VALUES. SOMETHING IS WRONG.
7. THE BIAS HAS BEEN ELIMINATED DELIBERATELY BY ADJUSTING THE SLOPE. HOW DO WE KNOW THIS WAS CORRECT? PERHAPS THE BLANK CORRECTION HAS BEEN INCORRECT?
8. NEW CONTROL LIMITS WERE FINALLY ESTABLISHED. THE CAUSE OF THE CYCLICAL DRIFT CANNOT BE DETERMINED AT THIS TIME BUT THE VARIATION IS TOLERABLE FOR THE INTENDED DATA USE.

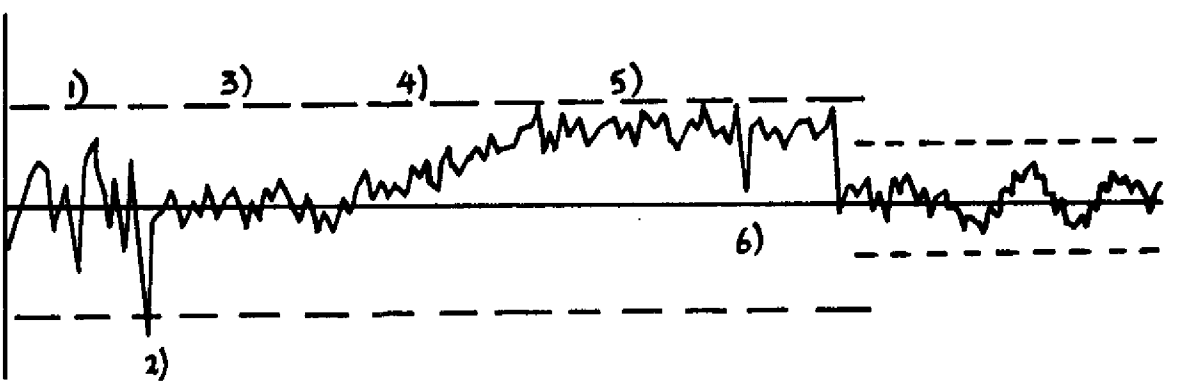


FIGURE 9: SEVERAL TYPES OF CONTROL PROBLEMS SEEM TO BE OCCURRING. ARE THEY WITHIN OR BETWEEN-RUN? ARE THEY BLANK, SLOPE OR SAMPLE RELATED? WERE PROPER ADJUSTMENTS MADE, AND ON WHAT BASIS?

When two control solutions are analyzed and measured it becomes possible to evaluate the nature of the change. If they differ in strength, so as to cover the bottom and top of the calibrated range, several factors can be evaluated over time by plotting a control chart, not just for the individual control standards but, more importantly, for their sum and difference values. If the variability of the high standard is greater than for the low standard, an overall lack of slope control is indicated. Intercept problems will be masked. Once this situation is controlled then;

1) Their difference monitors slope trend control as long as the variation in slope from day to day is controlled.

2) The variability of their difference monitors within-run precision as long as the slope is in-control.

3) The pattern of variation in the sum of the two control values will indicate between-run control problems. If the slope is in-control these problems will be related to intercept or blank determination errors.

4) If the blank is normal, an intercept problem suggests chronic curvature at the top end of an otherwise normally linear relationship.

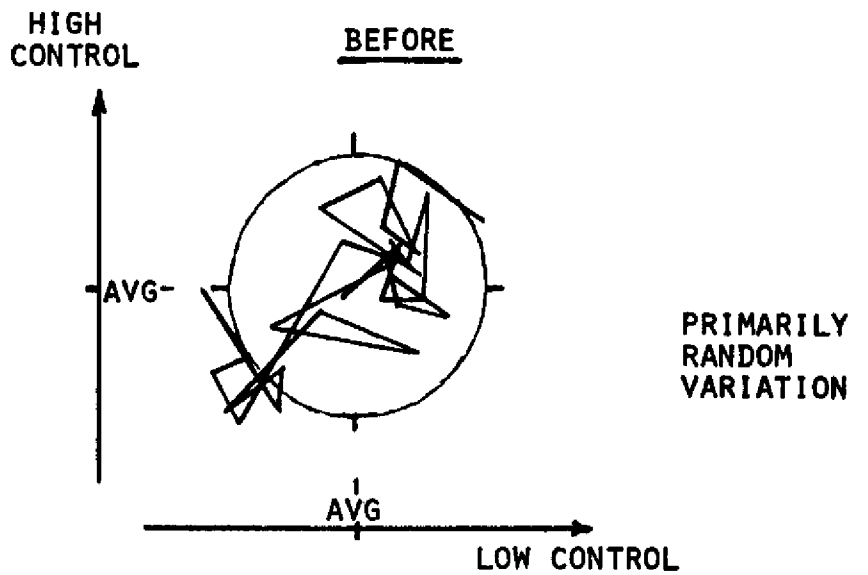
These patterns are demonstrated in figures 10 (and following). They reflect the calibration problems shown beside them.

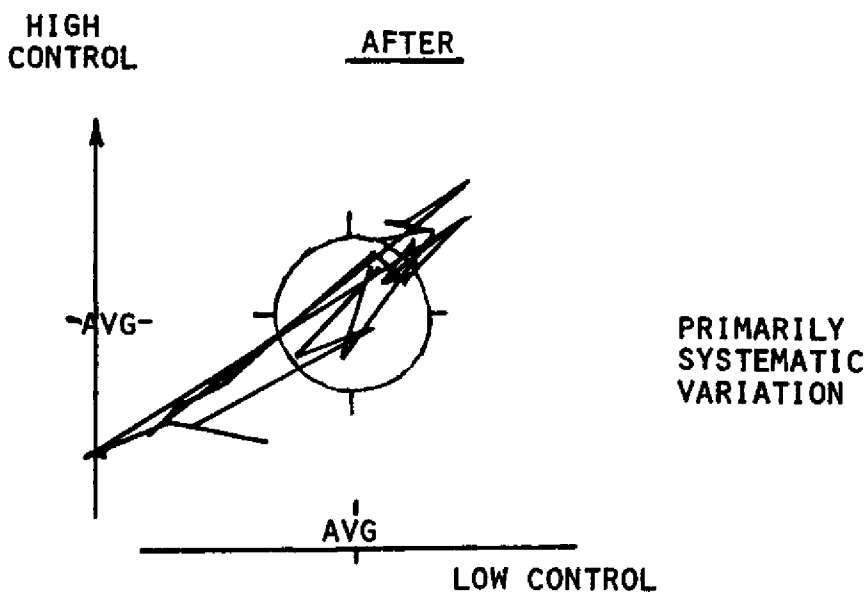
Accuracy Control

We have indicated that repeatability is essentially process controlled (3 times the within-run standard deviation). Further we have shown that between-run bias can be placed under control (based on a factor of 1.3 times the within-run limits or about 4 S_w.) However the question of accuracy control has not been resolved.

It is important to realize that between-run variability usually reveals trends, i.e. drifting of the average over time. While it is acceptable for individual points to

FIGURE 10: THE RESULTS FOR TWO CONTROL STANDARDS CAN BE PLOTTED VERSUS EACH OTHER. THE DOTS ARE JOINED IN SEQUENCE TO REVEAL SYSTEMATIC VERSUS RANDOM PATTERNS. SYSTEMATIC ERRORS CAUSE BOTH RESULTS TO INCREASE OR DECREASE TOGETHER CAUSING LINES FROM LOWER LEFT TO UPPER RIGHT. THESE TWO PATTERNS WERE OBTAINED FROM THE SAME SYSTEM BEFORE AND AFTER A MAJOR INSTRUMENT OVERHAUL.





CIRCLE REPRESENTS 2X CALCULATED IN-RUN STD. DEVIATION

FIGURE 11: TWO CONTROL STANDARDS OR SAMPLES CAN BE USED TO MONITOR THE TOP AND BOTTOM OF THE CALIBRATED RANGE. THEIR DIFFERENCE MONITORS SLOPE CONTROL AND IS USED TO ESTIMATE WITHIN-RUN STD. DEVIATION. THEIR SUM ACCENTUATES SYSTEMATIC CHANGES IF THEY ARE PRESENT AND CAN BE USED TO ESTIMATE THE BETWEEN-RUN STANDARD DEVIATION. IF THE SYSTEM IS IN-CONTROL THE RATIO OF THESE ESTIMATES SHOULD NOT EXCEED 1.3 TO 1.5

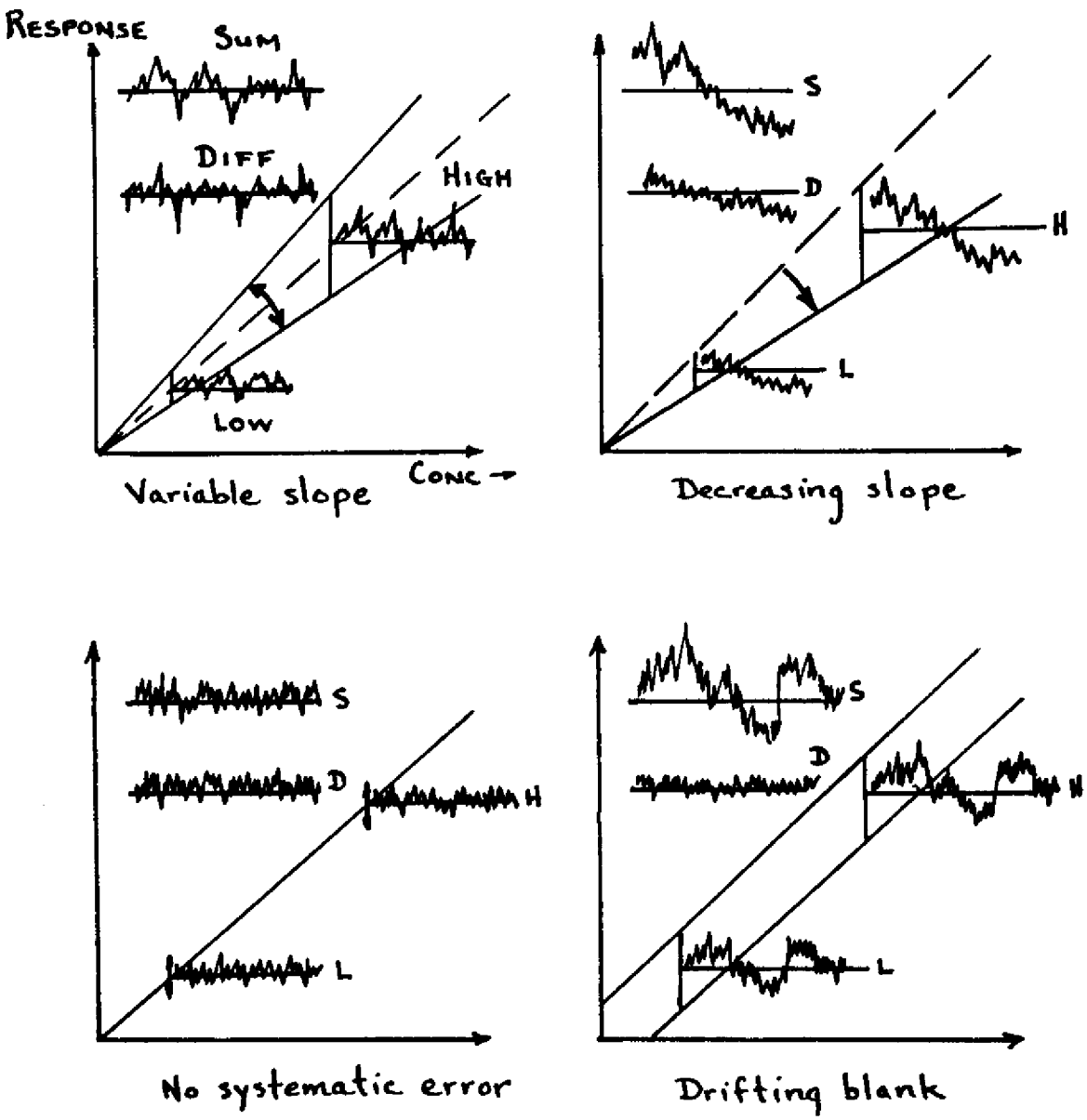
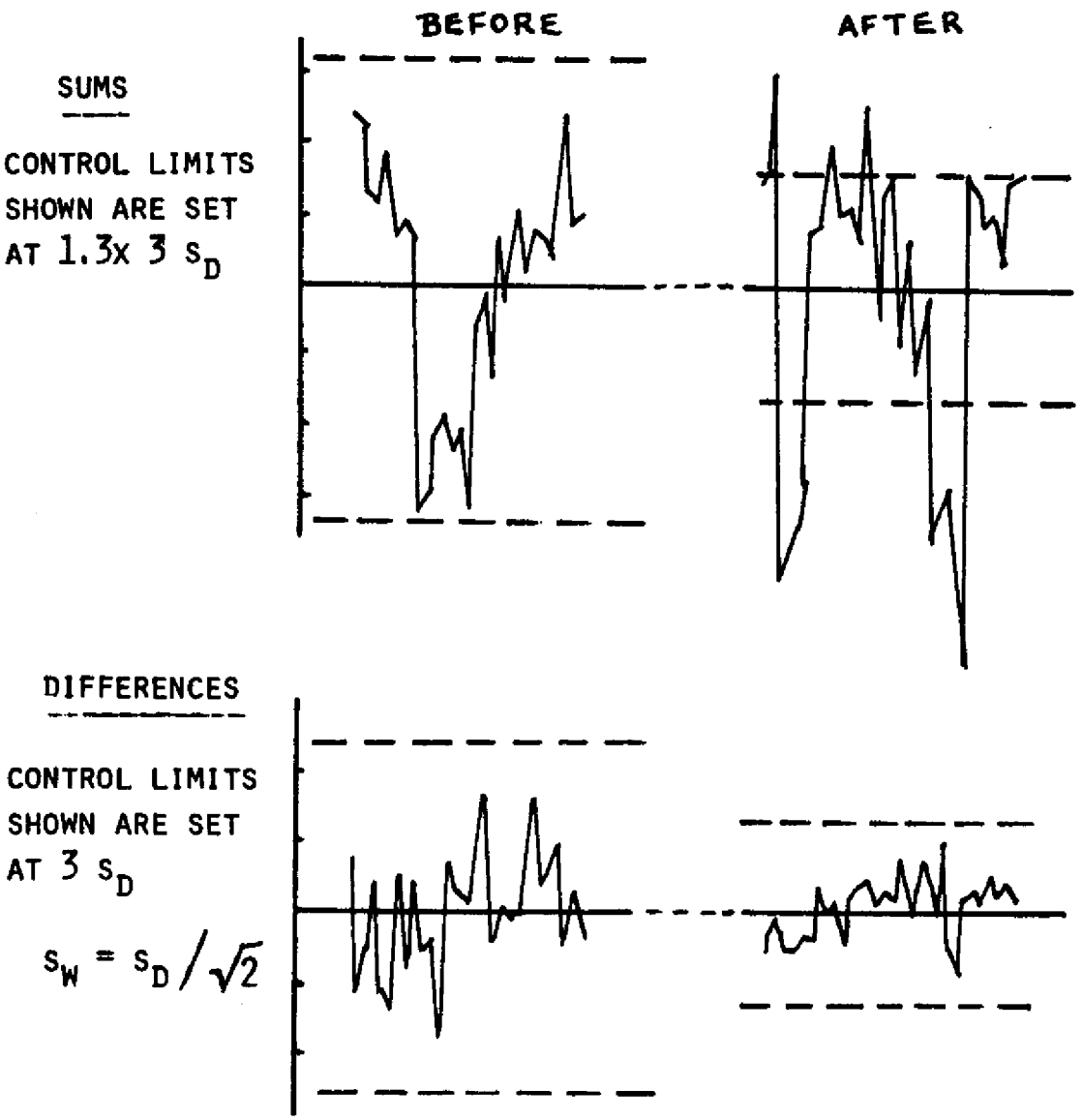


FIGURE 12: TIME SEQUENCE PLOTS OF THE HIGH AND LOW CONTROLS AND OF THEIR SUMS AND DIFFERENCES REVEAL THAT AFTER THE OVERHAUL THE SLOPE VARIATION HAS STABILIZED. THE LARGE VARIATION IN THE SUMS MUST BE DUE TO LACK OF CONTROL OVER THE BLANK OR INTERCEPT CORRECTION.



deviate by as much as 4 times S_w from expected, the average should not. Confidence in an average increases as the number (n) of values incorporated in it increases. The 'standard error' of an average is given by $s/\sqrt{n}$. Therefore, if the average is based on eight results, the standard error will be about 0.38 times the standard deviation.

