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United States Environmental Protection Agency

Office of Water Regulations and Standards
Industrial Technology Division

EIGHTH ANNUAL ANALYTICAL SYMPOSIUM

Norfolk, Virginia

April 3-4, 1985

FOREWORD

The Industrial Technology Division of the USEPA Office of Water Regulations and Standards sponsors the Annual Analytical Symposium to provide a forum where scientists and other interested parties can present new ideas and advances in methodology for the analysis of Priority Pollutants. The topics addressed during the Eighth Annual Analytical Symposium have deviated from strict analytical water chemistry; we have considered the role that quality assured biomonitoring techniques will play in compliance monitoring, as well as advances in high performance liquid chromatography/mass spectrometry analysis of environmental samples. In concert with the Division's responsibility to promulgate industrial effluent regulations, we feel that it is in the common interest to promote new technologies and ideas that will serve as analytical tools and assist us in this increasingly complex task.

W. A. Telliard

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P R O C E E D I N G S

MR. TELLIARD: Good morning. My name is Bill Telliard. I'm from EPA and I'm here to help you. Welcome to the Eighth Annual...boy, that's old...Analytical Meeting of the Office of Water. It's the first one for the Industrial Technology Division or the eighth one for the Effluent Guidelines Division, depending on how people score this one.

I'd like to open this morning's session with a few words from Dev Barnes, who I don't see. Dev? Dev is the Deputy Division Director for the Industrial Technology Division and I'd like to have him say a few words.

MR. BARNES: You found my hiding place, Bill. I'd like to welcome everybody here. It's a pleasure for the division to sponsor this every year. We look forward to this annual event and we think that it's paid a lot of dividends in terms of just getting everybody together and ironing out differences in various meetings that take place concurrently with this, dealing with things that our division directly deals with in terms of establishing guidelines. So I think this

meeting has a great deal of value to us. We hope that you also feel that way and we look forward to your continuing participation in it. So, I hope you all have a nice time today and tomorrow and we'll look forward to seeing you again next year, hopefully. Thank you.

MR. TELLIARD: A couple of announcements. Concurrent with last year's open sea disaster, we're repeating it again this year. We're going to try to get everyone out of here on time to catch the boat.

As the tradition holds, we have Tonie and Sandy from the County Court Reporters taking down every gem of knowledge that you're about to drop on us today. When you go to the microphone, again, please state your name, otherwise you will be beaten brutally about the knees; all except George Stanko because...just tradition.

In the back of the room you'll find some copies of previous years proceedings for all of you who really want to remember, and some copies of the new 1625, 1624 GCMS methodology with the QC stuff in it. They're available in the back of the room. There's also copies of 304(h); you remember that.

Breaking with tradition, our first speaker

this year is not Bob Medz. Bob didn't come because he's home being sued, but then again, it just shows he did a real good job.

To show that we're an open minded group this year, this morning will be spent discussing critters, both crawling and floating types, and most analytical chemists will find this a great eye opener. I particularly think it will make you feel warm and fuzzy when you look at standard deviations among star measurements and some of these guys.

So our first speaker this morning is Steve Bugbee from the Permits Office and Steve is going to talk about water quality type things. Steve.

STEPHEN L. BUGBEE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER ENFORCEMENT AND PERMITS

WATER QUALITY BASED TOXICS CONTROL

MR. BUGBEE: Good morning. The use of biological techniques to identify and control toxicity is not new, but as you will hear, it has been a very long and tortuous road to get where we are today.

The control of toxic pollutants is one of the Nation's most critical remaining water quality problems. For example, localized water quality standard violations or impairments of water uses have been widely reported by states in their biennial water quality reports to EPA. Because of the tendency of some toxics to accumulate in fish tissue, fishing bans and fish consumption warnings are in effect in many of the Nation's waters.

For a number of reasons, however, implementing controls for toxic pollutants has always been a difficult problem. These reasons include the sheer number of toxic compounds which are involved; insufficient monitoring data because of the high cost of laboratory analyses for toxics; and

uncertainties about the fate and effects of toxic substances and uncertainties about the fate and effects of these chemicals in the aquatic environment. EPA has developed a new approach which, together with advances in the field of biological monitoring, should help us deal more effectively with toxic pollution problems.

The Clean Water Act established two types of regulatory requirements to control pollutant discharges: technology-based effluent limitations which reflect the best controls available, considering the technical and economic achievability of those controls; and water quality-based effluent limitations which must be met so discharge permits reflect the more stringent of the two whenever there are differences. So, we have on one hand the technology-based approach which you're most familiar with, and then we have the water quality-based approach.

The technology-based requirements for discharges are currently being issued and will have a substantial affect in reducing toxic discharges. However, in some cases these controls will not be sufficient to eliminate water quality impacts and enable water quality standards to be met. In these cases, water quality-based controls are needed. Two technical

approaches are available for developing water quality-based effluent limits, the pollutant-specific approach and the biomonitoring approach.

EPA and the States have traditionally used the pollutant-specific approach. Pollutant-specific techniques are best used where effluents contain a few well quantified pollutants and the interactions and the effects of these pollutants are known. Thus, they have worked well for pollutants such as oxygen-demanding loads and nutrients and heavy metals. In addition, pollutant-specific techniques must be used where health hazards are a concern and/or if bioaccumulation is suspected.

In the case of toxic pollutants and complex effluents, however, it may be difficult in some cases to determine the attainment or the nonattainment of specific water quality use or water quality standards and to set the appropriate limits because of the complex chemical interactions which affect the fate and ultimate impact of these toxic substances in the receiving water. In many cases, chemical methods cannot easily be used to identify all potential toxic pollutants. Developing numerical water quality criteria and determining allowable loadings for all the wide variety of pollutants

found in effluents is also very time-consuming and resource intensive. In such situations, it would be very desirable to examine overall toxicity directly without identifying and analyzing every pollutant individually.

Biological methods, based on the direct measurement of the impact of whole effluents on biological test organisms and communities, provide this capability. Therefore, EPA is integrating this whole effluent biomonitoring into our programs to control toxic pollutants. The result is a two-fold approach. In certain situations, an example would be where potential human health impacts are a concern, we must rely upon the chemical-specific approach, measuring the individual toxicants and evaluating their specific toxic properties. In other situations, however, especially where we have complex effluents, multiple discharges, it is more appropriate to examine the harmful effects, or toxicity, of the whole effluent rather than trying to attempt to individually identify all the potential toxicants and understand the inherent chemical reactions. In other words, we're looking at both the whole effluent toxicity and the receiving water body toxicity.

This toxicity testing approach relies on newly developed methods and laboratory testing procedures while the two-fold approach employs both the chemical-specific and biological monitoring. The biological component deserves a special focus because it is the area that is developing most rapidly, both in scientific and programmatic terms. In fact, the scientific basis for using biological techniques has advanced so significantly in recent years that it is now an important aspect of the water quality-based approach for controlling toxic pollutants in the EPA's permit program.

The importance of biological monitoring, both as an ambient monitoring tool and as a potential regulatory tool, has long been recognized. We can point to a long history of field studies that have employed some type of biomonitoring activity. However, the success of biomonitoring as a regulatory tool has until now been limited because it has often proved difficult to translate the biological instream effects into effluent requirements that will be incorporated into a permit.

This is a very important point. Up until approximately last year, the Chemical Manufacturers Association, among others, had been very critical

of our toxicity-based approach in NPDES permitting. One of their major criticisms was the fact that we were unable to make the transition from the end of the pipe into the receiving water. In other words, if you limit the effluent using some type of toxicity limit, that limit would be very difficult to enforce because of defining receiving water impacts.

So to deal with this problem, we've conducted research over the last few years which has provided us with more effective biological monitoring tools that can help us screen for problems, assess impacts, set toxicity-based limits, and help identify ways to reduce toxicity. These toxicity tests are an outgrowth of those used in the laboratory to establish aquatic life criteria for specific chemicals. These tests include acute toxicity tests which measure short term exposure effects and chronic toxicity tests which measure long term exposure effects.

Through the work of EPA's Office of Water Regulations and Standards, the Office of Water Enforcement Programs, the Office of Research and Development, the EPA Regional Offices, the States and academia, we have taken these methods into the

field, employed them in site-specific situations, especially those involving complex effluents. We're primarily addressing the fresh water environs at the present time. However, we do have a similar program being developed which will deal with marine and estuarine toxicity.

The results of these applications have been promising. Not only do we have a better understanding of the behavior of toxics in the ambient conditions, but we also have learned that the various methods can be used in concert to complete the picture of the water quality impact. It now appears that effluent toxicity can be evaluated in conjunction with chemical and ecological data and can be very useful in developing regulatory requirements. We intend to continue these field applications of biological methods in FY86 and translate their results into useful guidance for the States to help in issuing water quality-based permits.