Therefore, given that the bias control limit for individual values is 4 times S_w, it can be determimed that the control limit for an average of eight results in sequence will be about 1.5 times S_w. If the recent trend of control data is such that the addition of today's result will cause the average to differ from expected by more than this amount, it is reasonable to conclude that long-term accuracy is being affected even if today's result is still in control.

Figure 13 reviews the various control limits that have been discussed. Their basis on the known within-run standard variation ensures control action will be taken, when necessary, to permit limited drift in bias while ensuring control of accuracy.

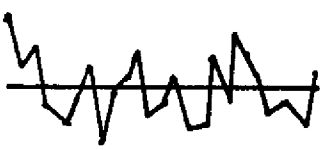
Conclusion

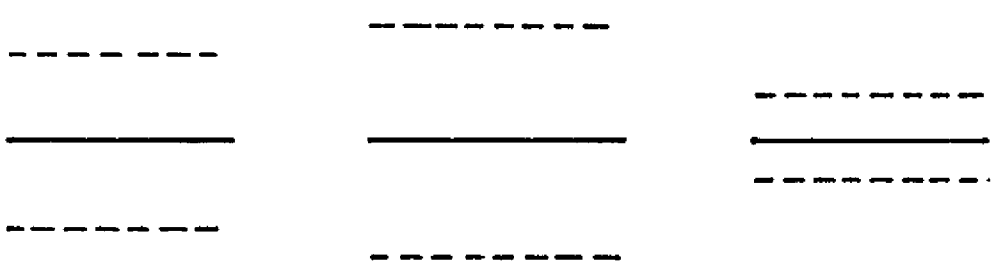
The important thing to keep in mind when considering use of control charts is not how they should be drawn, nor even how the control limits should be defined. If the objectives of a 'control chart' are not understood, it will not be plotted. These objectives include mechanisms for

1) determining when calibration errors between-run are affecting the accuracy of sample results.
2) characterizing the most significant sources of between-run problems.
3) limiting between-run variation and yet retaining control over it.
4) permitting trends and yet identifying unusual individual errors.
5) demonstrating the level and nature of control being maintained.

FIGURE 13: DIFFERENTIATION BETWEEN CONTROL LIMITS FOR PRECISION ($3s_w$), BIAS ($4s_w$), AND ACCURACY ($1.5\ s_w$).

A SEQUENCE OF IN-RUN REPLICATION REVEALS VARIATION USED TO ESTIMATE S_w.

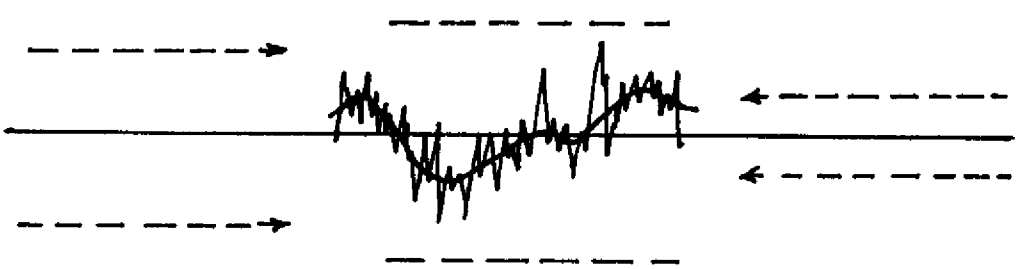




PRECISION IS SELF-CONTROLLED WITHIN $\pm 3\ S_w$

DAILY BIAS MUST BE LIMITED WITHIN $\pm 4\ S_w$

THE AVERAGE OF 8 VALUES IN SEQUENCE SHOULD REMAIN WITHIN $\pm 1.5\ S_w$



BETWEEN-RUN CONTROL STATUS?

C O N T R O L

PROPER ACTION TAKEN IN RESPONSE
TO THE OBSERVATION OF
UNEXPECTED BEHAVIOUR RELATIVE TO
DEFINED LIMITS ABOUT AN
EXPECTED VALUE OR MEAN

P R O P E R A C T I O N

ACTION BASED ON KNOWLEDGE
OF PROPER SYSTEM FUNCTION, AND
EXPERIENCE WITH RESPECT TO
PROBABLE CAUSES AND
MOST APPROPRIATE SOLUTIONS

If you don't know what went wrong.....
DON'T FIX IT.

If you don't have all the facts.....
DON'T MAKE DECISIONS.

If you're absolutely sure you can fix it...
THINK IT OUT AGAIN.

When you think it has been fixed.....
PROVE IT.

THEN..... DOCUMENT THE PROBLEM
AND YOUR SOLUTION

AND..... UPDATE THE CONTROL CHART.

The most important objective, however, is to become familiar with the characteristics of this particular analytical process as operated by you.

A control chart need not be complicated by statistics. Its primary purposes are to identify outliers and to detect trends. Obviously once this has occurred, a solution is required. But, blind action is to be avoided at all cost. It is far worse to take the wrong action than to take no action at all. The more information that is recorded to assist in finding the right solution the better. Two controls are much more informative and useful than one.

If resources are tight, precision control is less critical. It is already limited by the analytical process and technical proficiency. Accuracy however requires active control because of the nature of the human decision-making process. It needs you. It needs a control chart.

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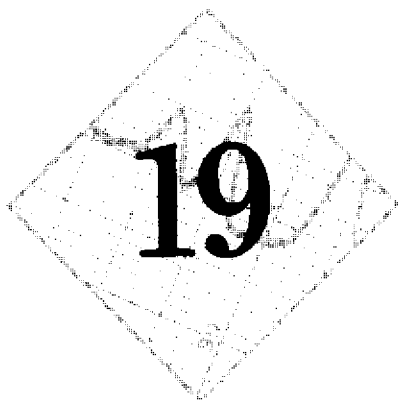

Principles of Environmental Analysis

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Principles of Environmental Analysis[1]

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PREFACE

Reliable analytical measurements of environmental samples are an essential ingredient of sound decisions involving many facets of society including safeguarding the public health, improving the quality of the environment, and facilitating advances in technology. In September 1978, the American Chemical Society's Committee on Environmental Improvement (CEI) directed its Subcommittee on Environmental Analytical Chemistry to develop a set of guidelines that would help improve the overall quality of environmental analytical measurements. In 1980, CEI published, "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry" (*1*).

In 1982 CEI decided to revise and update the 1980 publication and charged the restructured subcommittee, now named the Subcommittee on Environmental Monitoring and Analysis, with this task.

This new publication provides principles useful for many diverse applications. Its intent is to aid in the evaluation of the many options available in designing and conducting analytical measurements of environmental samples and in the intelligent choice of those that will meet the requirements of the situation at hand. These situations range from semiquantitative screening analyses to those involving strict quality assurance programs intended to document the accuracy of data for regulatory enforcement or legal purposes. In this

[1] This is a revision to the "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry", which appeared in *Anal. Chem.* 1980, *52*, 2242–2249.

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respect, these principles are a set of guidelines for making decisions—decisions for planning and executing analytical work or, conversely, for evaluating the usefulness of previously generated analytical measurements for a current need.

INTRODUCTION

The *Principles of Environmental Analysis* presented here are organized according to a general operational model for conducting analytical measurements of environmental samples. This document identifies elements needed to obtain reliable data as well as factors that have been shown to produce unreliable measurements. The principles, which appear in boldface type, are intended to encompass broadly the needs for both organic and inorganic measurements, although specific requirements for these two general categories differ widely. Thus, these principles are not a "recipe" for conducting specific determinations. They are, however, intended to provide guidance in identifying important elements in an analytical protocol that are necessary to meet the requirements of a specific need.

Many options are available when analyzing environmental samples. It must be recognized at the outset that differing degrees of reliability, dictated by the objectives, time, and resources available, influence the protocol chosen to meet the requirements of the problem at hand.

Analytical objectives for environmental samples differ from those for many other types of samples because reliable measurements at very low levels are frequently required. Often, specific analytes need to be measured at the parts-per-billion and even parts-per-trillion levels in complex matrices. Advances in analytical methodology continue to lower the levels at which reliable measurements can be made and, conversely, requirements for lower measurement levels provide an incentive for future analytical advances. At these levels, many factors that are of little or no concern in other analytical measurements are of critical importance in influencing the outcome and reliability of environmental analyses.

PLANNING

Good planning is an essential principle of environmental analysis. Inadequate planning will often lead to biased, meaningless, or unreliable results; good planning, on the other hand, can produce valid results. The intended use of the data should be addressed explicitly in the planning process. Intended results are those that answer a question or provide a basis on which a decision (for example, to adjust a process or to take regulatory action) can be made. The objective of planning is to define the problem and analytical program well enough that the intended results can be achieved efficiently and reliably.

It cannot be assumed that the person requesting an analysis will also be able to define the objectives of the analysis properly. Numerous discussions between the analyst and those who will use the results may be necessary until there is agreement on what is required of the analysis, how the results will be used, and what the expected results may be. The analytical methodology must meet realistic expectations regarding sensitivity, accuracy, reliability, precision, interferences, matrix effects, limitations, cost, and the time required for the analysis.

One of the most important elements in planning is to incorporate essential decision criteria into the overall analytical protocol. The protocol, which describes the analytical process in detail, should include the objective, a description of the

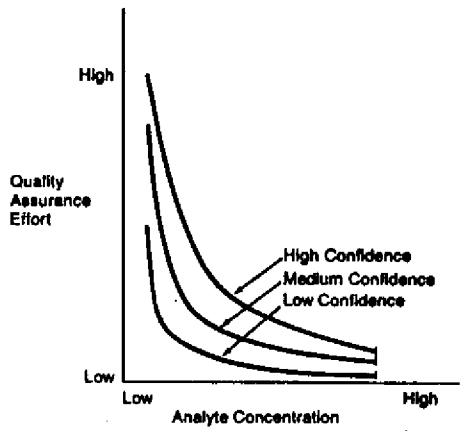

Figure 1. Qualitative relationship of confidence, concentration, and quality assurance effort. The quality assurance effort increases at a faster rate as the need for higher confidence increases and the analyte concentration decreases. Eventually a point is reached where the quality assurance effort increases rapidly as lower analyte concentration is approached and also where further quality assurance effort produces diminishing returns with increasing confidence.

quality assurance and quality control requirements, the sampling plan, analytical method(s), calculations, and documentation and report requirements.

Selection of the optimum analytical method is one of the most important factors influencing the reliability of resulting data (2). In addition to the obvious limitations of availability of equipment, amount of sample, time, and resources, other factors significantly affect the cost and reliability of the data obtained. These are decisions pertaining to:

(1) *The level of confidence required regarding the analyte's identity.* For example, is a low or a high level of confidence for correct identification required? The latter may be achieved at higher expense by confirmatory analysis with an independent measurement technique. A lower confidence level can be achieved at less expense by comparing the analyte's spectral, chromatographic, or other physical/chemical properties with values reported in the literature.

(2) *The analyte levels (both qualitative and quantitative) that need to be measured.* This decision often defines method selection, the amount of a sample to be taken, the degree and type of sample pretreatment (e.g., cleanup and concentration) to employ, and the method of analysis. Generally, the lower the levels of measurement that are required, the higher the cost of doing the analyses.

(3) *The degree of confidence needed (Figure 1).* This decision will influence method selection and the number of samples taken as well as the design of the quality assurance program. In general, the higher the degree of precision and accuracy needed, the more rigorous the quality assurance program must be and the higher the costs of analysis.

(4) *The degree of method validation that is necessary.* On the basis of the specifications developed in the first three items, the method must now be examined to determine whether it actually can produce the degree of specificity, precision, and "accuracy" required. If it does not, then either the method must be improved or another method must be chosen. The first stage of method validation ordinarily will involve only intralaboratory validation. If more than one laboratory will be involved in a measurement program (e.g., multilaboratory monitoring and surveillance or regulatory actions), interlaboratory validation may be required.

(5) *The degree of quality assurance that is necessary.* The use of validated methods, reference laboratories, and experienced personnel still does not assure the production of reliable analytical results. All analytical work must be monitored

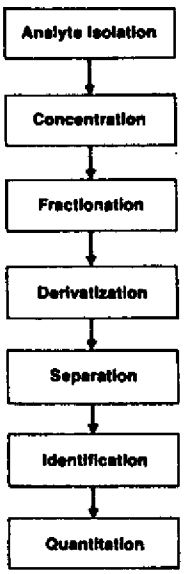

Figure 2. Technical decision framework.

by a system of quality assurance to verify that the results obtained have a high probability of being correct. The use of rugged methods, experienced personnel, and a history of low outlier production will justify a lesser degree of control than will the use of complex new procedures and inexperienced personnel. However, the most important factor determining the level of quality control is the consequences of being wrong.

If an analytical result is to be used in a screening program or to adjust a process parameter, an unvalidated analytical method may be sufficient and appropriate. On the other hand, if regulatory compliance is the reason for an analysis, a validated analytical method (especially one that has been upheld in the courts) is usually required. In addition to making decisions in a cost-effectiveness context, a separate set of decisions must be made within a technical decision framework (Figure 2) (*2*).

In selecting an analytical method, consideration also must be given to verifying that the method actually measures the analytes in question. A second analytical method is often used to confirm that the intended analyte is being measured by the first analytical method. For example, gas chromatography/mass spectrometry (GC/MS) is often used to confirm a gas chromatographic (GC) measurement.

QUALITY ASSURANCE AND QUALITY CONTROL

A quality assurance program is an essential part of a sound analytical protocol and should be used by individuals as well as by laboratory organizations to detect and correct problems in the measurement process or to demonstrate attainment of a state of statistical control. The objective of quality assurance programs for analytical measurements is to reduce measurement errors to agreed upon limits and to assure that the results have a high probability of being of acceptable quality.

Two concepts are involved in quality assurance: quality control, the mechanism established to control errors; and quality assessment, the system used to verify that the analytical process is operating within acceptable limits. Quality assurance of chemical measurements is reviewed in some detail in ref 3. General handbooks that discuss quality assurance are given in ref 4–8.

Each laboratory should have and use a quality assurance program. In addition, every monitoring program should contain an appropriate quality assurance plan and require that it be followed strictly by all participants. In each case, the quality assurance program and plan should be developed as a joint effort by all involved personnel, including statisticians as required.

The elements of a quality control program include the following: development of and strict adherence to principles of good laboratory practice; consistent use of standard operation procedures; and establishment of and adherence to carefully designed protocols for specific measurement programs. The consistent use of qualified personnel, reliable and well-maintained equipment, appropriate calibrations and standards, and the close supervision of all operations by management/senior personnel are essential components of a sound quality control system. When properly conceived and executed, a quality control program will result in a measurement system operating in a state of statistical control, which means that errors have been reduced to acceptable levels and have been characterized statistically.

Quality assessment describes those techniques used to assess the quality of the measurement process and the results. **The establishment of a system of control charts is a basic principle.** Control charts are plots of multiple data points from the same or similar samples or processes vs. time (*9*). They are used to determine if a system is in a state of statistical control. Control charts should be used to visualize or monitor the relative variability of repetitive data. Control charts also can be used with reference materials, spiked samples, and analysis of surrogates as a means of assessing the accuracy of measurements.