We are committed to a balanced and integrated approach; that is, one that applies both the chemical and biological techniques. This isn't to alarm you that we're suddenly shifting over to just the biological approach. I think one way of better illustration is the policy EPA published in the

Federal Register in March, 1984, that essentially underwrites this whole water quality-based approach for toxics control. So I'll run through a few of the basics of the policy for you.

SLIDE 1

The policy was published in the Federal Register March 9th, 1984.

SLIDE 2

Basically, the policy states, one, that we're going to be controlling pollutants beyond BAT, and EPA will use an integrated strategy consisting of biological and chemical methods. Secondly, through Section 308 of the Clean Water Act, EPA or a State may require a discharger to provide chemical, toxological, or ambient biological data to assure compliance with standards.

Third, through Section 402 of the Clean Water Act, EPA may develop NPDES permit limits based on effluent toxicity and require a toxicity reduction evaluation to eliminate unacceptable toxicity. The fourth point is that the EPA Regional Administrators will assure that each region has the capability to conduct both chemical, biological assessments and provide technical assistance to the States. This is an ambitious goal but we have been

Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants

o STATEMENT OF POLICY:

1) TO CONTROL POLLUTANTS BEYOND BAT, EPA WILL USE AN INTEGRATED STRATEGY CONSISTING OF BIOLOGICAL AND CHEMICAL METHODS;

2) THROUGH §308 OF THE CLEAN WATER ACT, EPA OR A STATE MAY REQUIRE A DISCHARGER TO PROVIDE CHEMICAL, TOXICOLOGICAL, OR AMBIENT BIOLOGICAL DATA TO ASSURE COMPLIANCE WITH STANDARDS;

3) THROUGH §402, EPA MAY DEVELOP NPDES PERMIT LIMITS ON EFFLUENT TOXICITY, AND REQUIRE A TOXICITY REDUCTION EVALUATION TO ELIMINATE UNACCEPTABLE TOXICITY;

4) EPA REGIONAL ADMINISTRATORS WILL ASSURE THAT EACH REGION HAS THE CAPABILITY TO CONDUCT BOTH CHEMICAL AND BIOLOGICAL ASSESSMENTS AND TO PROVIDE TECHNICAL ASSISTANCE.

building this into our work plans and budgets, and we've seen considerable success and progress in the Regions as well as the States in terms of developing toxicity testing capability.

SLIDE 3

Other issues addressed in the policy; one, the importance of whole effluent toxicity is stressed as an evaluation and control parameter, particularly for complex effluent permitting situations. In other words, toxicity can be used as a parameter just as BOD is used in NPDES permits. Second, effluent toxicity can provide a valid indication of receiving water impact.

The policy then goes on to respond to some of the issues raised in the NPDES permit regulations regarding the toxicity-based limits, in addition to outlining the benefits and the disadvantages of the chemical-specific versus the whole effluent approach. Obviously, when you're dealing with complex mixtures of chemicals, the whole effluent toxicity-based approach may be the way to go. The policy also stresses the importance of using toxicity testing when evaluating large publicly owned sewage treatment plants. Some of those effluents are extremely toxic.

OTHER ISSUES ADDRESSED:

- STRESSES THE IMPORTANCE OF WHOLE EFFLUENT TOXICITY AS AN EVALUATION AND CONTROL PARAMETER, PARTICULARLY FOR COMPLEX PERMITTING SITUATIONS;

- STATES THAT EFFLUENT TOXICITY CAN PROVIDE A VALID INDICATION OF RECEIVING STREAM IMPACT;

- IDENTIFIES THE "FREE FROM" WATER QUALITY STANDARD AS AN APPROPRIATE BASIS FOR BOTH CHEMICAL AND TOXICITY LIMITS.

The policy also asserts that toxicity testing methods are currently available. EPA is confident that there are adequate test methods which are reliable and reproducible. With these test methods available, we don't see any reason why this should prevent regulatory agencies from not using this approach.

In addition to the policy, we also have put out a technical support document which essentially supports the policy and provides technical guidance to the permit writers and water quality specialists.

Thus it is clear that in addition to the more traditional chemical-specific approach to evaluating water quality, whole effluent toxicity tests and other biological measures must now be systematically employed throughout the various steps of the regulatory process such as screening, impact assessment, setting limits and measuring compliance. These tests and measures must be related to instream factors such as zones of initial dilution, flow variability and sediment contribution so that the instream behavior of toxics can be fully understood as part of the decision-making process. Finally, the selection of tests and their application must be tailored to the specific site in question.

I would like to conclude with this observation. The history of the Nation's chemical and biological monitoring activities has experienced several significant shifts in emphasis. As you recall, in the early 60's the emphasis was on biological monitoring. We were looking at water quality standards, water quality wasteload allocation models, dealing with more of the traditional pollutants such as BOD and dissolved oxygen. Then in the '70's, with the event of the Clean Water Act, the shift was from out of the stream to the end of the pipe and we were following a technology-based approach which is essentially where we are today.

Now we are beginning to realize the real advantages of biomonitoring in dealing with complex effluents. However, chemical monitoring continues to have many advantages over biomonitoring, such as controlling human health hazards and in providing direct measures of operating treating process and evaluating compliance. Therefore, we must continue to view both chemical and biological monitoring not as separate entities but rather as integrated components of a balanced and effective water quality-based toxicity control program. Thank you.

MR. TELLIARD: We're going to do questions now. We're not going to let you off. Any questions?

QUESTION AND ANSWER SESSION

MR. DELLINGER: Bob Dellinger, Industrial Technology Division. It seems to me that probably the most critical thing in biomonitoring is selection of a test species or organism. I'm familiar with pulp and paper effluents and there's a wide range of effect depending on the test species chosen. Some are more sensitive than others. What would be the policy in that regard? Do we pick a very sensitive species or a relatively insensitive species, one in the middle, or how...

MR. BUGBEE: There really is no such thing as the most sensitive species. What we try to do is use several species to bracket the range of sensitivity which can occur.

The pulp and paper example is a good one because right now they're running acute tests using rainbow trout and people would think that rainbow trout would be the most sensitive species. Well, it turns out that these rainbow trout really do quite well in the kraft mill effluent.

The States of Oregon and Washington are now looking for more tests to use in order to regulate this toxicity. There is a chronic toxicity

associated with the kraft mill effluent, but the rainbow trout in the tests that they're using, does not pick this up. So if you would happen to use, let's say, an oyster-spat or a Ceriodaphnia test, these tests would show toxicity.

This is one of the reasons we're promoting the use of more than one species, preferably three to five species, and perhaps using both the acute and the chronic test combined.

MR. DELLINGER: Thanks.

MR. BUGBEE: Yes, sir. Jim?

MR. RICE: Jim Rice. I wanted to ask, Steve, you made some mention about part 136, I think. My recollection is that none of the tox tests methods that you're using are in part 136 now. I was wondering what your plans were in terms of publishing for notice and comment and the like, for these tox methods that you have under development now?

MR. BUGBEE: I'm glad you asked that question, Jim. We started in 1978, trying to get the acute toxicity tests for fathead minnows and daphnia into part 136, and the latest information I have, it will be March of 1986 when they propose

to get these acute methods into 136.

Corny Weber of ORD, Cincinnati, has put out the third edition of his Biological Methods Manual, which is a very fine manual for acute tests. He's going to also put out a separate manual just for some of the chronic tests. But that's what we're using right now, and also, I believe, in Standard Methods, there are some routine acute tests that can be used.

MR. TELLIARD: Thank you, Steve.

MR. BUGBEE: Your welcome.

MR. TELLIARD: Our next speaker, Dr. Duke, is going to talk a little bit about critters that I like better, which are shrimp.

Due to the fact that Industrial Technology Division always like to stay ahead or behind, depending on which view you take, we are, this year, going to for the first time, establish an effluent limitation guideline based on an LC50 killing critter number. This is for the offshore oil and gas industry. For those industries feeling slighted, we will do our best to get you an LC50 number as soon as possible.

Much of the work on our offshore oil and gas program has been supported by our agency laboratory, Gulf Breeze, and particularly by Dr. Duke. So Tom, this morning, is going to talk to you something about the biological monitoring program that they're conducting. Tom.

THOMAS W. DUKE, PH.D.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY

TOXICITY TESTS AND BEST AVAILABLE TECHNOLOGY (BAT) DETERMINATIONS FOR DISCHARGE FROM OFFSHORE OIL AND GAS PLATFORMS

(Revised presentation submitted.)