The attainment of statistical control is the first requirement that must be met before assessment of accuracy can be made. For this reason, and also to monitor ongoing precision, the development and maintenance of appropriate control charts are essential features of the quality assurance process. Control charts should be maintained and used in a real-time mode to the extent possible. The strategy of the decision process in their use and the corrective actions to be taken when lack of control is indicated should be planned and followed. Because all data obtained within the period of "last-known in control" and "first-known out-of-control" are suspect, laboratories must consider the risk involved when designing their quality assurance procedures (*10*).

Statistical criteria should be used in designing quality assessment programs, which include the kind of test samples to be used and the sequence of use, so that the establishment of statistical controls and an estimate of bias can be made (*11*). **Appropriate statistical concepts should be used in the design of the quality assurance program.**

Audits should be a feature of all quality assurance programs. A *systems audit* should be made at appropriate intervals to assure that all aspects of the quality assurance program are operative. *Performance audits*, in which a laboratory is evaluated based on the results of analyses of blind standard samples, also provide valuable quality assessment information. Participation in interlaboratory and collaborative test programs is another procedure a laboratory may use to assess the quality of its data output.

The terms repeatability, reproducibility, intralaboratory variability, and interlaboratory variability are terms sometimes used to describe various aspects of the measurement process. Repeatability describes the variation in data generated on a single sample by a single analyst and/or instrument over a short period of time while reproducibility refers to variation over an extended period of time and/or by various analysts or laboratories. Intralaboratory variability refers to the difference in results when a single laboratory measures portions of a common sample repeatedly. Interlaboratory variability refers to the difference of results obtained by different lab-

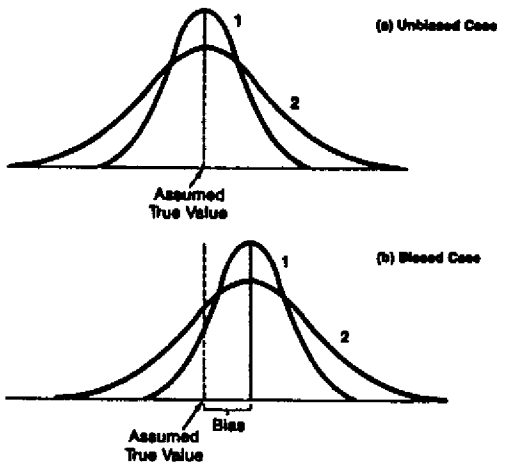

Figure 3. An illustration of precision and accuracy: (a) unbiased measures with (1) high precision (low dispersion) and (2) low precision (high dispersion); (b) biased measures with (1) high precision (low dispersion) and (2) low precision (high dispersion).

oratories when measuring portions of a common sample. Because of the nonspecific nature of this terminology, the experimental conditions must be specified whenever such terms are used.

Variabilities due to different operators, equipment, and conditions (but independent of sample variabilities) influence reproducibility and are indeed components of intralaboratory variability. Laboratory biases, when coupled with differences in the capabilities of various laboratories, result in an interlaboratory variability that is often very much larger than intralaboratory variability. Well-designed and well-executed quality assurance programs help reduce both intralaboratory and interlaboratory variability.

VERIFICATION AND VALIDATION

Verification is the general process used to decide whether a method in question is capable of producing accurate and reliable data. Validation is an experimental process involving external corroboration by other laboratories (internal or external) or methods or the use of reference materials to evaluate the suitability of methodology. Neither principle addresses the relevance, applicability, usefulness, or legality of an environmental measurement. A review on this subject is covered in ref 12. Confirmation, a type of verification, is a process used to assure that the analyte in question has been detected and measured acceptably and reliably.

The reliability and acceptability of environmental analytical measurements depend upon rigorous completion of all the requirements stipulated in a well-defined protocol. Such protocols should prescribe the documentation requirements of the study including sampling procedures, measurements, verification, and validation. In addition, all results should be reviewed critically. If questions arise during the review, additional confirmatory tests should be conducted, including the use of methods other than those applied previously. In situations where large numbers of samples are analyzed with widely accepted and well-documented analytical systems, unusually high results, or unexpected low ones, on critical samples should be checked by a repeat analysis of a duplicate subsample by using the same method and a third subsample analyzed by a different analytical method. Agreement of the three results indicates that the analyte has been measured correctly; disagreement requires careful study of its cause including analysis of additional samples. This requires prior planning for the collection of sufficient amounts of sample at the beginning of the program or for resampling if necessary.

Confidence in the measurement process is strengthened considerably by interlaboratory comparisons and is one of the most effective elements of a quality assurance plan. **Collaborative testing should be a prerequisite for analytical methods used in major decision-making processes.**

> Analytical systems that are part of major decision-making processes should utilize collaboratively studied methods. Confidence in the measurement process is strengthened considerably by participation in check sample programs conducted by external organizations. Such programs are some of the most effective elements of a quality assurance program.

Qualitative identification should be confirmed. This confirmation should be based on a measurement principle or on analytical conditions that are distinctly different from those used in the initial method. The procedure chosen should be highly selective and should refer to a different unambiguous property that is characteristic of the analyte.

PRECISION AND ACCURACY

Precision describes the degree to which data generated from replicate or repetitive measurements differ from one another. Statistically this concept is referred to as dispersion. Accuracy refers to the correctness of the data. Unfortunately, in spite of its importance, there is no general agreement as to how accuracy is evaluated. Inaccuracy results from imprecision (random error) and bias (systematic error) in the measurement process. As illustrated by Figure 3, high precision does not imply high accuracy and vice versa.

Unless the true value is known, or can be assumed, accuracy cannot be evaluated. Bias can only be estimated from the results of measurements of samples of known composition. Standard reference materials, when available, are ideal for use in such an evaluation. Bias is often estimated from the recovery of spiked samples. It should be remembered that such samples may not fully simulate natural samples so the recovery information should be interpreted with this in mind. The accuracy of individual analytical measurements is discussed in ref 13.

Despite the use of validated methods, principles of good laboratory practices, and systematic quality assurance procedures, outlier data points can frequently appear in sets of analytical measurements. Outliers are analytical results that differ so much from the average as to be highly improbable. Statistical techniques may be used for their identification. **Zero or negative measurements are often considered to be outliers, but when working near the limit of detection, a certain number of analyses by chance alone are expected to be zero (*14*).** When outliers are discarded from a data set, it is important that they are identified and that the statistical or operational reasons for their deletion are given.

SAMPLING

The quality and utility of analytical data depend critically on the validity of the sample and the adequacy of the sampling program. Sampling, including the development of sampling plans, is often complex and may require special expertise such as statistical input. Guidance for developing adequate programs is contained in ref 15 and 16.

The purpose of sampling is to obtain specimens that represent the situation being studied. Sampling plans may re-

quire that systematic samples be obtained at specified times and places; or simple random sampling or stratified sampling may be necessary. Generally, the sample should be an unbiased representative of the population of interest. This means that the sample obtained is related in probability to all other samples that could be selected from the target population under the specified conditions; this can only be accomplished through appropriate randomization schemes.

All aspects of a sampling program should be planned and documented in detail, and the expected relationship of the sampling protocol to the analytical results should be defined. A sampling program should include reasons for choosing sampling sites, the number of samples, the timing of sample acquisition, and the expected level of fluctuations due to heterogeneity. These reasons should be based on the decisions made in the planning phase. A detailed description of sampling sites and procedures is necessary and should include the sampling methodology, labeling, container preparation, field blank preparation, storage, and pretreatment procedures. An acceptable sampling program should include at least the following: (1) a sampling plan that takes into account the goals of the studies and the expected uncertainties associated with the number of samples collected and the population variability; (2) instructions for sample collection, labeling, preservation, and transport to the analytical facility; and (3) training of personnel in the sampling techniques and procedures specified.

The quality assurance program should include a means to demonstrate that the sample container and storage procedures do not alter the composition of the sample in a way that would affect the concentration or the identification of the analyte being determined. Special transportation procedures (such as refrigeration or exclusion of light) need to be specified if they are required to ensure the integrity of the sample. Detailed guidelines for sampling designs in some special situations are available in ref 6, 11, and 15–18.

Samples should be safeguarded from loss, tampering, or misidentification. This requires use of a unique sample identification system, safe storage facilities, and a sample management system in which all steps are fully documented. **Samples that could be involved in potential litigation should be protected with a chain-of-custody documentation system in addition to the above safeguards.**

Sampling Requirements. Because environmental samples are typically heterogeneous, a large number of samples ordinarily must be analyzed to obtain meaningful compositional data. (Measurement of a single sample can tell nothing about environmental patterns, but only about the sample itself.) The number of individual samples that should be analyzed will depend on the kind of information required by the investigation. If an average compositional value is required, a large number of randomly selected samples may be obtained, combined, and blended to provide a reasonably homogeneous composite sample from which a sufficient number of subsamples are analyzed. If composition profiles or the variability of the sample population is of interest, many samples will need to be collected and analyzed individually.

In general, the number of samples and the quality of the sampling procedure must be planned to facilitate characterizing the population of interest and enhance the utility of the final results. If the sampling plan is not imposed (for example, by regulation), the investigator will need to decide what error and confidence levels are tolerable. Once these are determined, the minimum number of samples necessary for specific confidence limits that satisfy the requirements of the protocol can be estimated. Several approaches for defining the number of such samples may be used.

A statistical approach to determine the number of samples to be taken is possible when the distribution and standard deviation of the population are known or can be assumed. It is usually assumed that most chemical data are approximated by a Gaussian or normal distribution so that well-known, common statistical techniques can be used. This assumption is not always correct particularly with the very low concentrations (ppb, ppt) encountered in environmental analyses. In such cases, a log-normal distribution may be more suitable. In a log-normal distribution, the logarithms of the concentrations follow a Gaussian or normal distribution (*19*, *20*). One consequence of the assumption of a log-normal distribution is that the common arithmetic average is *not* a suitable way to estimate the mean of a set of data. Instead, the geometric average (the average of the logarithms) should be used. The discussion that follows is applicable to any statistical distribution once it is assumed. It is, however, important for the investigator to verify that the statistical distribution being used is appropriate.

A relationship that may be used to calculate the required number of samples for a given standard deviation and for a given acceptable error is

$$N_s = \left(\frac{z\sigma_p}{e}\right)^2 \qquad (1)$$

where N_s is the number of samples, z is the value of the standard normal variate (see ref 21, Table A-2, page T-3, for example) based on the level of confidence desired, σ_p is the standard deviation of the sample population, and e is the tolerable error in the estimate of the mean for the characteristic of interest.

For illustration, assume that the sample population is expected to have a mean concentration of 0.1 ppm with a standard deviation of 0.05 ppm and that the tolerable error in the stated value of the mean at the 95% confidence level (z = 1.96) is not to exceed 20% (0.02 ppm). A further assumption made is that the measurement error is small in comparison with the measured values and can be neglected in the calculation. With the above values, the approximate number of samples required will be

$$N_s = [(1.96 \times 0.05)/0.02]^2 \approx 24 \qquad (2)$$

Unfortunately, environmental analyses often are done where the expected levels and the standard deviation of the population are not known in advance and where the measurement error cannot be predicted adequately, nor can it be assumed to be negligible. Nevertheless, the measurement error must be estimated from the analytical results. In this case, the measured values of similar samples can be used to calculate an overall standard deviation, σ_o, which is related to the standard deviation of measurement, σ_m, and the standard deviation of the sample population, σ_p, by the expression

$$\sigma_o^2 = \sigma_m^2 + \sigma_p^2 \qquad (3)$$

An estimate of σ_m can be obtained by a pooling process, using the differences in the measured values of duplicate samples (see ref 22, p 316). Then the population standard deviation, σ_p, can be estimated. Unless such calculations are based on a sufficient number of measurements (at least seven), the standard deviations may be significantly overestimated or underestimated. To minimize this problem, the appropriate value of the Student's t distribution (ref 21, Table A-4, page T-5) should be used and t values should be substituted for z in eq 1 and similar expressions.

An equation of the form of eq 1 may be used also to estimate the number of replicate measurements, N_m, required on a homogeneous sample to achieve a mean value within a given confidence interval E

$$N_m = \left(\frac{z\sigma_m}{E}\right)^2 \quad (4)$$

In this case, σ_m represents the standard deviation of the measurement process. Unless N_m is large, the Student's t distribution should be used in place of the standardized normal variate z.

An empirical approach to sampling is used sometimes. For example, the *N–N–N* concept (*18*) has been suggested where equal numbers (*N*) of samples, blanks (a no-treatment control sample), and spiked blanks are to be analyzed. This concept was first used in U.S. Department of Agriculture pesticide residue studies as the "10–10–10 rule". In an extensive monitoring program, the number of field blanks (blanks from a similar source that do not contain the analytes of interest) and blanks spiked with the analytes at known concentrations can be substantially less than the number suggested by the *N–N–N* approach.

Blanks. If field blanks are not available, every effort should be made to obtain blank samples that best simulate a sample that does not contain the analyte. In certain circumstances, a simulated or synthetic field blank is the only alternative.

In addition, measurements should be made to ascertain whether and to what extent any analytical reagents or solvents used contribute or interfere with the measurement results. Good laboratory practices are required to control the level and variability of artifacts and interferences in reagent and solvent blanks, and a sufficient number of reagent and solvent blanks must be measured to evaluate potential interferences with sufficient confidence (*23*, *24*).

Use of Control Sites. When environmental measurements are made to investigate localized contamination (e.g., at a hazardous waste site or a point source discharge), measurement of concentrations at sites recognized as uncontaminated (control sites) is usually required. Such sites and the numbers of samples to be analyzed must be chosen carefully and be adequate to establish clearly the significance of any apparent differences that may be indicated. As the concentration levels in samples from test and control sites approach each other, the analysis of control site samples becomes increasingly important. The planning of environmental analytical programs and interpretation of the results of such monitoring programs will require utilization of statistical principles.

Field Control Samples vs. Laboratory Control Samples. The recovery of spikes is used frequently to evaluate analytical methodology. Uncontaminated samples from control sites that have been spiked with the analytes of interest provide the best information because they simulate any matrix effects. Although commonly referred to as "spiked blanks", when a known amount of a reference material is spiked into a liquid, it becomes a "standard solution"; so "spiked blank" is incorrect terminology and its use is discouraged. Simulated or synthetic field blanks may be spiked when suitable blanks from control sites are unavailable. When feasible, isotopically labeled analytes spiked into samples provide the greatest accuracy since they are subjected to the same matrix effects as the analyte. Exceptions may occur with some solid samples (including biological samples).

The object of spiking a blank or a sample "in the field" (field control samples) at the time samples are collected vs. spiking a sample or blank in the laboratory (laboratory control samples) prior to analysis is to determine if there are any matrix effects caused by time and/or the conditions under which the sample is taken, transported, and stored prior to actual analysis. Although field control samples are a useful type of spike, they are also the most difficult to prepare because of special technical procedures that must be devised to prepare them accurately. Because of this, most spikes are made after samples have been returned to a laboratory, possibly hours, days, or even longer periods of time after the samples were taken.

The recovery of both laboratory and field control samples must be interpreted with due consideration for possible differences of behavior between naturally incorporated substances and those added artificially. For example, if an analyte is strongly associated with any other component in the sample matrix, it may not be possible to recover it as efficiently as from the appropriate blank matrix that has been spiked and then immediately extracted.

MEASUREMENTS

Measurements should be made with properly tested and documented procedures. Furthermore, every laboratory and analyst must conduct sufficient preliminary tests, using the methodology and typical samples, to demonstrate competence in the use of the measurement procedure (*12*). **Procedures should utilize controls and calibration steps to minimize random and systematic errors.** When possible, procedures should provide the required precision, minimum artifact contamination, and the best recovery possible. Contamination can be introduced from sampling containers, equipment, reagents, solvents, glassware, atmosphere and added surrogates, or internal standards. Keeping the number and complexity of operations to a minimum will lessen contamination possibilities from these sources.

To establish confidence in the analytical measurements, data obtained on sensitivity, accuracy, precision, and recovery should be comparable, when possible, to literature values obtained on similar problems. "State-of-the-art" analytical techniques are not always necessary but the results and methods used should be able to stand the test of peer review.

Definition of the Data Set. Environmental analytical measurement programs should be designed to obtain quality assurance data from the following types: calibration standards; field samples; field blanks; spiked field or laboratory blanks; and reference samples. Field sample data are the objectives of an investigation. Field blanks are necessary to account for the presence of spurious analytes, interferences, and background concentrations of the analyte of interest. Spiked field or laboratory samples are required to establish recovery. Reference samples are required as specified in the quality assurance program and project plans to document the quality of the field sample measurements. Calibration standards are required to provide a basis for quantitating the analytes of interest. The frequency and order for measuring a sequence of these various samples and blanks should be defined in the protocols developed in the planning stage of the program.

Preparation of Samples. After a sample has been obtained, the analytical protocol often requires one or more treatments prior to actual measurement of the analytes. Sample preparation may involve physical operations such as sieving, blending, crushing, drying, and/or chemical operations such as dissolution, extraction, digestion, fractionation, derivatization, pH adjustment, and the addition of preservatives, standards, or other materials. These physical and chemical treatments not only add complexity to the analytical process but are potential sources of bias, variance, contamination, and mechanical loss. Therefore, sample preparation should be planned carefully and documented in sufficient detail to provide a complete record of the sample history. Further, samples taken specifically to test the quality assurance system (i.e., quality assurance samples) should be subjected to these same preparation steps.

The analyst must recognize and be aware of the risks that are associated with each form of pretreatment and take ap-

propriate preventative action for each. This may include reporting, correcting for, or possibly removing interferences from the analytes of interest by modifying the protocol. All changes in the protocol must be documented.

Calibration and Standardization. Calibration is the process for determining the correctness of the assigned values of the physical standards used or the scales of the measuring instruments. Typical calibrations include standards for mass, volume, and length; also, instruments that measure temperature, pH, and chemical composition can be calibrated. The term standardization is used frequently to describe the determination of the response function of analytical instruments.

Calibration accuracy is critically dependent on the reliability of the standards used for the required intercomparisons. Likewise, chemical calibrations or standardization depends critically upon the quality of the chemicals used to provide the necessary standard solutions and the care exercised in their preparation. Where possible, calibration should be performed by suitable regression analysis of the net signal on the analyte concentration. **At least three different concentrations of calibration standards should be measured in triplicate, but more than three different concentrations are recommended.** The concentrations of the calibration standards must bracket the expected concentration of the analyte in the samples. **No data should be reported beyond the range of calibration of the methodology.** The calibration data, when plotted graphically, are referred to as a calibration curve. The calibration must be done under the same instrumental and chemical conditions as those that will exist during the measurement process, and the frequency of calibration depends on the accuracy requirements of the investigation and the stability of the instrument used for the measurements.

Internal standardization involves the addition of a reference material to the sample. External standardization involves use of a reference material separately. The internal standard material is chosen to simulate the analyte of interest; the external standard material is usually the analyte being measured. The ratio of the response of the internal standard to the analyte response is called the response constant and is used to calculate analyte concentration.

Surrogates are sometimes incorrectly termed internal standards. Compounds closely related chemically to the analyte of concern may be used as spikes; in this document, this technique is known as "surrogate spiking". The difference is that an internal standard is the reference material against which the signal from the analyte is compared directly whereas the signal from a surrogate is not used directly for quantitation. Both types of reference materials are chosen to simulate the analyte of interest. Surrogates may be used indirectly for quantitation of analytes as, for example, in determining recovery efficiency during sample pretreatment.

Another technique is one of "standard addition" where successive, increasing known amounts of analytes are added to the sample or aliquots of it. In each of these techniques, the spiked sample is analyzed under exactly the same conditions as the actual sample, including all phases of pretreatment. Recoveries of the analytes of interest are inferred from those found when using spiked materials.

It is essential to demonstrate that the spiking procedure is valid. It must be shown either that the spiked chemicals equilibrate with the corresponding endogenous ones, or that the recovery of the spiked chemicals is the same as the recovery of the endogenous chemicals, within experimental error, over the full range of concentration levels to be analyzed (*24*).

Recovery. Recovery of analytes is influenced by such factors as concentration of the analytes, sample matrix, and time of storage. Because recovery often varies with concentration, **the spike and the analyte concentrations should be as close as practical.** When the spike and/or analyte concentrations are close to the background concentration, recoveries can be highly variable.

Matrix effects can cause wide variability in recoveries, especially with organic compounds. Therefore, to be valid, **recoveries of a spike standard must be determined in the same matrix as the sample.** It is not unusual to find significant recovery differences for organic standards spiked into samples of industrial wastewaters that have been taken only days (or even hours) apart because the composition of wastewater samples often changes significantly with time. Another consideration is the amount of time a sample has been stored before sample pretreatment and analysis. Analyzing a sample that has been stored for a long period of time will sometimes give different values for the analytes than when the sample is analyzed while fresh. This difference may be caused by changes in the matrix and/or the analytes. Therefore, if it is anticipated that a sample is going to be stored for an appreciable period of time before analysis, it should be demonstrated that significant changes have not occurred. What an "appreciable period of time" is depends on the analytes, sample matrix, and other chemical and physical factors that are determined in the planning phase. An appropriately designed quality assurance program can assist in determining the potential effects of storage time on analyte loss and will specify maximum holding times as necessary.

Variable recovery is sometimes resolved by using the isotope dilution technique in which isotopes of the analytes being measured are spiked directly into the sample. Both radioactively and nonradioactively labeled compounds or elements can be used. This principle is based on the assumption that the labeled compound or element will behave in an essentially identical manner to the unlabeled analyte of interest.

Generally, analytical values should be reported as measured (uncorrected for recovery) with full and complete supporting data involving recovery experiments. If the measurements are reported as "recovery-corrected", all calculations and experimental data should be documented so that the original uncorrected values can be derived if desired. In carrying out recovery studies, the analyst should recognize that an analyte added to a blank sample may behave differently (typically, showing higher recovery) than an analyte in a field sample. In such a case, the method of standard addition tends to lead to erroneously low values. Whenever possible, testing should include experiments on homogeneous working standards containing known amounts of naturally incorporated analyte. Unfortunately, the frequent lack of such environmental standard reference materials is a major limitation in analyses and is a more acute problem with organic standards than with inorganic or elemental standards.

Interferences. Because of the complexity of environmental samples and the limited selectivity of most methodologies, interferences are common during analysis. **Appropriate controls and experiments must be included to verify that interferences are not present with the analytes of interest or, if they are, that they be removed or accommodated.** Interferences that are not accommodated cause incorrect analytical results, including false positives and false negatives.

Interferences arise from two sources that may occur simultaneously: constituents that are inherent in the sample; and artifacts or contaminants that have been introduced during the analytical process. **A good measurement plan and quality assurance program, incorporating an appropriate experimental design and field and method blanks, is critical to identifying the sources of interferences in a sample and differentiating between interferences and artifacts.** Interferences are generally removed by modifying the methodology. Artifacts are avoided

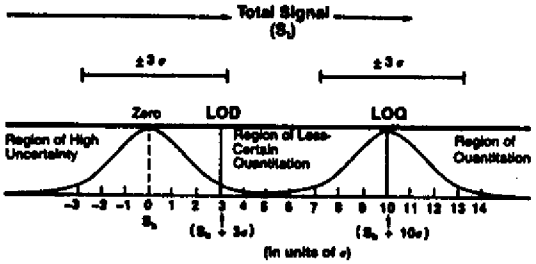

Figure 4. Relationship of LOD and LOQ to signal strength. The LOD is located 3σ above the measured average difference in the total (S_t) and blank (S_b) signals; the LOQ is 10σ above S_b.

or identified by an appropriate, comprehensive quality assurance program.

Limit of Detection (LOD). The limit of detection (LOD) is defined as the lowest concentration level that can be determined to be statistically different from a blank. The concept is reviewed in ref 25 together with the statistical basis for its evaluation. Additional concepts include method detection limit (MDL), which refers to the lowest concentration of analyte that a method can detect reliably in either a sample or blank, and the instrument detection limit (IDL), which refers to the smallest signal above background noise that an instrument can detect reliably. Sometimes, the IDL and LOD are operationally the same. In practice, an indication of whether an analyte is detected by an instrument is sometimes based on the extent to which the analyte signal exceeds peak-to-peak noise. The IDL and especially the MDL are important parameters for comparing and selecting instrumentation and methodology. The experimental determination of the MDL is discussed in ref 26.

The question of detection of a given analyte is often one of the most important decisions in low-level analysis. The question that must be answered is whether a measured value is significantly different from that found for the sample blank. Let S_t represent the total value measured for the sample, S_b the value for the blank, and σ the standard deviation for these measurements. The analyte signal is then the difference $S_t - S_b$. It can be shown that for normal distributions $S_t - S_b > 0$ at the 99% confidence level when that difference $(S_t - S_b) > 3\sigma$. The recommended value of LOD is 3σ. LOD is numerically equivalent to the MDL as S_b approaches zero.

Limit of Quantitation (LOQ). The limit of quantitation (LOQ) is defined as the level above which quantitative results may be obtained with a specified degree of confidence. Confidence in the apparent analyte concentration increases as the analyte signal increases above the LOD. The value for LOQ = 10σ is recommended, corresponding to an uncertainty of ±30% in the measured value ($10\sigma \pm 3\sigma$) at the 99% confidence level.

The LOQ is most useful for defining the lower limit of the useful range of measurement methodology. This range extends from this lower value to an upper value where the response is no longer linear and sometimes is referred to as the limit of linearity.

The LOD and LOQ are shown graphically in Figure 4. The base scale is in units of standard deviation of the measurement process which is assumed to be the same for all of the measurements indicated.

Considerations of the above guidelines for reporting low-level data are given in Table I. In using the table, it should be remembered that the concentration levels indicated refer to interpretation of single measurements.

Signals below 3σ should be reported as "not detected" (ND) and the limit of detection should be given in parentheses: ND (LOD = value). Signals in the "region of less-certain quantitation" (3σ to 10σ) should be reported as detections with the limit of detection given in parentheses. The practice of using the symbols "T" or "tr" for amounts and the term "trace" and similar statements of relative concentration should be avoided because of the relative nature of such terminology, the confusion surrounding it, and the danger of its misuse.

Table I. Guidelines for Reporting Data

analyte concn in units of σ ($S_t - S_b$)	region of reliability
$< 3\sigma$	region of questionable detection (and therefore unacceptable)
3σ	limit of detection (LOD)
3σ to 10σ	region of less-certain quantitation
10σ	limit of quantitation (LOQ)
$> 10\sigma$	region of quantitation

Data measured at or near the limit of detection have two problems. The uncertainty can approach and even equal the reported value. Furthermore, confirmation of the species reported is virtually impossible; hence the identification must depend solely on the selectivity of the methodology and knowledge of the absence of possible interferents. These problems diminish when measurable amounts of analytes are present. Accordingly, **quantitative interpretation, decision-making, and regulatory actions should be limited to data at or above the limit of quantitation.**

It must be emphasized that the LOD and LOQ are not intrinsic constants of the methodology but depend upon the precision attainable by a laboratory when using it, which can be very diverse. Attainable precision can also vary according to the matrix analyzed. Published values of LODs must be considered only as typical. Each laboratory reporting data must evaluate its own precision and estimate its own LOD and LOQ values when they are important aspects of the data.

Analytical chemists must always emphasize to the public that **the single most important characteristic of any result obtained from one or more analytical measurements is an adequate statement of its uncertainty interval** (*14*). Lawyers usually attempt to dispense with uncertainty and try to obtain unequivocal statements; therefore, an uncertainty interval must be clearly defined in cases involving litigation and/or enforcement proceedings. Otherwise, a value of 1.001 without a specified uncertainty, for example, may be viewed as legally exceeding a permissible level of 1.

DOCUMENTATION AND REPORTING

Documentation of analytical measurements should provide information sufficient to support all claims made for all the results. Documentation requires all information necessary to (1) trace the sample from the field to the final results, (2) describe the methodology used, (3) describe the confirmatory evidence, (4) support statements about detectability, (5) describe the QA program and demonstrate adherence to it, and (6) support confidence statements for the data.

Data, including all instrumental output, must be recorded in laboratory notebooks or other suitable media and should include complete sample documentation, transfers and movement, sample number, initial sample weight, extraction volume, final weight and volume analyzed, instrument response, sample calculations, and concentration of sample as appropriate. The time that "spiked" samples and blanks were run relative to measurement of the analyte is of utmost importance when measurements are attempted close to the limit

of detection. **Laboratory records should be retained in a permanent file for a length of time set by government, other legal requirements, or the employing institution, whichever is longer.** Bound notebooks are preferred to looseleaf-type notebooks.

Electronic data handling, reduction, storage, and transmission systems greatly facilitate data handling and help minimize errors due to misreading, faulty transcription, or miscalculations. However, the performance of the data system must be tested periodically with known data that have already been calculated; this should be part of the quality assurance program. These tests must have sufficient diversity and rigor to provide a reliable test of the data handling system.

The analytical chemist is responsible for fully describing and interpreting the data and reporting it in an appropriate manner.

Measurement results should be expressed so that their meaning is not distorted by the reporting process. The public at large will not be able to recognize that 10000 ng/kg and 10 μg/kg are the same.

Data should be reported only to the number of significant figures consistent with their limits of uncertainty. When appropriate, the relationship between individual sample values, blanks, recoveries, and other supporting data should be shown.

If possible, and within the scope of desired results, a number of measurements sufficient for statistical treatment should be made. When this is not the case, an explanation is necessary including complete details of the treatment of the data.

Reports should make clear which results, if any, have been corrected for blank and recovery measurements. Any other limitations should also be noted.

Conclusions as to whether a signal is detected, whether a positive signal is confirmed to be an analyte, how much uncertainty is contributed by the sampling, and the risk of systematic error are best made by those involved in the study and should be included in any report. **Reports should contain sufficient data and information so that users of the conclusions can understand the interpretations without having to make their own interpretations from raw data.**

If a published methodology is used, it should be cited. Any modification, as well as any new methodology or new approach to the making of measurements or interpreting the results, must be described in detail, including test results and details of its validation.

Raw data for each sample, along with reagent blanks, control, and "spiked" samples should be suitably identified if included in a report. **If average values are reported, an expression of the precision, including the number of measurements, must be included.** Details should be written with the standard deviation of the mean and presented showing that the averaging process accounts for sample heterogeneity as well as observed imprecision among replicate measurements of homogenized samples (*1*).