Toxicity Tests and Best Available Technology Determinations for Discharges from Offshore Oil and Gas Drilling Platforms

T.W. Duke and P.R. Parrish

The Environmental Protection Agency is charged with regulating the discharge of materials from offshore oil and gas drilling platforms. This is accomplished through Best Available Technology (BAT) and through Section 403(c) of the Clean Water Act. BAT is promulgated on a national basis and Section 403(c) includes guidelines for testing indigenous species and for more complex chronic and community tests. The proposed BAT guidelines for drilling fluid discharge from offshore platforms contain a requirement for a toxicity test to estimate potential adverse biological effect of a particular drilling fluid.

This paper discusses the toxicity test specified in the proposed guidelines and how test results can be applied to regulatory activities.

Drilling Fluid Used and Characteristics

Before discussing the toxicity test, it is important to describe drilling fluids (muds) and how the fluids are used before discharge. Drilling fluids are a complex mixture of chemicals and clays that are forced through the drill pipe, through the rotating bit, and are returned to the surface through the space between the drill string and casing. During this process, drilling fluids lubricate the bit, coat the bore hole with an impermeable cake to prevent fluid loss, reduce corrosion, transmit hydraulic power to the bit, and remove cuttings (Ayers, 1981).

Four basic components -- barite, bentonite, lignite, and lignosulfonate -- comprise about 90 percent of the materials used in drilling fluids (Table 1).

Barite, the most common weighting agent, is used to increase the density of the fluid. Bentonite clay is used to thicken the drilling fluid; lignite and lignosulfonates are used to ensure that the fluid does not become too viscous. Speciality additives, including diesel or other oils to increase lubricity and biocides to control microorganisms, also may be added as the drilling process requires (Perricone, 1980).

Discharge of Fluids

After a drilling fluid is pumped from the well to the drilling platform, it is passed through solids-control equipment and the separated solids (cuttings) are discharged into the sea. This discharge occurs continually, as long as drilling is in progress. Fluid passing through the solids-control equipment is returned to a holding tank for possible treatment and recirculation into the well. When the fluid becomes too viscous for use or if it no longer functions as desired, a portion is intermittently discharged into the sea. All of the used drilling fluid is discharged at the completion of drilling of exploratory wells. The volume of solids continuously discharged varies from 3,000 to 6,000 barrels per well (one barrel equals 42 U.S. gallons), and the volume of used drilling fluid intermittently discharged varies from 5,000 to 30,000 barrels per well (National Research Council, 1983).

The fate of drilling fluids discharged into the sea is determined by physical, chemical, and biological processes. A portion of discharged drilling fluids contains larger particles that sink to the bottom relatively near the well site, depending upon their density and upon current velocitites and other environmental factors. A lesser amount of solids, as well as soluble components, remains in the water column and can be transported away from the well by ambient currents (Ayers et al., 1980a; Ayers et

al., 1980b; Brandsma et al., 1980). Thus, there is potential interaction between discharged drilling fluids and biota in the water column and on the bottom.

Toxicity Test

The proposed guidelines for BAT include a toxicity test, the purpose of which is to obtain a general idea of the toxicity of a specific drilling fluid or fluid component to marine organisms. This static, acute toxicity test may lead to more complex tests, but it is not intended to provide detailed toxiciological data as may be required under 403(c). Rather, the test is intended to provide a basis for comparing the toxicity of drilling fluids.

In order to be an effective test for BAT purposes, methodology should be simple, the test organism should be relatively sensitive and readily available and transportable, and results should be amenable to statistical analysis. For these and other reasons, a 96-hour test with mysids, *Mysidopsis bahia*, was chosen as the BAT toxicity test. Mysids have been used as a reliable and sensitive test organism for test materials other than drilling fluids and a substantial data base exists. In addition, the mysid test has been used to evaluate the toxicity of many drilling fluids; therefore, new results can be compared with the existing drilling fluid data base. Although the test described here is only four days in duration, mysids can be used for long-term tests to study the effects of materials on partial or full life-cycle stages (Nimmo et al., 1977).

Mysids can be cultured by those performing the test or purchased from a supplier. All mysids used in the drilling fluid test should be 5 ± 1 days old at the beginning of the test and fed *Artemia salina* nauplii during culture and testing.

Details of the test method are documented by Petrazzuolo (1983) and Duke et al. (1984). Natural or artifical seawater can be used to prepare the suspended particulate phase (SPP) of the fluids. The SPP is prepared by adding one part fluid to nine parts seawater (volume/volume) and stirring. The mixture is allowed to settle for one hour and the material that remains in suspension, the SPP, is the test material. It is added to seawater (volume/volume) to prepare the test solutions. Dissolved oxygen (DO) and pH of the SPP are controlled during preparation. For range finding tests, 10 mysids are added to each of four concentrations -- 100%, 50%, 10% and 1% SPP and a seawater control, none of which is replicated. For definitive tests, 20 mysids are added to a seawater control and each of five concentrations that are based on the results of the range-finding tests. Three replications give a total of 60 animals per treatment. All treatments are aerated during the 96-hour exposure. Water quality (DO, pH, and and salinity) are measured at 24-hour intervals; temperature is measured continuously.

After 96 hours, the number of live organisms is determined in each drilling fluid concentration and in the control. Mortality data from the drilling fluid test and a reference toxicant test that must be conducted at the same time are subjected to statistical analyses. A 96-hour LC50 (the concentration lethal to 50% of the test animals after 96 hours of exposure) and its 95% confidence limits are calculated for each drilling fluids (if the mortality data are amendable) by using probit analysis (Finney, 1971) or other suitable methods (Stephan, 1977).

Application of Toxicity tests Results

The purpose of the toxicity test is to obtain general information on the comparative toxicity of a specific drilling fluid or component. The

test is considered a first-tier test because other tests may be required to determine the sensitivity of other organisms or communities if the toxicity of the tested material warrants.

An example of how the tiered system works and how the results of toxicity tests can be used to protect the environment through product substitution resulted from a recent evaluation of drilling fluids by the Environmental Resarch Laboratory at Gulf Breeze (ERL/GB). A cooperative program (Duke and Parrish, 1984) was conducted to determine the effects of 11 used drilling fluids on selected marine organisms. The fluids were collected from operating platforms that were drilling wells of various depths at different locations in the Gulf of Mexico. Drilling fluids were tested at ERL/GB to determine their effect on mysids and were analyzed for specific metals and hydrocarbon content by other laboratories. The tests with mysids indicated that several of the 11 drilling fluids were more toxic than fluids tested in the past (Petrazzuolo, 1981 and Ayers et al., 1983). Toxicity of whole muds to mysids ranged from 26 to >1,500 µl/l (ppm) and toxicity of the SPP phase from 726 to >50,000 ppm (Gaetz et al., in press).

Subsamples of the fluids were provided to other laboratories to determine toxic effects on several marine organisms. The toxicity pattern of whole muds to grass shrimp (*Palaemonetes pugio*) was similar to that for mysids. Grass shrimp were not as sensitive as mysids, but the relative sensitivity of grass shrimp and mysids to the 11 fluids was similar. The 96-hr LC50s for grass shrimp ranged from 142 to >100,000 ppm (Conklin and Rao, 1984).

The results of the mysid and grass shrimp tests suggested the need to test other organisms to confirm the effect of the petroleum hydrocarbons

in the used fluids. Fertilized eggs of hard clams (Mercenaria mercenaria) were exposed to the SPP phase of the fluids for 96 hr. The number of clams exposed to the drilling fluids that reached straight-hinge or "D" stage larvae was compared with the number of clams that attained this stage that were maintained in sea water without the fluids. The 96-hr EC50s (the effective concentration that prevented 50 percent of the clams from reaching the straight-hinge stage) were from 64 to >3,000 ppm (New England Aquarium, 1984).

A comparison of the diesel content of the 11 used drilling fluids and their toxicity to mysids, grass shrimp, and clam embryos indicated that the higher the diesel content, the greater the toxicity. The correlation between toxicity and diesel content was significant, according to the Spearman rank order method (Simpson et al., 1960). Subsequent results obtained at the University of West Florida by testing the toxicity of drilling fluids to larval grass shrimp, P. intermedius, before and after addition of diesel oil and mineral oil also confirmed that addition of petroleum hydrocarbons increased toxicity of the muds tested (Conklin and Rao, 1984).

Thus, the results of toxicity tests that began with a modification of the first-tier mysid test described in this paper revealed a component in the used drilling fluids that contributed greatly to the toxicity of the fluids to marine organisms. Under use conditions, results such as these would justify excluding the toxic material from a drilling fluid and adding a less-toxic substitute that would also perform the necessary function.

TABLE 1. Some chemical ingredients in drilling fluids[1]

Ingredient	Use
Barite Bentonite Attapulgite	Weighting Agents and Viscosifiers
Sodium Tetraphosphate Modified Tannin Chromium Lignosulfonate Calcium Lignosulfonate Lignite	Dispersants and Thinners
Starch Cellulose	Fluid Loss Reducers
Detergents Non-ionic Emulsifier Processed Hydrocarbons, including Diesel Oil	Lubricants and Emulsifiers
Aluminum Stearate Paraformaldehyde	Defoamers, Bactericides
Sodium Chromate Sodium Hydroxide Potassium Hydroxide	Corrosion Inhibitor pH Control pH Stability

[1] After Perricone (1980).