ACKNOWLEDGMENT

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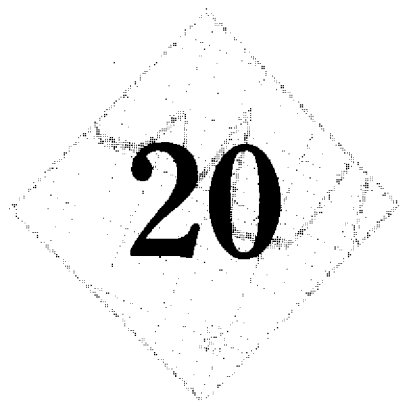

Quality Control in Water Analyses

Cliff J. Kirchmer

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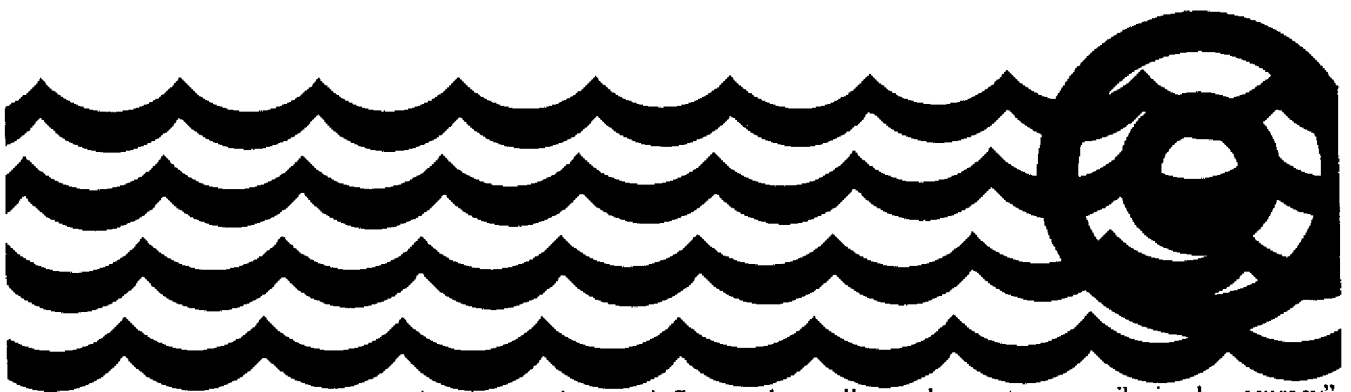
Quality control in water analyses

The definitions and principles underlying the practice of quality control need to be critically evaluated



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Until the late 1960s, there was only limited official recognition of the need for analytical quality control in water analyses. In January 1967, the Federal Water Pollution Control Administration (FWPCA) held its first meeting on the subject and established the Committee on Methods Validation and Analytical Quality Control. An analytical quality control laboratory was subsequently established in the FWPCA's Division of Research (the FWPCA was later replaced by the Environmental Protection Agency). This laboratory has evolved into what is now the Environmental Monitoring and Support Laboratory. Throughout its existence, it has played an important role in providing the necessary leadership for developing analytical quality control procedures and encouraging their use in environmental laboratories.

In 1971, the EPA published the "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," which has since undergone several revisions. It provided the first comprehensive discussion of factors that can influence the quality of data in water analyses and was written so that it could be used by a laboratory for self-evaluation. Also, recent editions of "Standard Methods for the Analysis of Water and Wastewater" have included expanded sections that have contributed to the development of criteria and methodology for analytical quality control.

Even though the importance of quality control has been recognized, some of the more commonly used definitions in this subject area need to be clarified, and principles that have been established but are not uniformly applied in practice need to be emphasized. This article discusses these definitions and principles and makes specific recommendations for their application.

Accuracy

The expression "accuracy and precision" is used commonly to characterize the performance of analytical methods. There is no general agreement regarding the meaning of this phrase, however. Churchill Eisenhart referred to this problem when he wrote: "It is most unfortunate that in everyday parlance we often speak of 'accuracy and precision' because accuracy requires precision, but precision does not necessarily imply accuracy" (*1*).

R. B. Murphy compared the situation to a marksman aiming at a target: "We would call him a precise marksman if in firing a sequence of rounds, he were able to place all his shots in a rather small circle on the target. Any other rifleman unable to group his shots in such a small circle would naturally be regarded as less precise. Most people would accept this characterization whether either rifleman hits the bull's-eye or not" (*2*). Precision in water measurements has been defined by the EPA as "the degree of mutual agreement among individual measurements made under prescribed conditions" with a "single test procedure" (*3*). While there is general agreement about the meaning of precision, there is less agreement about the meaning of accuracy and its relationship to precision.

The problem in defining the term "accuracy" is the discrepancy between the accuracy of "individual analytical measurements" and the accuracy of "average values" obtained from a number of replicate measurements. In terms of the bull's-eye analogy, Murphy states that "one school of thought on the subject of accuracy insists that if a marksman hits the bull's-eye 'on

the average,' then he is accurate even though [he] may have a wavering aim so that his shots scatter. The point is that accuracy in this sense is determined solely by the behavior of the long-run average of the shots. The position of the average shot is assumed, of course, to be the centroid of the bullet holes in the target: few shots might actually hit or nearly hit the bull's-eye.

"The second school of thought on accuracy would insist that if the [marksman] is unlikely to be very close to the bull's-eye, he should be termed an inaccurate shot. That is, the second school holds to the belief that accuracy should imply that any given shot is very likely to be in the bull's-eye or very near to it" (*2*).

It is difficult to say which of these definitions is more correct, but it may be possible to decide which is more useful when applied to water analysis. Reported results of analyses are usually based on "individual analytical measurements" rather than "averages" of replicate determinations. Duplicate determinations are made on perhaps 5–10% of the samples as a quality control measure, but extensive replication is not economically feasible. Thus, we are concerned with the "accuracy" of these individual analytical measurements.

It is interesting to note that while statisticians in the U.S. have clearly recognized the two ways of defining accuracy, the definition that equates accuracy with bias or systematic error has been commonly used in water analyses. For example, the EPA has defined accuracy as "the difference between an average value and the true value when the latter is known or assumed" (*3*).

In contrast, the Water Research Centre in England bases its definition of accuracy on individual analytical measurements. The error, E, of an analytical result, R, is defined as: $E = R - \tau$ where τ is the true value. Accuracy is then defined as the total error of a result; that is, accuracy represents the combined random and systematic errors of results and is said to improve as the total error decreases (*4*).

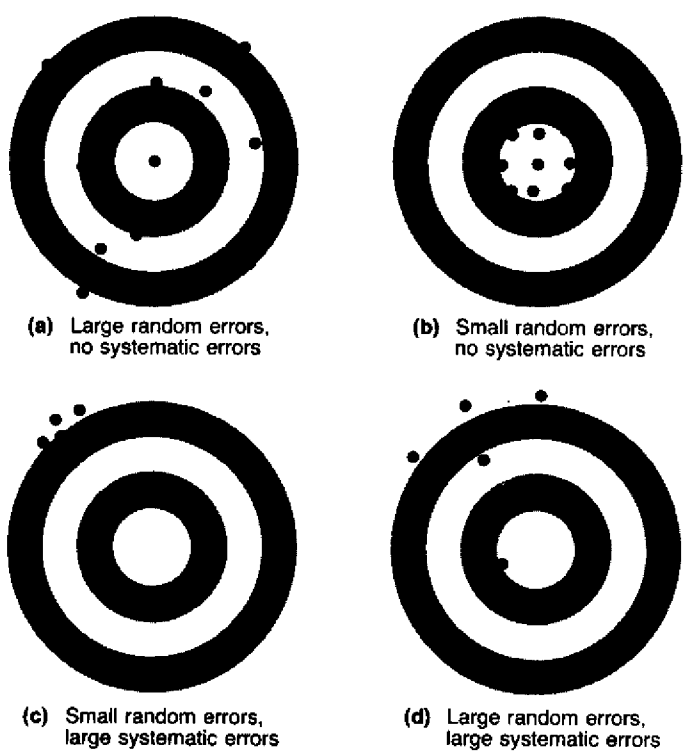
The Water Research Centre defines systematic error as follows. The mean of n analytical results on the same sample approaches a definite value, μ, as the number of results is increased indefinitely. When μ differs from the true value, τ, results are said to be subject to systematic error of magnitude B, where $B = \mu - \tau$. The term "bias" is used synonymously with systematic error.

Note that in the Water Research Centre definition, precision (which is a measure of random error) is a part of accuracy. One then speaks of precision and bias (the two components of accuracy) rather than precision and accuracy. Also, under this definition, it is impossible to speak of measurements being accurate but not precise, since precision is a component of accuracy.

In statistical terminology, common practice in the U.S. has been to define accuracy by comparing the mean of n measurements with the true value, and to define precision separately as the standard deviation, s, of n analytical measurements (*3*, *5*). The Water Research Centre approach is more realistic in that it defines accuracy as the difference between individual analytical measurements and the true value, this difference corresponding to the sum of bias (systematic) and random errors.

The bull's-eye analogy described by R. B. Murphy has been used in both the U.S. and Great Britain to graphically illustrate the types of error that

FIGURE 1
Water Research Centre interpretation of the bull's-eye analogy for describing analytical error



Source: Reference 4

can occur in water analysis. The first school of thought has dominated the interpretation of this bull's-eye analogy in the U.S. and has been included even in government training manuals (*6*). In contrast, the Water Research Centre subscribes to the second school of thought; its interpretation of the bull's-eye analogy is illustrated in Figure 1 (*4*). The major difference of interpretation is shown in Figure 1a, which represents "accurate but imprecise" data in the U.S. (*6*). The Water Research Centre avoids the terms accuracy and precision entirely, referring only to random and systematic errors.

Using an appropriate definition of accuracy has important practical consequences. With the definition commonly used in the U.S., methods giving very imprecise results can be characterized as accurate, when individual analytical measurements are clearly not accurate. A definition of accuracy based on individual analytical measurements, which includes the effects of random as well as systematic errors, is clearly more useful.

Analytical method

Before discussing the subject of bias in water analysis, it is useful to define "analytical method." The following definition has been proposed by A. L. Wilson: "An analytical method is to be regarded as the set of written instructions completely defining the procedure to be adopted by the analyst in order to obtain the required analytical result" (*7*).

Wilson states that this definition has two important consequences: A distinction must be made between the errors of analytical methods and the errors of results, and great emphasis must be placed on specifying methods accurately and completely. Under this definition, it is clear that one should not make such statements as "the precision of the method is. . . ." The correct form would be "when the method was followed, analytical results were obtained with a precision of. . . ."

A number of terms have been used to indicate the status of a method (i.e., "standard" method, "tentative" method, "approved" method) or hierarchy of a method (i.e., technique, method, procedure, protocol). There is probably a place for these terms within the classifications of standard methods devised by committees or regulatory agencies. The relative merits of these terms will not be discussed further, however, since the aim of this article is to emphasize the importance of the performance of individual laboratories.

The Water Research Centre has emphasized the value of supplying quantitative evidence about the performance of an analytical method. It recommends that a summary of performance characteristics, using clearly defined criteria, be included at the beginning of each published method. With this approach, methods are not classified arbitrarily, and one is able to answer the basic question: Does this method meet my analytical requirements? A representative tabulation of performance characteristics used by the Water Research Centre is given in Table 1 (*8*).

Bias

It has been stated that there are six possible sources of bias or systematic error in water analyses (*4*):

- unrepresentative sampling,
- instability of samples between sampling and analysis,
- interference effects,
- biased calibration,
- a biased blank correction, and
- inability to determine all forms of the determinand (that which is to be determined). For example, if "total iron" is to be measured, the method should give "total iron" and not soluble ferrous iron.

Because they are more directly associated with the problems of definitions and principles in analytical quality control, the third, fourth, and fifth items are the only ones discussed here.

Calibration bias. Related to the definition of analytical method is the Water Research Centre's insistence that blanks, standards, and samples be analyzed by exactly the same procedure. Failure to do this may result in either a biased blank correction or a biased calibration. Different procedures are acceptable only if there is experimental evidence that they produce results that differ by a negligible amount.

A survey of "standard methods" and "government-approved methods" of water analyses in the U.S. indicates that the possibility of biased calibration curves or biased calibration factors resulting from the use of different analytical procedures for standards and samples is not widely recognized. It is common for calibration standards

TABLE I

Example of tabulation of performance characteristics used by the Water Research Centre

1. Substance determined	Those forms of manganese reacting with formaldoxime after collection of the sample into acid.	
2. Type of sample	Fresh and treated waters.	
3. Basis of method	Manganese reacts with formaldoxime to form a colored complex whose concentration is measured absorptiometrically.	
4. Range of application[a]	Tested over the range 0–0.5 mg Mn/L.	
5. Calibration curve[a]	At 450 nm the curve is linear to at least 1.0 mg Mn/L	
6. Total standard deviation (S_t)[a,b]	Manganese concentration (mg Mn/L)	Total S_t (mg Mn/L)
	0.05	0.002
	0.1	0.004
	0.2	0.008
	0.5	0.011
	1.0	0.022
7. Criterion of detection[a]	0.001 mg Mn/L.	
8. Sensitivity[a]	0.5 mg Mn/L corresponds to 0.19 optical density units.	
9. Bias	No bias detected except when interferences occur.	
10. Interferences	Large concentrations of ferrous and ferric iron may interfere.	
11. Time required for analysis	For six samples, the analytical and operator times are both approximately 75 min.	

[a] These data were obtained at the Water Research Centre using a Hilger Uvispek spectrophotometer with 40-mm cuvettes at 450 nm.
[b] The data refer to standard solutions in distilled water; certain samples may tend to give worse precision.

to be subjected only to the final step in an analytical procedure. This is justified as being the only "practical" approach to analysis, particularly for those methods involving long and complex concentration and separation procedures. According to A. L. Wilson, however, these methods present the greatest possibility for bias due to improper calibration procedures (*9*).

While "bias in the calibration procedure" is usually not recognized by that name in the U.S., procedures have been recommended to estimate this error. They are usually termed "procedures for estimating recovery." For example, the recently published "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry" states that "the recovery of a method is derived from the measurement of spiked blanks" (*10*). The difference between the actual recovery and theoretical recovery of 100% corresponds to the bias in the calibration procedure. For methods that give very low recoveries, changes in the calibration procedure should be made whenever possible to correct for this source of bias.

An alternative approach would be to use recovery estimates to correct for the calibration bias. Unfortunately, many methods prohibit this type of correction, although it would clearly permit a better estimate of the true concentration in the water sample. For example, EPA Method 608 for organochlorine pesticides and PCBs says to "report results in micrograms per liter without correction for recovery data." A similar restriction in EPA Method 615 for chlorinated herbicides states that one should "report results in micrograms per liter as the acid equivalent without correction for recovery data." These methods seem intended to measure the quantities of pollutant in the final extracts, rather than in the original water samples.

While not as critical to the analytical results, the use of the increasingly common term "spiked blank" is not recommended. When a known amount of an element or compound is added to a known amount of water, this is a standard solution, not a "spiked blank." Standard solutions can be used either for calibration (calibration standards) or as a check on precision or calibration bias (check standards). Thus, instead of writing that "the recovery of a method is derived from the measurement of spiked blanks," it is preferable to state that the calibration bias of a method can be estimated by the analysis of check standard solutions (where the check standard solutions are analyzed exactly as the samples are analyzed).

Blank correction bias. To obtain accurate analytical results, particularly when performing trace analyses, it is necessary to make a blank correction of sample responses. O'Haver has stated that "in strictest terms, the blank is a solution that contains everything in the sample solution except the analyte" (*11*). Blank correction of individual samples—that is, subtracting a separately determined blank response from each sample response—is the recommended procedure based on statistical considerations, and it is generally accepted in principle, although sometimes ignored in practice. At a minimum, a blank should be analyzed along with each batch of samples. (Subtracting blank response from sample response is valid only for procedures in which calibration is of the form $A = \alpha + \beta C$ where A = response, C = concentration, and α and β are constants. Only these types of procedures are considered in this article.)

It is equally important to recognize that blank determinations should be made by exactly the same procedure as that used for the samples. For example, according to O'Haver, "in analytical procedures involving sample preparation, separation, or preconcentration steps, it is almost always essential that a blank be carried through the entire procedure" (*11*). If this is done, then blank-corrected results for water analyses should not present any problem of bias due to the blank, provided that the concentration of the determinand in the water used for the blank is negligible. On the other hand, when the concentration of the determinand in the water used for the blank is not negligible, it is essential to determine its concentration and make an appropriate correction in the blank value.

Bias due to interference. As indicated previously, the term "recovery" has been used in the methods for measuring the bias of the calibration procedure. More commonly (and correctly), the term recovery has been used to indicate the percent "recovered" when a sample is spiked with a known amount of compound. Recovery is the difference between the analytical results before and after spiking, divided by the known amount of spiking compound and multiplied by 100 to convert to percentage. The difference between the actual recovery and theoretical recovery (100%) is considered to be caused by interference. In other words, the recovery test is used to determine the presence of bias due to interference. The term recovery should be limited to this usage only and not to the evaluation of bias in the calibration procedure.

The recovery test is not very powerful in a statistical sense. The experimental recovery is obtained from the difference between two measurements (sample and spiked sample), each of which is subject to random error. Even in the absence of interference effects, significant deviations from 100% recovery are common. For example, Water Research Centre Technical Report 66 indicates that if the standard deviations of spiked and unspiked samples are equal and the amount of spiking material is 10 times the standard deviation, the expected recovery would lie between 72% and 128% at the 95% confidence level, even in the absence of interference (*4*). In addition, the recovery test does not detect interfering species whose effects are independent of the determinand concentration. This can be another important limitation.

Control charts

A common procedure for analytical quality control is to analyze check standards, duplicates, and spiked samples in amounts corresponding to approximately 10% of the samples analyzed (*3*). As subsequent data are accumulated, these analyses should be used to prepare control charts defining accuracy and precision. As already noted, when referring to tests for interference, accuracy control charts are better termed bias control charts.

Because laboratories operate under budget constraints and limited time, some order of priority should be assigned to the different kinds of control tests. In some European laboratories (*4, 12*), first priority has been given to precision control charts based on the analysis of standard solutions. (These standard solutions should be prepared independently of the calibration standards to provide a truly independent check, including the accuracy and stability of the stock calibration standard solution.) The next priority is the preparation of precision control charts based on duplicate analyses of actual samples. Bias control charts based on the recovery of spiked samples are the third priority. Finally, control charts based on blanks can be plotted to detect changes in the quality of reagents, etc. The latter are not true control charts since there are no control limits. Using this priority listing contrasts sharply with the common practice of devoting equal time to the analysis of

standards, duplicate samples, and spiked samples.

It should be added, however, that the order of priority for control charts depends somewhat on the determinand. For example, one may not be able to prepare appropriate standard solutions for "suspended solids" and biochemical oxygen demand; for such determinands, the other types of control tests may be more important.

Several kinds of quality control charts have been recommended, including Cumulative-Summation (CuSum) and Shewhart charts (*3*). But if the emphasis is on the accuracy of individual analytical results, it is sufficient to simply plot the measured values on a chart in which $\mu \pm 2\sigma$ defines the "warning limits" and $\mu \pm 3\sigma$ defines the "action limits," where μ is the arithmetic mean and σ is the standard deviation. This type of chart has been described in "Standard Methods" (*5*), and detailed instructions for construction of the four previously mentioned categories of charts have been given in a publication by the World Health Organization Regional Office for Europe (*12*). An example of the most commonly used control chart, based on the use of standard solutions, is given in Figure 2. Note that the control limits in this chart are based on estimates of standard deviations and, as such, must be updated periodically by "pooling" preliminary and recent estimates. Note also that the warning and action limits in this case are plotted symmetrically around the expected mean concentration. This is because the true concentration of the solution is known. Any trend indicating that the statistical mean is higher or lower than the expected mean would signify that calibration bias probably affects the measurements.

Method validation

In the U.S., tests are often performed before a laboratory begins to use a method for routine sample analyses. These tests are commonly called "method validation" procedures. Few guidelines have been written, however, that provide optimal experimental designs for method validation.

In general, a good experimental design would enable the laboratory to estimate precision for the analysis of both standard solutions and real samples, to estimate bias due to interference in the analysis of real samples, and to estimate a lower limit of detection for the method.

Wilson has proposed an experimental design using analysis of variance to give a preliminary estimate of precision (*13*). This design was later expanded to include estimates of some sources of bias as well as limits of detection (*4*). The design specifies that analyses be performed in several batches over a period of one to two weeks. Each batch consists of replicate analyses of blanks, standard solutions, samples, and "spiked" samples. In general, to estimate precision over a range of concentrations, at least two standard solutions are recommended with the upper and lower concentrations of interest. This is necessary because it is common for precision to worsen with increasing concentration, and therefore the standard deviation at one concentration will not necessarily be the same as the standard deviation at another. A detailed discussion of experimental design is beyond the scope of this article, but clearly there is a need to apply these techniques more often in our laboratories and to be more critical of the approaches to "method validation" now being used.

Limit of detection

Many definitions of the term "limit of detection" or "detection limit" have been proposed in the literature. In recent years, however, there have been indications that some consensus is being reached. It is generally agreed that in qualitative terms, limit of de-

FIGURE 2
Example of a control chart for a standard solution

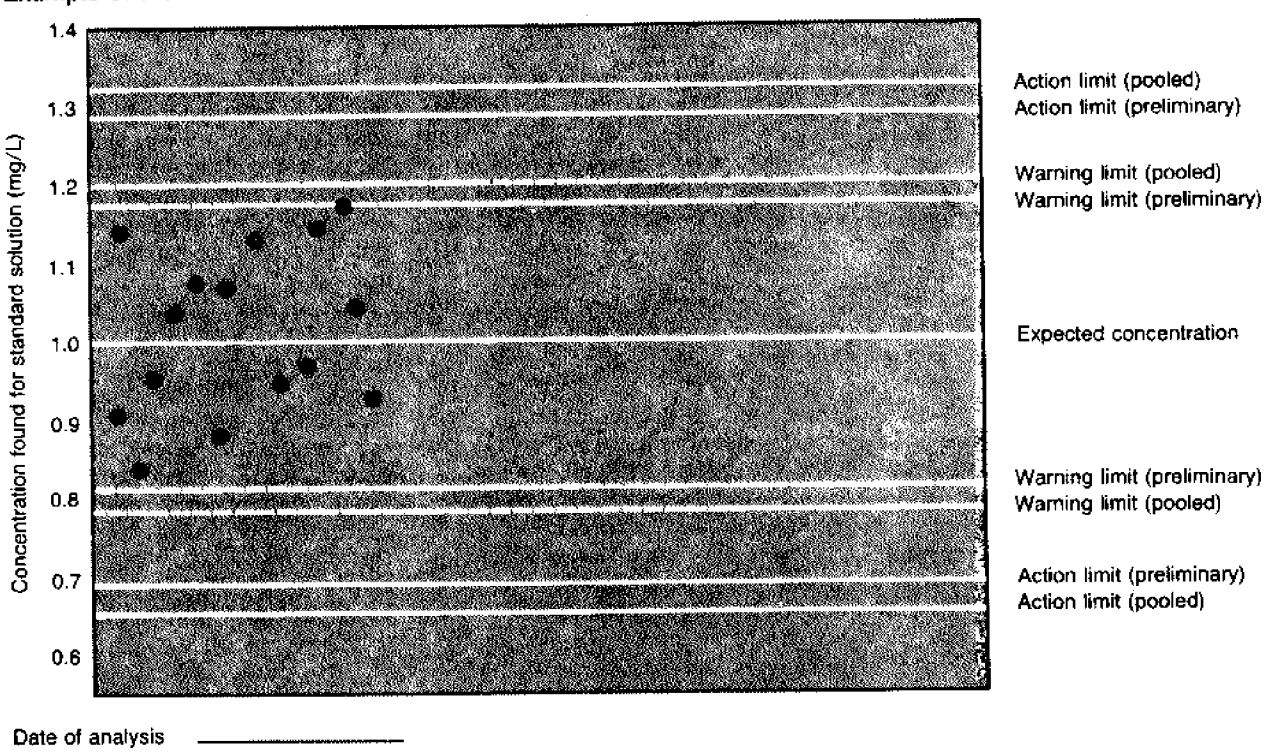
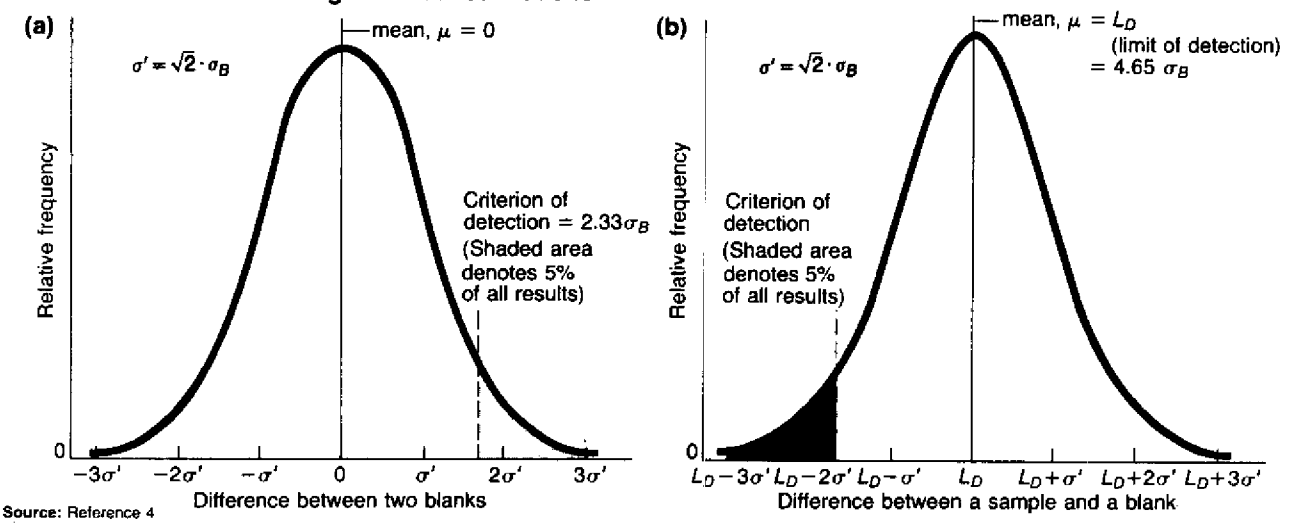


FIGURE 3
Statistical basis for detecting small concentrations



Source: Reference 4

tection is the lowest concentration of determinand that the analytical process can reliably detect. Because an analytical result is generally equal to the difference between the responses obtained for sample and blank, the variability of the blank response has been recognized as the determining factor for estimating the limit of detection for many methods.

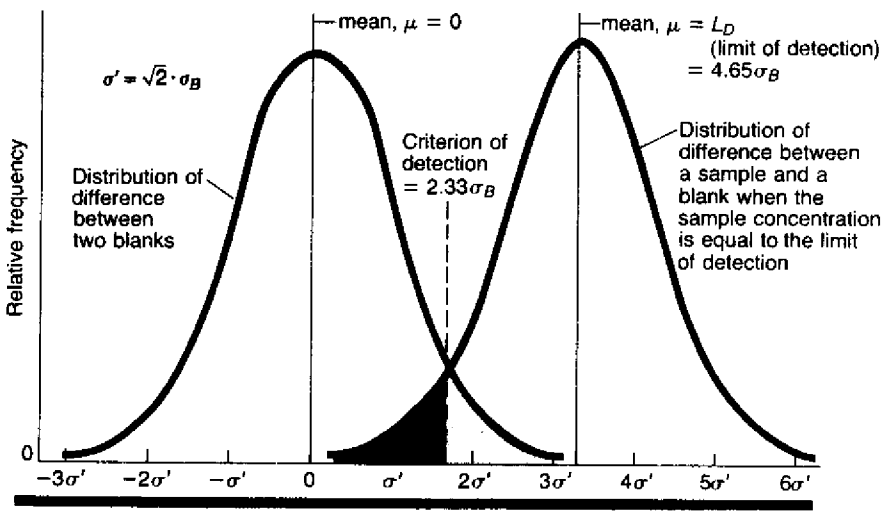
The following general equation has recently been accepted by several authors for defining the limit of detection (*10*, *14*, *15*):

$$L_D = K_d \sigma_B \qquad (1)$$

where L_D = limit of detection, K_d = constant obtained from a statistical evaluation of blank responses, and σ_B = the within-batch standard deviation of the blank.

When the limit of detection is treated statistically, it is assumed that analytical results follow a normal distribution. Figure 3a illustrates the distribution of results for the differences of pairs of blank determinations, each pair of blanks being measured in the same batch of analyses. The distribution has a mean of zero and a standard deviation of $\sqrt{2}\ \sigma_B$. (Note: the property of additivity of variances results in the standard deviation of the differences between blank determinations being $\sqrt{2}$ greater than the standard deviations of the blank responses themselves.) Thus the difference of $+1.65\ \sqrt{2}\ \sigma_B$ ($=2.33\ \sigma_B$) will be exceeded, on the average, only once in every 20 occasions. The analytical result (R) is obtained by subtracting the result of a blank determination (B) from that of a sample (S), i.e., R = S − B. If we now analyze a sample and a blank in the same batch and the difference (S − B) is greater than 2.33 σ_B, there is less than a 5% chance that the sample contains the same concentration of the determinand as the blank. This value, 2.33 σ_B, has been called the criterion of detection and is based on an evaluation of the risk of an *error of the first kind* (that is, the error of accepting an apparent effect arising by chance as a real effect).

FIGURE 4
Illustration of the relationship between the criterion of detection and the limit of detection



There is also the *error of the second kind*, corresponding to the error of failing to recognize a real effect. In the problem under consideration, this would correspond to concluding that the sample contains the same concentration of the determinand as the blank, when in reality the determinand concentration is greater in the sample.

If we assume a case where the sample concentration is equal to the previously defined criteria of detection, it is apparent that there is a 50% chance of an error of the second kind.

The limit of detection is defined such that the error of the second kind has a lower value (that is, in Figure 3b, the significance level associated with the error of the second kind is equal to 5%) and the limit of detection is 4.65 σ_B. Both Currie (*14*) and Wilson (*15*) suggest this value for defining the detection limit.

Figure 4 summarizes and illustrates the relationship between the criterion of detection and the limit of detection.

Values other than 5% have been chosen for the significance levels associated with errors of the first and second kinds, resulting in different values for K_d in the general equation for limit of detection. For example, the

American Chemical Society's Subcommittee on Environmental Chemistry has recommended using a value of 3 for K_d. This treatment is apparently based, however, on the use of a "well-known" blank rather than paired observations; for paired observations, $K_d = 4.23$. This value is said to imply definite risks of 7% for false positives and false negatives. (The precise value is 6.68%.)

When standard deviation values for a population are known, it is not critical whether a 5% or a 7% risk level is chosen. But in real situations, the limit of detection (L_D) must be estimated from a restricted number of blank measurements, and the following equation is recommended:

$$L_D = 2\sqrt{2}t\,s_B \qquad (2)$$

where t = the 5% point of the single-sided t statistic and s_B = estimated within-batch standard deviation of the blank.

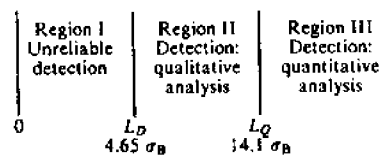
While t values corresponding to the 5% confidence level are usually listed in statistical tables, those corresponding to the 7% confidence level are not. Thus, it would appear that the detection limit expression of Currie and Wilson is more easily applied in practice. It is important to recognize the weaknesses of this definition. These include the following assumptions:

- The within-batch standard deviations of both the blank and samples containing very small concentrations of determinand are the same.
- The analytical response is not zero for finite concentrations of the determinand.
- The sample and blank are not biased with respect to each other, (that is, there are no interfering substances in the sample or the blank).

If any one of these assumptions is not true, then the detection limit cannot be calculated using the equations given previously.