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INTRALABORATORY VARIATION

SAMPLE	96-HOUR LC50	95% CONFIDENCE LIMITS
1	2.9 SPP	2.4-3.5% SPP
2	2.5%	2.3-2.3%
3	1.3%	0.5-2.2%
4	2.1%	1.7-2.4%
5	2.4%	2.1-2.8%

SOURCES OF VARIATION

1. MYSID CONDITION
2. TREATMENT OF SPP
   A. DEFINITION
   B. SEPARATION FROM SOLID PHASE
   C. AERATION TO INCREASE DO

BASICS OF METHOD

* 1:9 DILUTION OF DRILLING FLUID
** pH ADJUSTMENT
*** SUSPENDED PARTICULATE PHASE
**** POSITIVE CONTROL INCLUDED

SENSITIVITY OF MYSIDS

GENERIC MUD	TOXICITY[A] (96-HOUR LC50)			
	MUD ALONE	MINERAL OIL ADDED (%,v/v)		
		1	5	10
#2	51.6	13.5	1.8	0.49
#8	29.3	7.1	0.90	0.76

[A]CONCENTRATIONS GIVEN AS PERCENT (v/v) OF SUSPENDED PARTICULATE PHASE IN SEAWATER.

INTERLAB RESULTS

TEST LAB	GENERIC MUD	TOXICITY (96-HOUR LC50)	95% CONFIDENCE LIMITS
GULF BREEZE	#1	2.7% SPP	2.5-2.9% SPP
	#5	NO MEDIAN EFFECT	---
NARRAGANSETT	#1	2.8% SPP	2.5-3.0% SPP
	#5	NO MEDIAN EFFECT	---

TERESA NORBERG-KING

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY

BIOLOGICAL ANALYSES OF COMPLEX EFFLUENTS

MRS. NORBERG-KING:

Actually, Norb told me to pass a message on to Bill. He told me that you owe him one. I got to come here today and explain some ideas of biological methods to chemists.

When Dale Rushneck asked me to present the paper on fresh water toxicity tests in relation to the testing approach that's being used for the permitting process that's currently underway, I said a tentative yes and it depends on who can pay my travel. Then he explained it was for an analytical symposium and I was pretty sure he wouldn't get me to go. Finally he said I didn't have to discuss policy because Steve Bugbee was going to, and in that case, I said sure. I don't like to talk about policy. Biologists never like to.

First, what I want to discuss is how EPA got involved in the toxicity testing developed for complex effluents. Then quickly describe the test

methods that we're using, and then to take some of our test results and compare them to the biological survey results.

First I just want to give you some quick background that explains and again reiterates some of what Steve Bugbee was saying. Under the Public Law 92-500, the procedures for regulating pollutants were laid out and a national pollutant discharge system, the NPDES Permit System was introduced. Permits were originally written to incorporate standards for toxicants. Yet these were not successfull and EPA chose to establish effluent limitations for the "priority pollutants." The limitations were to incorporate the Best Available Technology (BAT) that was economically feasible and to do so on a chemical-by-chemical basis.

Toxicity information on many compounds is limited, if nonexistent, and the interactions of mixtures are poorly understood. Consequently, a move to effluent toxicity testing in order to assess the overall toxicity of effluents has been under way.

The priority pollutant approach has not been useful in effluent testing because they are just not a concern to aquatic organisms. With the

exception of a few metals and pesticides, barely any priority pollutants are in effluents above the detection level. In fact, a recent article by Staples et al (1985) addressed the issue of priority pollutants in effluents. The authors evaluated EPA's STORET computerized data base of water quality information that's collected by the states and the regions. Their review pointed out that 80 percent of organic priority pollutants are not detectable in industrial effluents and that 78 percent of the priority pollutants are not found in the average waterway.

Effluent testing for aquatic organisms has been going on for years, but it's only recently with the water quality-based permit policy (EPA, 1985) that the renewed interest has been displayed. With this new-found interest, the emphasis for sub-lethal or subchronic tests to supplement the short-term acute toxicity has increased.

This is where the research efforts at the Environmental Research Laboratory at Duluth (ERL-Duluth) Minnesota, have been expended for the last three to four years. The goals of the complex effluent program are to validate the ability of the laboratory toxicity tests to predict the community

impact of the industrial and municipal effluents. To do so, we developed two short-term, subchronic tests which are both run for seven days. We use a cladoceran, Ceriodaphnia dubia, and a small fish, the fathead minnow, Pimephales promelas. We run a set of various effluent dilutions and we run samples of the receiving water. Both tests that we run are static with daily renewal of the test solutions. Most often, these tests are conducted on-site.

The Ceriodaphnia test end-points are reproduction and survival to estimate the effect of the level of the effluents. The fathead minnow test end-points are growth and survival to estimate the effluent effect levels. We have generally found the mean number of young produced per female for the Ceriodaphnia and that the mean weights for the fathead minnows are more sensitive end-points than survival in each seven day test.

Briefly, the Ceriodaphnia test uses newborn young (0-4 hours old) to start the test. The young are placed in individual test cups containing 15 mls. test solution each. As an aside, most people are familiar with Daphnia magna. So, the first thing people always ask me is how small is the Ceriodaphnia compared to the Daphnia. I can

see it with my naked eye; some people like to use a microscope, and we don't have to use electromicroscopy to read these results. Ceriodaphnia produce three broods in seven days which is an end-point for toxicity tests following the OECD guidelines. They produce an average young of 18 to 30, depending on what source of water is used in the test.

The fathead minnow test is a daily renewal static test and is initiated using less than 24 hour old post-hatch larvae. In seven days we have found they typically increase their weight five to seven times that of their initial weight. This is a quick overview of what the test methods are. If you want more information on the methods, see Mount and Norberg, 1984; and Norberg and Mount, 1985.

In addition to the effluent dilution tests, a set of tests that we have termed the ambient toxicity tests, are run (Mount et al, 1984). These are run with water that is collected directly from the stream. Typically the locations of these stations are established above and below each discharger and the water collected for the ambient tests allows us to evaluate what's actually happening when the effluent and the receiving water mix. The results

of the Ceriodaphnia and fathead minnow ambient tests are then used for comparisons with the stream community data.

For these ambient tests, the concentrations of effluents in the stream are not important, but rather whether the water in the stream has an observable effect in the toxicity tests and on the stream community. So what you are actually getting with these ambient tests is a measure of toxicity of various stream stations which we can then use to compare to the condition of the biological community at the same stations.

Steve Bugbee mentioned the work that has been going on for these sites. It can be intensive laboratory and field work. We have completed eight site studies around the country covering Connecticut (Mount et al, in press), Alabama (Mount et al, 1985), Oklahoma (Norberg-King and Mount, in press), Maryland (Mount et al, in press), West Virginia (Mount and Norberg-King, in press; and Mount et al, in press), and Ohio (Mount et al, 1984; Mount and Norberg-King, 1985).

Along with the ambient tests, the field surveys of the biological and hydrological conditions are done. These consist of fish collection, benthic

sampling, periphyton and zooplankton sampling or artifical substrates where appropriate.

What I want to discuss now, in the remainder of this talk, is how these biological analyses of the effluents and the ambient toxicity tests compare. This discussion will present the analysis of effluents by means of the ambient toxicity tests compared to the stream community for just three out of eight field sites.

The first site I want to discuss was conducted on a very small prairie stream in Oklahoma called Skeleton Creek. It has a very low gradient and the stretch of river studied covered approximately 27 kilometers. There are three dischargers that are located on this small creek directly. They are a Publicly Owned Treatment Works (POTW), a refinery and a fertilizer plant.

SLIDE 1

What you see on this slide, along the bottom, are the stream stations that we plotted, and along the left hand side is the mean fathead minnow weights. The data is the mean individual weight at the end of the seven day test, which is a dry weight. Please note these stations were not equal distances apart on the stream as they are depicted here.

Again, just to quickly explain what the ambient tests are, they are the daily renewal tests with a grab or a composite sample from each river station, that are tested to determine if chronic toxicity occurs instream. Note with this site, we saw substantial toxicity observed at Station 5.

SLIDE 2

Next are the results of the Ceriodaphnia and the fathead minnow ambient tests plotted together. These show that there were similar responses of each species up to Station 5 and yet there was a dramatic drop in the Ceriodaphnia young production evident at Stations 7 and 8. Irrigation flow entered the creek at various points, but the first known entering above Station 7. In fact, the water from Stations 7 and 8 had chronic activity levels that were three times those of upstream stations. We have data that show the Ceriodaphnia to be much more sensitive to salinity than the fathead minnows, which may account for this. You may also note that the slight inhibition of young production and weight values at the most upstream station, which was Station 2, was removed after the POTW and the refinery effluents were mixed in the stream.