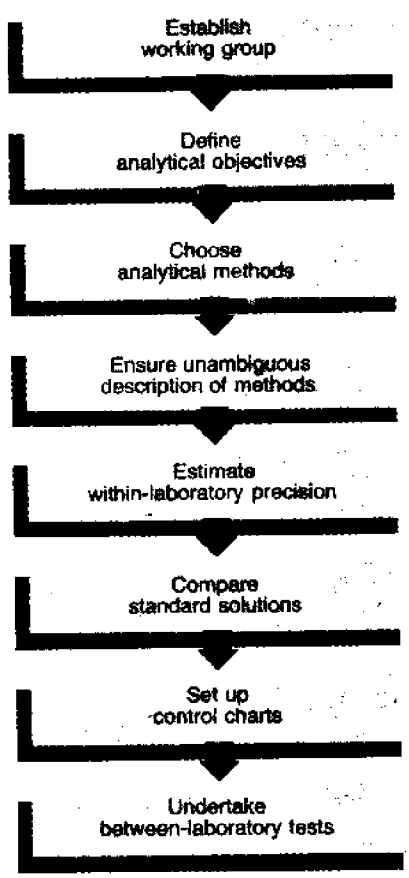
It has been stressed that the limit of detection should apply to a complete analytical procedure and not to a given instrument or instrumental method (*11*, *16*). O'Haver also states that a concentration at the detection limit can only be detected, as the term "detection limit" implies, and not measured quantitatively (*11*). In fact, when using Currie and Wilson's definition, the random error at the limit of detection is equal to approximately 66% of the limit of detection at the 95% confidence level.

As a consequence of this high random error for concentrations at or approaching the limit of detection, L_D, Currie suggested the use of another term, the determination limit, L_Q, for which the relative standard deviation is 10% (that is, $L_Q = 14.1\ \sigma_B$). For practical purposes, the following three principal analytical regions defined by Currie can be applied in water analyses:



In using these definitions, when a measured value is below the limit of detection, it is reported as such (i.e., $<L_D$). When the measured value is between the limit of detection and the limit of quantification, it is reported as being qualitatively detected but no

FIGURE 5
Flow chart for achieving comparable analytical results from a group of laboratories



Define the determinand, limit of detection, and accuracy required.

Choose analytical methods with satisfactorily small sources of bias and adequate precision. When suitable methods are not available, improved methods should be developed.

Ensure that the chosen methods are completely and unambiguously specified and that they will be followed as far as possible by all laboratories.

Estimate the standard deviation of analytical results and, if necessary, improve the precision until the target value is achieved.

Ensure that the standard solutions used by all laboratories are in satisfactory agreement.

Establish a control chart and regularly analyze solutions of known concentration to ensure that the precision remains adequate.

Estimate the bias of each laboratory and, if necessary, improve until the target value is achieved.

value is given. If the measured value exceeds L_Q, it is reported as such—that is, the quantitative result is reported.

When it is essential to provide quantitative estimates at low concentrations, the method recommended by the Water Research Centre probably supplies the most information (*4*). The Centre suggests reporting the actual analytical results together with their 95% confidence limits because this gives all relevant information.

Precision of measurement has been described by A. L. Wilson as one of the "performance characteristics" of an analytical method. It is widely recognized that a laboratory using a given method may obtain one precision when applying a method to the analysis of standard solutions and another when applying it to real samples. It should not be surprising, therefore, that the detection limit as determined from repeated measurements on blanks may not always be the same as that obtained when real samples are analyzed. However, while it is fairly easy to determine precision on real samples, it is not so easy to determine the limit of detection on real samples when, for example, the sample contains interfering substances (that is, the sample and blank are biased with respect to each other). Despite the limitations of the limit-of-detection concept in practice, it is still useful, just as determining the precision of measurements of standard solutions is of use.

Finally, it is important to place the limit-of-detection problem into perspective in terms of real analytical needs. Wilson has recommended that analytical objectives be established for each measurement program. These goals include the need to define the determinand, the required accuracy, and the required limit of detection. In other words, the required limit of detection should be distinguished from the experimental limit of detection. In many cases, it will be clear that the method used in the laboratory is capable of measuring below the required limit of detection, whether that be on standard solutions or real samples. In this case, in order not to expend resources in obtaining information unrelated to the objectives of the measurement program, all results less than the required limit of detection can simply be recorded as such (i.e., less than the required L_D).

Between-laboratory control

The concepts discussed thus far relate principally to within-laboratory quality control (also known as intralaboratory or internal quality control). Accurate analyses depend primarily on the implementation of a well-conceived within-laboratory quality control program involving two stages (*4*):

- preliminary error estimation (i.e., method validation), and
- routine quality control through the use of appropriate control charts.

Between-laboratory quality control (also known as interlaboratory or external quality control) is also useful for several purposes. From the standpoint of the individual laboratory, the analysis of standard solutions or samples prepared by another laboratory (for example, EPA quality control samples or standard reference materials of the National Bureau of Standards) can serve as a check on the efficiency of its within-laboratory quality control program. From a broader perspective, between-laboratory collaborative tests may be necessary for several reasons: to evaluate analytical methods or to determine individual and group laboratory performance, for example.

Between-laboratory tests should involve two stages (*4*):

- comparison of standard solutions used by each laboratory with a standard distributed by a coordinating laboratory and, when all standards are in satisfactory agreement, and
- collaborative analysis of samples distributed to all laboratories by a coordinating laboratory.

It is important to stress that between-laboratory quality control can only complement, and not be a substitute for, an efficient within-laboratory quality control program. Figure 5 illustrates the approach to analytical quality control recommended by the Water Research Centre (*4*). Note that in this step-wise approach, between-laboratory quality control is initiated after the individual laboratories have completed within-laboratory tests. Essentially the same approach is used to control the accuracy of a single laboratory and to achieve comparability of results from a group of laboratories by controlling the accuracy of each.

Summary

Although quality control has become an accepted and even required practice in water analysis laboratories, the definitions and principles underlying the practice of quality control still need to be critically evaluated. In evaluating quality control practices in the U.S., the experience of European laboratories, particularly the Water Research Centre in Great Britain, appears most useful.

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Separation Function for Measuring the Information in Complex Chromatograms

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Separation Function for Measuring the Information in Complex Chromatograms

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The separation function for use in the optimization of complex separations relates the information content of a chromatogram to the number and the extent of resolution between adjacent peaks. Each fully resolved peak is arbitrarily assigned 2.0 bits of information while partially resolved peaks are assigned 1 to 2 bits depending upon the depth of the valley between them. Pairs that give no valley but are detectable using a second-derivative method are assigned 0.5 bit. No a priori information is needed so the separation function may be easily incorporated into existing optimization schemes.

Recently, the optimization of chromatographic separations has moved away from conventional methods in which a chromatographer uses experience and intuition to judge the quality of a complex separation. Systematic methods are now being used which provide a more objective optimization of separations and are amenable to computer control. Laub and Purnell (*1*) searched for conditions that gave the highest minimum α value (ratio of capacity factors) for all pairs of adjacent peaks. Later Glajch et al. (*2*) used resolution in a similar manner. Morgan and Deming (*3*) suggested the use of a peak separation factor (*4*) as a quality measure in their simplex optimization procedure. Later Watson and Carr (*5*) added a weighting factor for the time of analysis as well as a consideration of the actual peak separation compared to the desired peak separation.

Wegscheider et al. (*6*) developed a response function that operates differently than those mentioned above, i.e., as a product rather than a summation, and it takes into account the noise level of the chromatogram. In addition, there have also been peak-counting algorithms. Spencer and Rogers (*7*) proposed a separation number using a procedure that obtained information from badly overlapped peaks, even before a valley appeared. Later, Berridge (*8*) introduced a response function in which the resolution of each pair of adjacent peaks was summed up to a maximum value of 2.0. In addition, the number of peaks was weighted as were the analysis times for the first and last peaks in the chromatogram.

A new response function has been developed to overcome some of the limitations of the previous functions. For example, those that utilize the natural logarithm of a fraction will approach zero at the optimum. However, one cannot determine whether a large number of peaks has been fairly well separated or a few have been poorly separated. In addition, peak crossing causes problems since the function is zero for totally overlapped pairs of peaks, causing false optima. The function of Wegscheider et al. (*6*), even though it operates as a product of the individual peak separations rather than the sum of natural logarithms, suffers from the same problems. Furthermore, for all of the above functions, the number of components being separated should be known in order to ensure proper operation of the function. Berridge's response function (*8*) does keep track of the number of peaks detected during each separation. One drawback of his function is the need for calculating the resolution of each adjacent pair of components. In practice, unsymmetrical and severly overlapped peaks may present problems. In contrast, the separation number of Spencer and Rogers (*7*) is essentially a peak-counting algorithm that can provide information about overlapped peaks and does not require the calculation of resolution. However, the information for an isolated peak depended upon its shape and retention time. Furthermore, the function lacked general applicability because it was limited to isothermal or isocratic separations.

In the present study, a new response function has been tested which incorporates some aspects of the others. It operates as a peak-counting function that assigns a value between 0.5 and 2.0 for each component that is detected. It is applicable to the optimization of all chromatographic methods and requires no a priori information. Furthermore, it can easily be expanded to include additional weighting terms similar to those of Watson and Carr (*5*) and Berridge (*8*). Both simulated and real chromatograms have been examined in this study.

EXPERIMENTAL SECTION

Chemicals. All of the chemicals were reagent grade or better and were used without further purification. Ethanol (U.S. Industrial Chemicals Co., New York) was used as the solvent for the gas chromatographic test mixture. This mixture was composed of 2,3,3-trimethylbutane, 2-pentanol, 2-methylheptane, cycloheptane, *n*-octane (Aldrich Chemical Co., Inc., Milwaukee, WI), tetrahydrofuran, *m*-xylene (J. T. Baker Chemical Co., Phillipsburg, NJ), 2-pentanone, *o*-xylene (Eastman Kodak, Rochester, NY), and cyclohexane (Fisher Scientific Co., Norcross, GA).

The compressed gases used to operate the gas chromatograph were all obtained from the same source (Selox, Inc., Gainesville, GA). They were first passed through a gas purifier (Alltech Associates, Norcross, GA) which contained Drierite and molecular

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sieve 5A. Nitrogen was the carrier gas while hydrogen and compressed air were used with the flame ionization detector.

Apparatus. A Hewlett-Packard 5880A Level 4 gas chromatograph was used to generate the chromatograms. This instrument was configured with a split/splitless capillary inlet system and a flame ionization detector. A Hewlett-Packard 0.2 mm × 12.5 m fused silica capillary column coated with cross-linked dimethylsilicone was used to effect the separations. Injections were made with a Hamilton 701-N 10-μL syringe.

The digital output from the 5880A was converted to an analog signal by using the Hewlett-Packard analog output board option. A 0–10 V range was selected on the output board and the analog signal was then passed through an operational amplifier circuit. The purpose of this circuit was to create a bipolar analog signal, −5.12 V to +5.12 V, for input to the analog-to-digital converter (A/D).

A MINC laboratory computer (Digital Equipment Corp., Maynard, MA) was used for acquisition and storage of the gas chromatographic data. The MINC contained a full complement of I/O accessories including a MNCAD module which provided 16 channels of A/D input and a MNCKW programmable real-time clock which was used for initiating A/D conversions. An RT-11 foreground/background operating system was used to run the data acquisition programs which were written in Fortran IV. In addition, extensive use was made of the REAL-11/MNC library subroutines supplied with the MINC. The data were stored on 8-in., double density, floppy disks.

A PDP 11/23 computer (Digital Equipment Corp.) running an RSX-11M operating system was used for the analysis of the chromatographic data and for chromatographic simulations.

Procedures. *Simulations.* Peaks for the simulated chromatograms were generated from either the equation for a normalized Gaussian

$$Y = \frac{N}{\sigma(2\pi)^{1/2}} \exp\left[-\left(\frac{X_i - \bar{X}}{2\sigma^2}\right)\right] \tag{1}$$

or that for an exponentially modified Gaussian (*10*)

$$Y = \frac{N}{\tau\sigma(2\pi)^{1/2}} \int_0^\infty \exp\left(-\left[\frac{(X_i - \bar{X} + \tau - t')^2}{2\sigma^2}\right]\right) \exp\left[\frac{-t'}{\tau}\right] dt' \tag{2}$$

where Y is the response, N the scale factor, σ the standard deviation, X_i the sample time, $\bar{X}$ the peak mean, τ the time constant of the exponential decay, and t' the dummy variable of integration. Gaussian peaks were used for the simulations involving comparisons between the SEP functions and the others. Exponentially modified Gaussian peaks were used to examine the contribution of peak tailing.

Several inputs were necessary for the generation of a simulated chromatogram. The number of theoretical plates, P_t, in a hypothetical column was needed to determine the standard deviation of the peaks from the equation

$$\sigma = \bar{X}P_t^{1/2} \tag{3}$$

The sampling rate or the time between data points as well as the threshold value were also specified. The threshold refers to a response value below which no information was generated. For tailing peaks, a skewing factor (τ/σ) was entered from which the τ value could be determined. Finally, the number of peaks to be generated in the chromatogram was entered. For each peak, the peak mean, $\bar{X}$, and the peak-height scaling factor, N, were input. Unless otherwise stated, standard values for the input parameters were a 5000-plate column, sampling rate of 0.1 time units, scale factor of 1.0, threshold value of 0.0001, and peak mean for a single peak of 30.0 time units.

Calculations of Response Functions. Resolution, R, was defined as

$$R = \frac{\Delta T}{2(\sigma_1 + \sigma_2)} \tag{4}$$

where ΔT is the time between two peak means and σ_1 and σ_2 are the standard deviations of the two peaks. The chromatographic response function, CRF, as specified by Morgan and Deming (*3*), was calculated from

$$\mathrm{CRF} = \sum_{i=1}^{n} \ln (f_i/g_i) \tag{5}$$

where n is the number of adjacent peak pairs, f_i is the distance from a line connecting the peak maxima to the valley between the peaks, and g_i is the distance from the connecting line to the base line. The CRF was derived from Kaiser's peak separation, f_i/g_i (*4*), which is equal to 1.0 for completely resolved peaks.

The separation number, SN, is a measure of the total information in a chromatogram, i.e., it is simply the sum of information from all of the data points (*7*). That is

$$\mathrm{SN} = -\sum \log_2 Q \tag{6}$$

where Q is defined below. This information is calculated from the difference between a predicted value and the actual value for a datum. Predictions are based upon knowledge of the preceding data values and the near Gaussian nature of chromatographic peaks. The expected or predicted value is compared to the actual value using the equation

$$Q = X/(2X - Y) \tag{7}$$

where Q is the error in prediction, X is the larger of the predicted and actual value, and Y is the smaller of the two values. Since Q will take values between 1/2 and 1, the negative logarithm (base 2) of Q will range between 0 and 1. Thus, depending upon the size of the error in prediction, any one datum may contain between 0 and 1 bit of information.

Two forms of the separation function, SEP, were used in the comparison studies. They were

$$\mathrm{SEP}_p = N_p + \sum_{i=1}^{n} P_i + 0.5N_s \tag{8}$$

$$\mathrm{SEP}_v = N_p + \sum_{i=1}^{n} (1 - V_i) + 0.5N_s \tag{9}$$

where N_p is the number of peaks detected in the chromatogram, P_i is Kaiser's peak separation as defined previously, N_s is the number of shoulders as determined by a second-derivative method, and V_i is the valley-to-peak ratio (*10*). The valley-to-peak ratio is simply the height above base line of the valley between two peaks divided by the height of the smaller peak. This ratio becomes zero when the two peaks are fully resolved.

In the present study, a second-derivative operation was performed on the data and the number of minima was determined. The number of peak shoulders in the chromatogram corresponded to the number of minima minus the number of peaks detected. Each peak shoulder was arbitrarily assigned a value of 0.5 bit of information. This corresponds well with its relative importance to the overall separation since a barely resolved peak contains just over 1.0 bit of information.

In the first comparison, a group of six peaks was simulated starting at 30 time units and having one time unit between each peak mean. The time between peak means was incremented by 0.5 time unit each cycle to determine its effect on the SN, resolution, CRF, and both of the separation functions.