SLIDE 3

Next is the fathead minnow weight data and the dotted line is the number of benthic taxa. The results of the benthic taxa show us they were unaffected over the entire study area. If toxicity observed in the ambient tests was due to ammonia from the fertilizer plant, or from one discharger--I'm trying not to point fingers here--the benthic population may not have been affected due to their lack of sensitivity to the ammonia.

SLIDE 4

Next, is the dash line, is the number of the fish taxa plotted against the mean fathead minnow weights, and these data match extremely well. You can see that the number of fish taxa show a response at Stations 7 and 8 that were not displayed in the fathead minnow ambient test.

SLIDE 5

But when we plot the Ceriodaphnia data together with the fish taxa as the dash line, you can readily see more similar responses. For this site, an invertebrate test organism is much more predictive of the stream fish population as the Ceriodaphnia test results correlate extremely well with the number of fish taxa collected in the biological

survey. This all is aimed at trying to answer the question of which species to use and whether it's a sensitive species, or how do we choose which species we're testing for effluents.

The second site I want to discuss is Five Mile Creek in Alabama. It is a foothill, spring-fed creek with a high gradient in the upper end. The study area covered a 42 kilometer stretch of river. In this area there are two coke plant dischargers in the upper stretch and a POTW discharge farther downstream, and a few tributaries entered the stream.

SLIDE 6

What you see here are the mean weights of the fathead minnows plotted against the stream stations for Five Mile Creek. Substantial toxicity was observed at Station 5. Again, to reiterate, what I'm showing you are chronic test results and it's important to keep that in mind. We feel we are able to make better predictive value judgments based on chronic data than we can on acute data.

SLIDE 7

Here is one of my favorite slides. Tom had one of his favorite slides and I have one of mine. I'd like to just leave this one up for a while and figure

out something else to talk about so you can stare at this one because this is one of our better slides. The values for the fathead minnow weights and the number of the fish taxa at every station, show the response is the same, with the sharp decline of the taxa correlating extremely well with the measurable toxicity of the fathead minnow ambient toxicity test. Even the slight upstream effect that was observed at Station 1, the ambient toxicity test, was also observed in the number of fish taxa. This is better agreement than we had expected or we had hoped for in our comparisons.

SLIDE 8

So, if we take the same fathead minnow ambient test data and we plot the number of benthic invertebrates, there's good agreement. At the upper three stations the agreement was not as good, but because of those stations at the upper end, what actually happens is that we would under-predict the impact at those stations.

SLIDE 9

The Ceriodaphnia toxicity test results also show some of that same inhibition at the upper end, yet when the tributary flow entered at Station 3, just after 2A, and after the coke plant's effluents

entered, what we saw was increased young production. Only after the POTW discharge entered the stream did we see additional toxicity in Ceriodaphnia young production.

These dramatic result differences between the fathead minnow weights and the Ceriodaphnia young production indicates the value of running toxicity tests with more than one test species. We may have missed something with just running one species, but we gained a lot with running two species. Now, how to make them mesh is another question.

SLIDE 10

We took that same Ceriodaphnia data and we plotted the zooplankton density in the stream with the mean number of young per female for the Ceriodaphnia at each ambient station. We shifted the zooplankton response in the stream upstream to match the upstream stations and allow for the drift that would be occuring in the stream. There is really good agreement and the same decrease in the upper reaches is there with the zooplankton in the stream that we saw with the Ceriodaphnia ambient test.

So with this site, the Five Mile Creek site, we can sum the results of the toxicity testing and

the field survey as follows. The fathead minnow tests and the fish test results correlate extremely well. My favorite slide pointed that out. The Ceriodaphnia and the zooplankton tests correlate extremely well when one allows for the drift. The benthic taxa does not agree well with the fathead minnow tests at a few stations and the reasons for this are too numerous to postulate here. However, completely different responses of the two laboratory toxicity tests again points to the value of testing more than one species.

The last site I am going to discuss is the Naugatuck River in Connecticut. It is a very high gradient stream with large rubble boulders in the upper reaches. This was a complex site and it covered 64 kilometers of river. There were also numerous tributary inputs that occurred. A total of 27 discharge outfalls are located on the stream and on the tributaries. Most of the industrial discharges are metal plating operations with similar plant designs and the effluents contain mostly the same heavy metals.

SLIDE 11

Again, please note on this slide, I've plotted where the tributary input enters the stream across

the top as well as the POTW inputs. Again, on this slide as on the other one, the stream stations are not equal distances apart as the slide appears to indicate. Most of the plating operations and the other industrial discharges went directly into two main tributaries. Also, there was a reservoir above Station 4.

This same slide shows the results of the fathead minnow ambient test and this site, being so much more complex, it's difficult to interpret. The toxicity at Stations 10 and 11 was not chronic but it was rather known to be due to a spill in the river that occurred early during the testing. When such an event occurs, it results in an overestimate of the toxicity at those two stations. We are not able to start the test again after a spill happens because then the animals are older or a different age group or something.

The two single data points shown by the arrows are the results of fathead minnow ambient tests with the source of that test water being collected directly in the mouth of the tributary with the large amount of dischargers on it. Essentially what was measured was an estimate of the combined effluent effects in each tributary prior to mixing

with the river. We saw toxicity in the tributary sample above Station 6. We saw some decrease in growth in ambient Station 6 water. We also saw toxicity in terms of reduced growth above Station 8 with the tributary sample, and we saw a sharp decline in the ambient toxicity test weights of the fathead minnows at Station 8.

SLIDE 12

If you take the periphyton diversity and plot it against the fathead minnow test results, there's good agreement on these two curves. There is a problem here in that periphyton diversity was used rather than taxa, but this is done because periphyton have not been identified as to species as well as many other groups such as the fish or the benthic macroinvertebrates.

SLIDE 13

The plot of the number of fish taxa with the fathead minnow ambient test data does not show any definite trend except possibly for lesser numbers of fish taxa downstream. We feel we could not discern any clear area of impact.

SLIDE 14

The benthic invertebrate taxa plotted against the fathead minnow ambient test shows a general

decline of taxa from the headwaters with a substantial and consistent drop over Stations 3 through 7. Except it appears to recover at Station 9 as the number of benthic taxa increase, and increased weights were obtained at Station 9 in the ambient toxicity test.

SLIDE 15

The Ceriodaphnia ambient toxicity data is plotted in dotted line, and the dash line is the zooplankton density in the stream. When one corrects for the drift of the zooplankton and moves the response of the Ceriodaphnia upstream to account for drift, again we see good agreement.

So, what this all sums up for the Naugatuck River is that there is a good agreement from the periphyton, the benthic macroinvertebrates, the zooplankton to the laboratory ambient toxicity test data, while the fish taxa data did not show any real clear trend of impact with the toxicity tests in this instance.

From these studies and from this approach to complex effluent testing, the following conclusions can be made. One, that in every study the toxicity was shown by one or both test species which correlated with an adverse impact in the community. This says that no one test species represents one group of

the community, but rather some group in the community, albeit fish, benthic macroinvertebrates or the periphyton, and that the impacted component or components of the community correlates with the toxicity found in the lab. Two laboratory test results differ from site to site. Therefore, the test species cannot be considered a surrogate for any one component of the community.

Finally, to say that this has been a brief summary of the lab to field data. We have other projects we are considering now such as negative interactions, species sensitivity, and the frequency and cause of reverse toxicity curves. This data is only for three out of the eight sites. We have reports on all of the sites in near final form. It appears that toxicity tests will be used as permits limits soon. We know of at least two states that already have implemented toxicity testing into their state policies. Also, we may need to start looking for the toxic components in an effluent in order to advise industries where their problem is. However, when that happens, we feel as the biologists, that we really must incorporate toxicity testing in that the analytical methodology cannot provide the whole picture.

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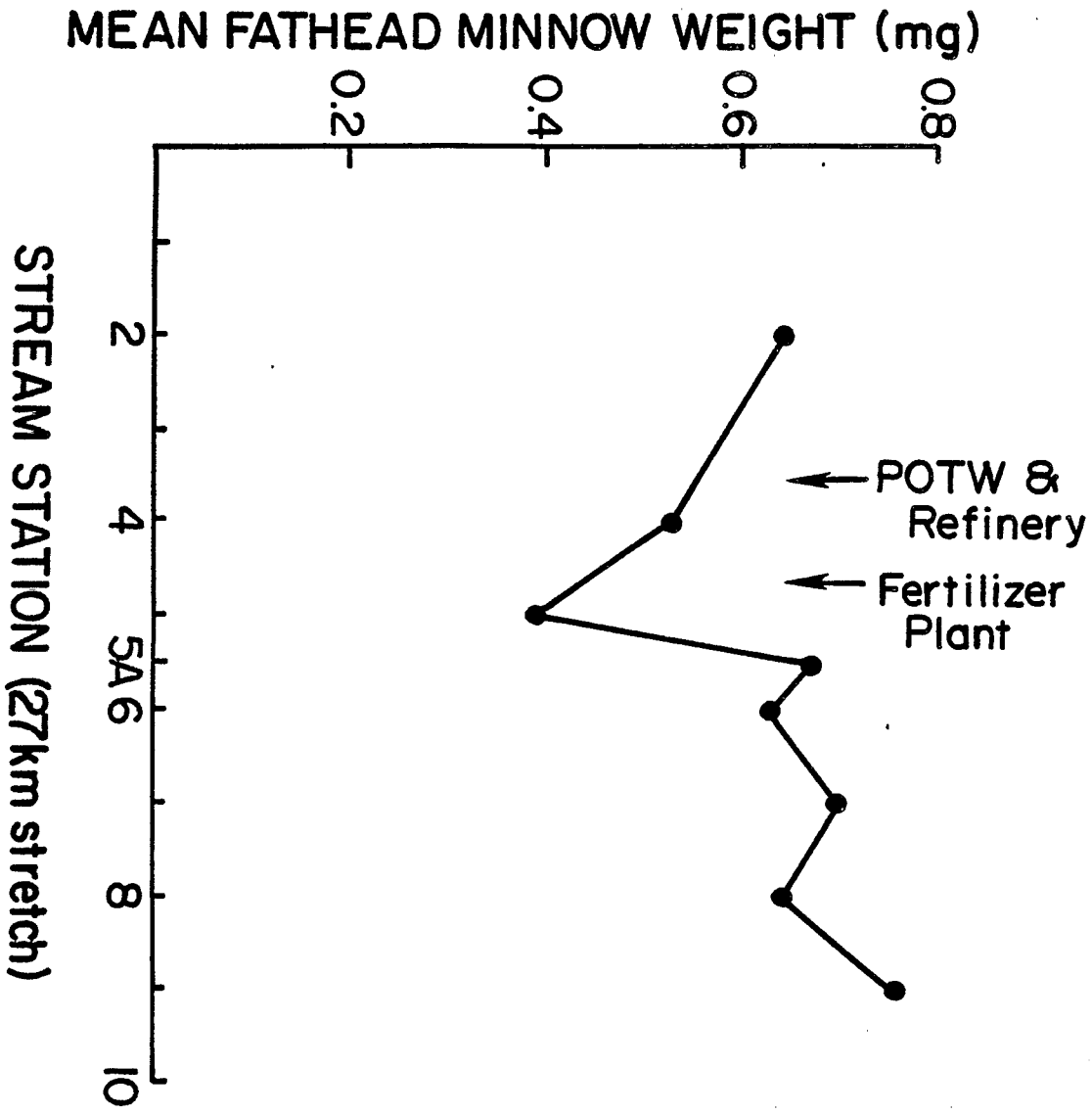
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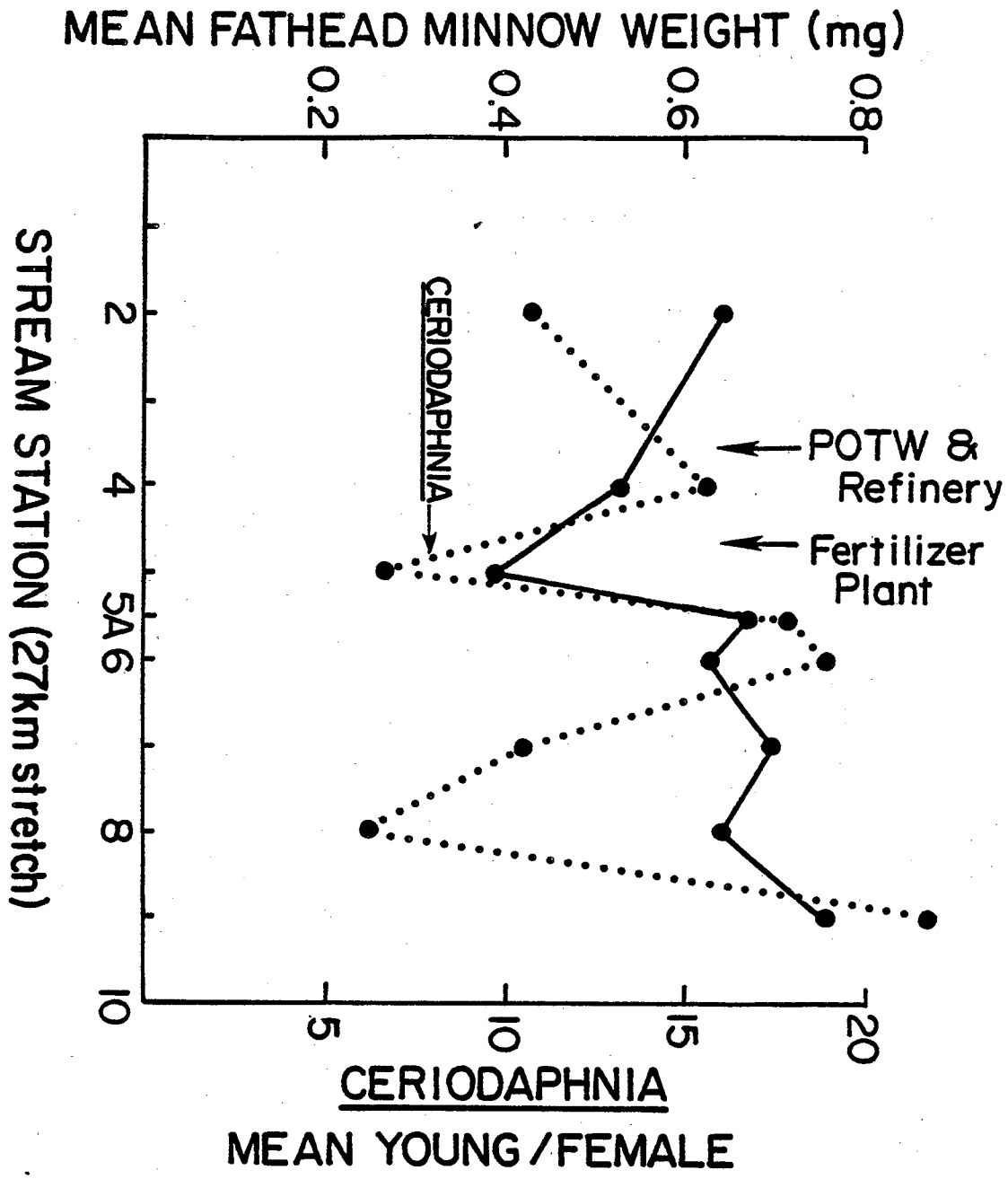
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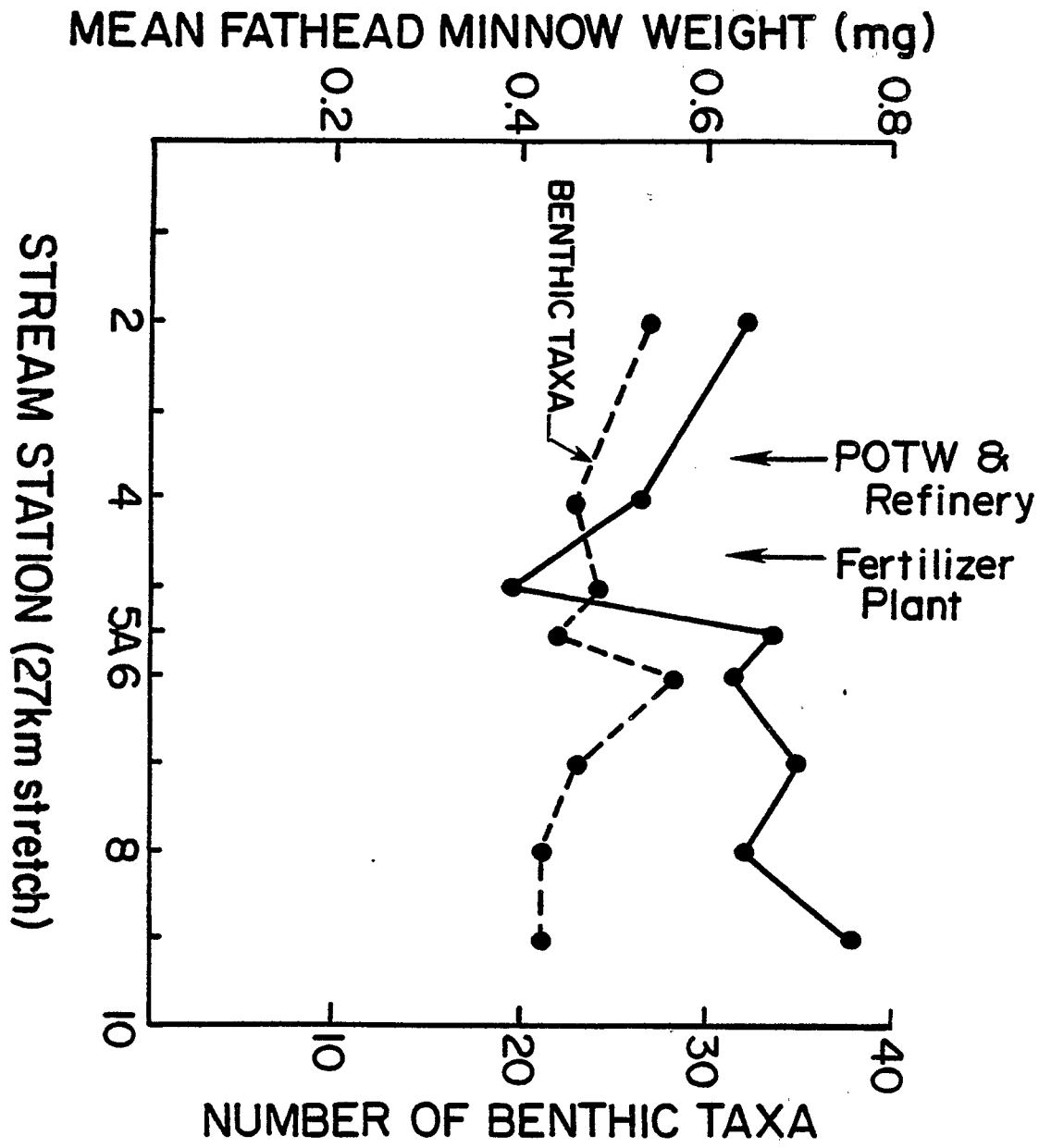