In the second comparison, optimization of a four-component mixture was simulated in the manner of Debets et al. (*10*). The retention times of the components were changed linearly as a function of the hypothetical solvent composition. The retention times ranged from 20 time units to 29 time units and a 2000-plate hypothetical column was used to obtain chromatograms that closely resembled those obtained by Debets et al. The simulation was performed over 41 different solvent compositions and the effects on the response functions were determined.

Real Analyses. Typical GC operating conditions were as follows: injection port temperature, 250 °C; detector manifold temperature, 275 °C; nitrogen carrier gas flow rate, 2.0 mL/min at 70 °C; nitrogen makeup gas flow rate, 30 mL/min; and split ratio, 75:1. The flame ionization detector was set up in accordance with the manufacturer's specifications. The analyses were run isothermally with the oven temperature ranging from 40 °C to 100 °C in increments of 10 °C.

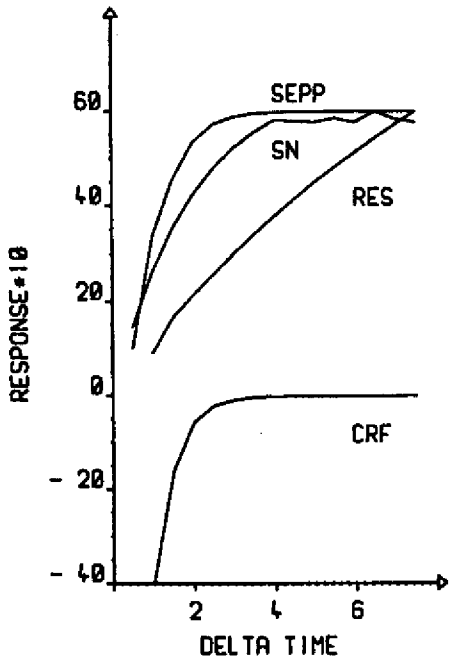

Figure 1. Separation function (SEPP), separation number (SN), total resolution (RES), and chromatographic response function (CRF) vs. the time between peak means for a six-peak separation.

A test mixture was prepared containing 2% (v/v) of each of the following compounds in 100% ethanol: 2,3,3-trimethylbutane, 2-pentanol, 2-pentanone, cyclohexane, cycloheptane, 2-methylheptane, *n*-octane, *o*-xylene, *m*-xylene, and tetrahydrofuran. The sample size used was 0.5–1.0 μL.

The analog output from the gas chromatograph was updated at a rate of 8 Hz. Sampling of this analog signal by the MINC A/D occurred at a rate of 80 Hz so that 10 successive values were averaged, thus minimizing fluctuations in the analog signal. The averaged data values were stored in 480 point arrays with each array corresponding to 1 min of output from the gas chromatograph. The first 100 points of data after injection were used to determine the base line and threshold values. This corresponded to a peak-free region of the chromatogram before the solvent peak was eluted. The threshold was taken as 10 times the standard deviation of the first 100 points and the base line used was equal to the mean value over this region.

The response functions were calculated from both raw and smoothed data. The data were smoothed twice in succession using either a five-point or a nine-point cubic quadratic smooth. Peak shoulders were determined by a second-derivative method in which the presence of peaks and peak shoulders was related to the magnitude of the negative peaks from the second derivative of the data. Both five-point and nine-point second-derivative operations (*11*) were examined as a function of the signal-to-noise ratio of the second-derivative peaks.

RESULTS

Simulation Studies. A comparison between the resolution, CRF, SN, and the separation functions, SEP_p and SEP_w, is shown in Figure 1 for six overlapped peaks. Delta time, plotted on the *X* axis, refers to the time between each of the peak means. The resolution increased linearly as the time between the peak means increased, even after the peaks were completely resolved. The CRF started at a large negative number and became zero at base-line resolution. This occurred at a delta time of 4 units. The SN increased more gradually than the CRF but reached a maximum value at the same delta time. SEP_p and SEP_v gave identical values for this simulation so only SEP_p was plotted. The shape of the separation function closely followed that of the CRF while being shifted to positive values. The maximum value for SEP_p was also reached at 4 delta time units. The results from this simulation showed that, while resolution was not suited to multiple peak separations, the other response functions were. They all reached a maximum value at a base-line separation corresponding to a delta time of 4. In addition, SN approached its maximum response more gradually than either CRF or SEP_p, both of which used Kaiser's peak separation as a determining factor. This explains the similarity in the shapes of the curves for these two functions.

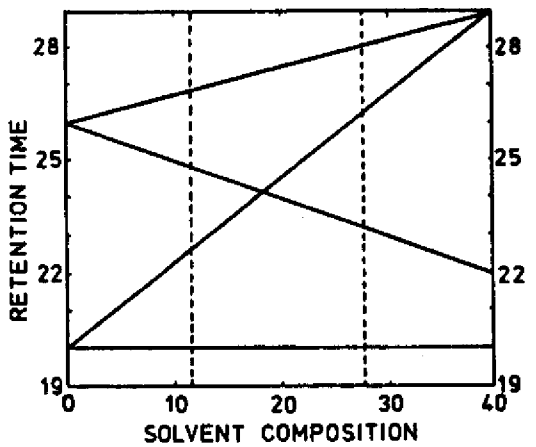

Figure 2. Retention time vs. solvent composition used for modeling the optimization of a four-peak separation.

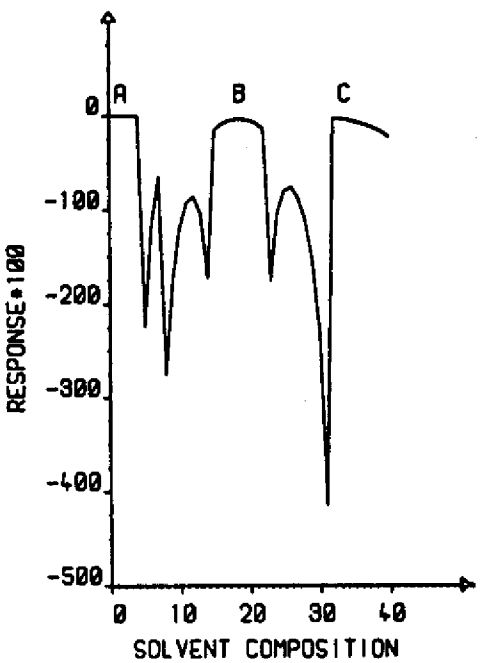

Figure 3. CRF vs. solvent composition for the four-peak separation. A, B, and C correspond to the solvent compositions that give optimal values for the CRF.

The effects of solvent composition on retention times (Figure 2) and their effects on the CRF, SEP_p, and SEP_v are shown in Figures 3 and 4 for a four-peak separation. Figure 3 shows the behavior of the CRF which found three optimum areas corresponding to regions A, B, and C. However, the chromatogram for region A has only two peaks while B and C have only three. This confirms what Debets et al. (*10*) found for the CRF when no a priori information was given about

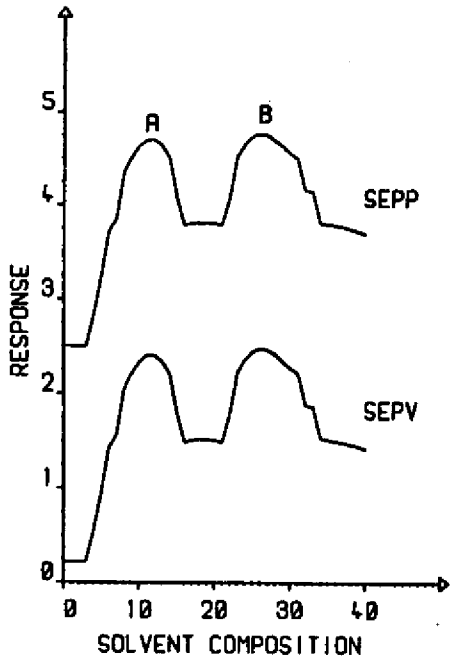

Figure 4. SEP_p and SEP_v vs. solvent composition for the four-peak separation. A and B correspond to the solvent compositions that give optimal values for the separation functions.

the number of components present. This is because the CRF cannot distinguish between a single peak and two highly overlapped peaks.

SEP_p and SEP_v gave identical results for the optimization simulations as shown in Figure 4. Two optimum regions were found and correspond to the solvent compositions represented by the two dotted lines in Figure 2. The behavior of the separation functions compared favorably with that found by Debets et al. (*10*) for the CRF and other response functions when information concerning the number of components in the mixture was provided. The separation functions, however, needed none of that information since the number of peaks detected at any solvent composition was included in the calculation of the functions. Although Figure 4 shows no significant difference between SEP_p and SEP_w other simulations showed SEP_v to be less affected by large differences in peak height. Hence, SEP_v is the preferred function.

Real Analyses. The effect of different second-derivative operations was examined as a function of the signal-to-noise ratio, SNR. The results are shown in Table I for the *o*-xylene peak run at 40 °C. The *o*-xylene peak was chosen because it was the most strongly retained and, thus, the most broadened of the peaks in the test mixture. Since the magnitude of the negative peak from the second derivative was inversely proportional to the peak width, a worst-case example was used. The noise level used for the SNR was equal to three times the standard deviation of the base line after derivitization. For both types of smoothing, the nine-point second derivative gave much better SNR values than the five-point second derivative. In addition, the nine-point smooth gave better SNR values than the corresponding five-point smooth. However a five-point smooth was found to produce less peak distortion; it was used with the nine-point second derivative to determine the separation number of real chromatograms.

The response functions were next calculated for the separation of the ten-component test mixture at various GC oven temperatures. Figure 5 shows a plot of the SN, CRF, and SEP_v as a function of oven temperature. Essentially base-line

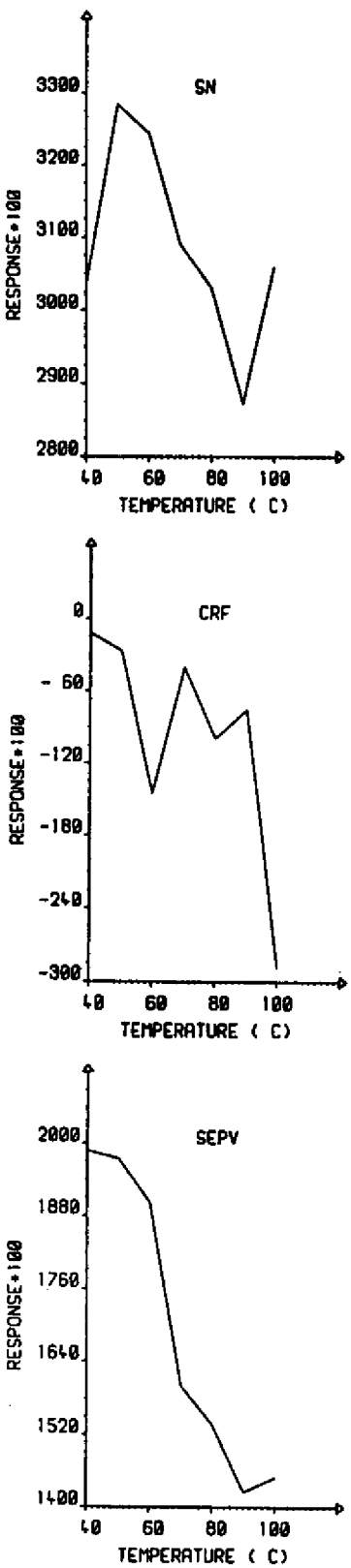

Figure 5. SN, CRF, and SEP_v vs. GC oven temperature for the separation of the ten-component test mixture.

Table I. Effect of Smoothing Real Data on the Signal-to-Noise Ratio of Second Derivative Minima

no. of pts in smooth	no. of pts in derivative	S/N
5	5	3.6
5	9	9.7
9	5	10.6
9	9	14.5

resolution of all components occurred at an oven temperature of 40 °C. This corresponded to a SN of 30.3. At 50 °C, the peaks for cyclohexane and 2-pentanol started to overlap, yet the SN rose to 32.8. From 50 °C to 90 °C, the SN decreased to a value of 28.7 before increasing at 100 °C to 30.6. Since the SN is supposed to be an absolute measure of the quality of a separation (7), these results indicated that the best separation occurred at oven temperatures in the 50 °C to 60 °C region rather than at 40 °C. Further examination of the SN data showed that the retention time of a peak played an important part in determining the SN, especially if the peak was non-Gaussian. Peaks at short retention times gave more information than peaks at long retention times. This caused the SN algorithm to shift the optimum separation to shorter analysis times. Thus, at 50 °C, the partial overlap of two peaks was more than offset by the 32% reduction in analysis time. At 60 °C, however, the additional peak overlap had a greater effect than a 28% reduction in analysis time and the SN decreased slightly from 32.8 to 32.4. As the oven temperature was raised from 60 °C to 90 °C, the overlapping peaks caused the SN to decrease to 28.7. However, a 100 °C the SN again increased to a value of 30.6. This increase was due to a slight decrease (14%) in analysis time coupled with partial resolution of the cycloheptane–*n*-octane peak pair which appeared as a single peak at 90 °C.

Next the CRF was examined as a function of oven temperature. As expected, the highest value for the CRF occurred at 40 °C where the peaks were nearly base-line resolved. The CRF, however, showed erratic behavior by increasing to relatively high values at 70 °C and 90 °C. These increases occurred at points where partially resolved peaks became totally overlapped and is a well recognized trait of the CRF (7).

Finally, since all of the peaks in the chromatograms were about the same height, SEP_p and SEP_v were identical and thus only SEP_v was plotted. The largest value for SEP_v was attained at 40 °C with relatively large values also at 50 °C and 60 °C. Over this region, all ten peaks were still separated to some extent. At 70 °C, there was a large decrease in the value of SEP_v from 19.02 to 16.01. This occurred due to the total overlap of two pairs of peaks such that eight peaks and one peak shoulder were detected. SEP_v decreased further to 14.25 at 90 °C where seven peaks and one peak shoulder were detected. Finally, at 100 °C there was a slight increase in SEP_v to 14.49 due to the partial resolution of an overlapped peak pair. As in the case of the SN, the partial resolution of the cycloheptane–*n*-octane peak pair more than offset the poorer resolution of the other peaks.

DISCUSSION

This study has shown once again that the CRF alone does not provide an accurate indication of the quality of a separation especially when peak crossing occurs (*10*). Either the number of peaks to be expected must be known or the number of peaks present in the chromatogram must be tracked. Otherwise, optimal values for the response may occur when some peaks are strongly overlapped. SEP_v does not suffer from that problem. In addition, SEP_v enjoys one advantage of SN, i.e., it provides an indication of badly overlapped peaks well before a valley forms between them. Hence, it overcomes one limitation of the overall function reported by Berridge (*8*). The importance of analysis time and weighting of peaks can easily be added to the SEP_v algorithm so as to incorporate those factors as done either by Watson and Carr (*5*) or by Berridge (*8*). Furthermore, other refinements could be added such as weighting small regions of a chromatogram in order to maximize the importance of a few peaks of interest.

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Appendix B
Workshop Agenda

METHODS FOR ANALYSIS OF ORGANIC COMPOUNDS IN THE GREAT LAKES, II

Sponsored by the
University of Wisconsin Sea Grant College Program
and National Marine Pollution Program Office,
National Oceanic & Atmospheric Administration,
U.S. Department of Commerce

October 10-11, 1985
Wisconsin Alumni Center
Madison, Wisconsin

◆

Thursday, October 10

Welcome and Workshop Overview

Introduction to Workshop Sessions:

Low Level Organics in Water
D. Swackhamer, Indiana University

Pattern Recognition of Complex Mixtures
D. Stallings, U.S. Fish & Wildlife Service

Mass Spectrometry Techniques
D. Kuehl, U.S. Environmental Protection Agency

New Quality Control Issues
D. King, Ontario Ministry of the Environment

Individual Workgroup Discussions

◆

Friday, October 11

Final Workgroup Sessions

Sharing of Workgroup Findings

Wrap-Up

Adjournment

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