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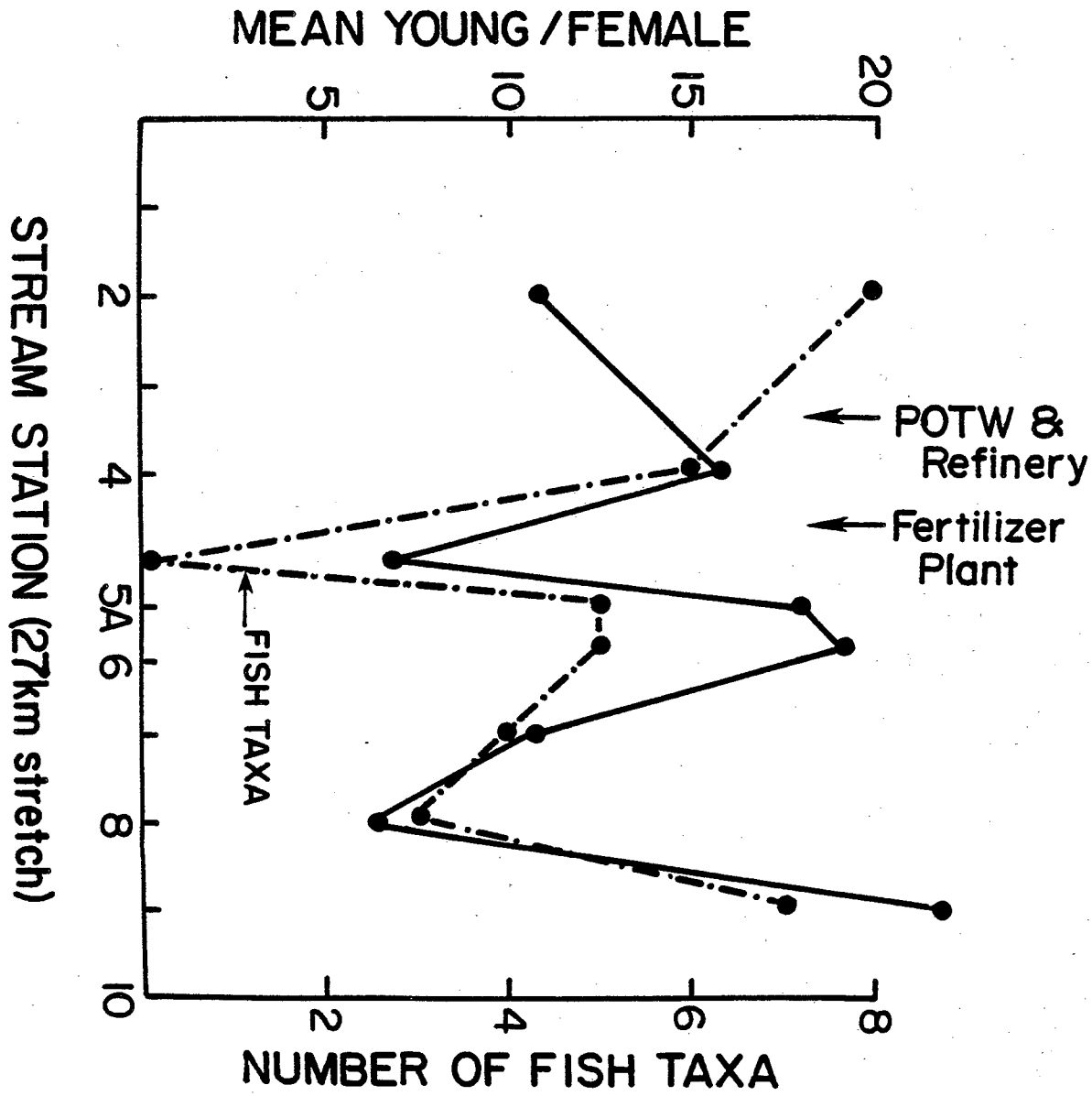
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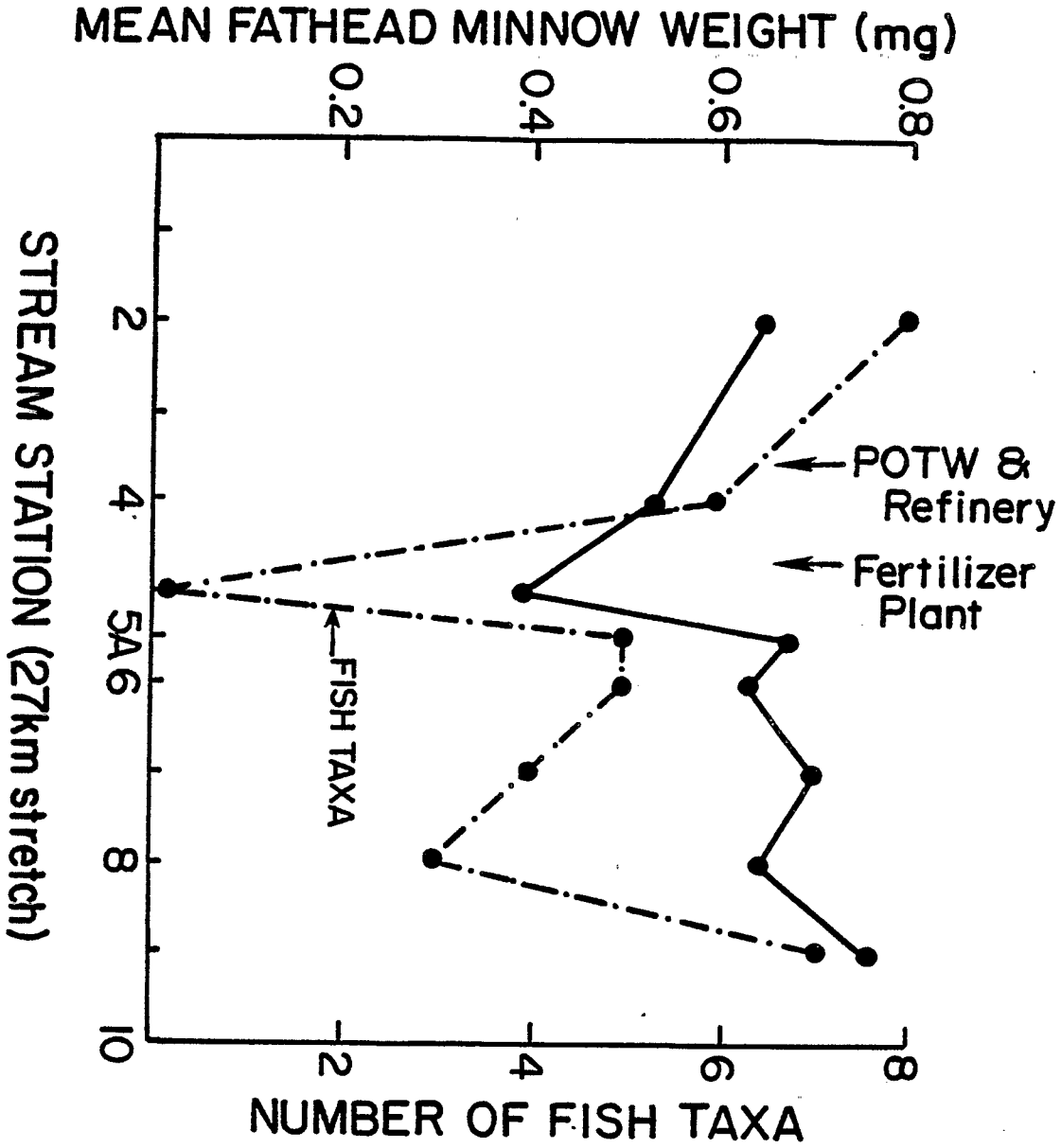
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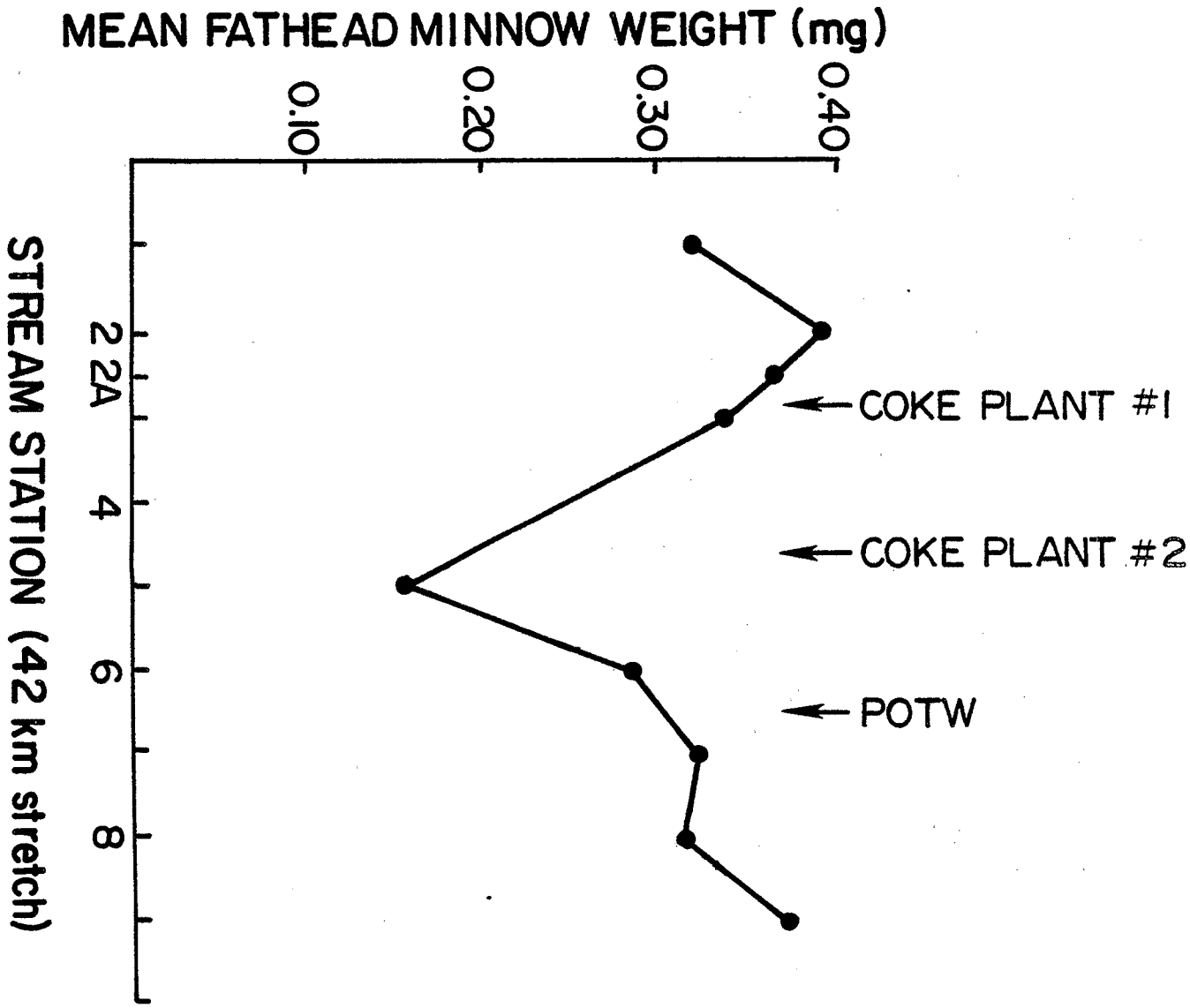
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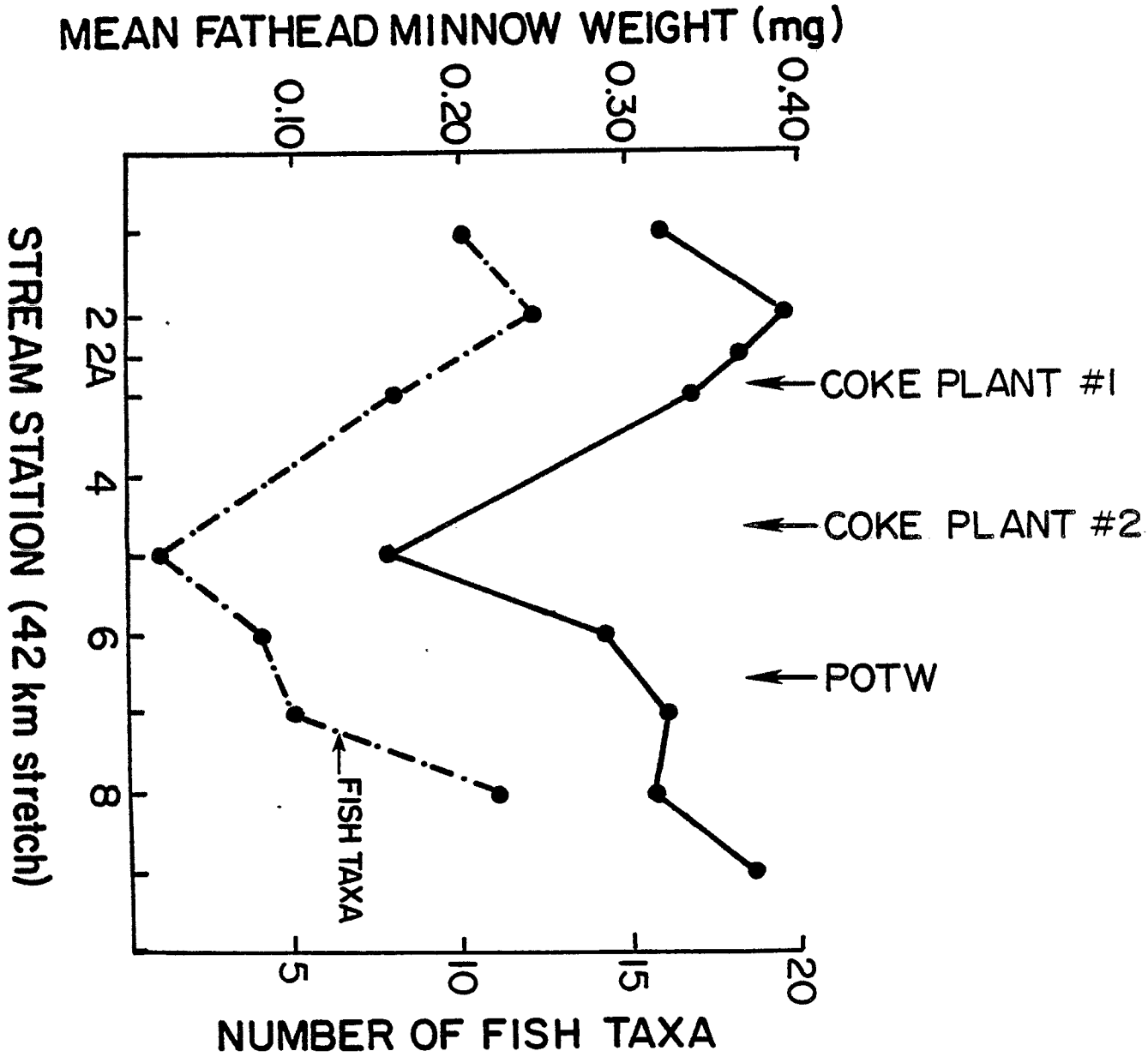
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0.10
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POTW
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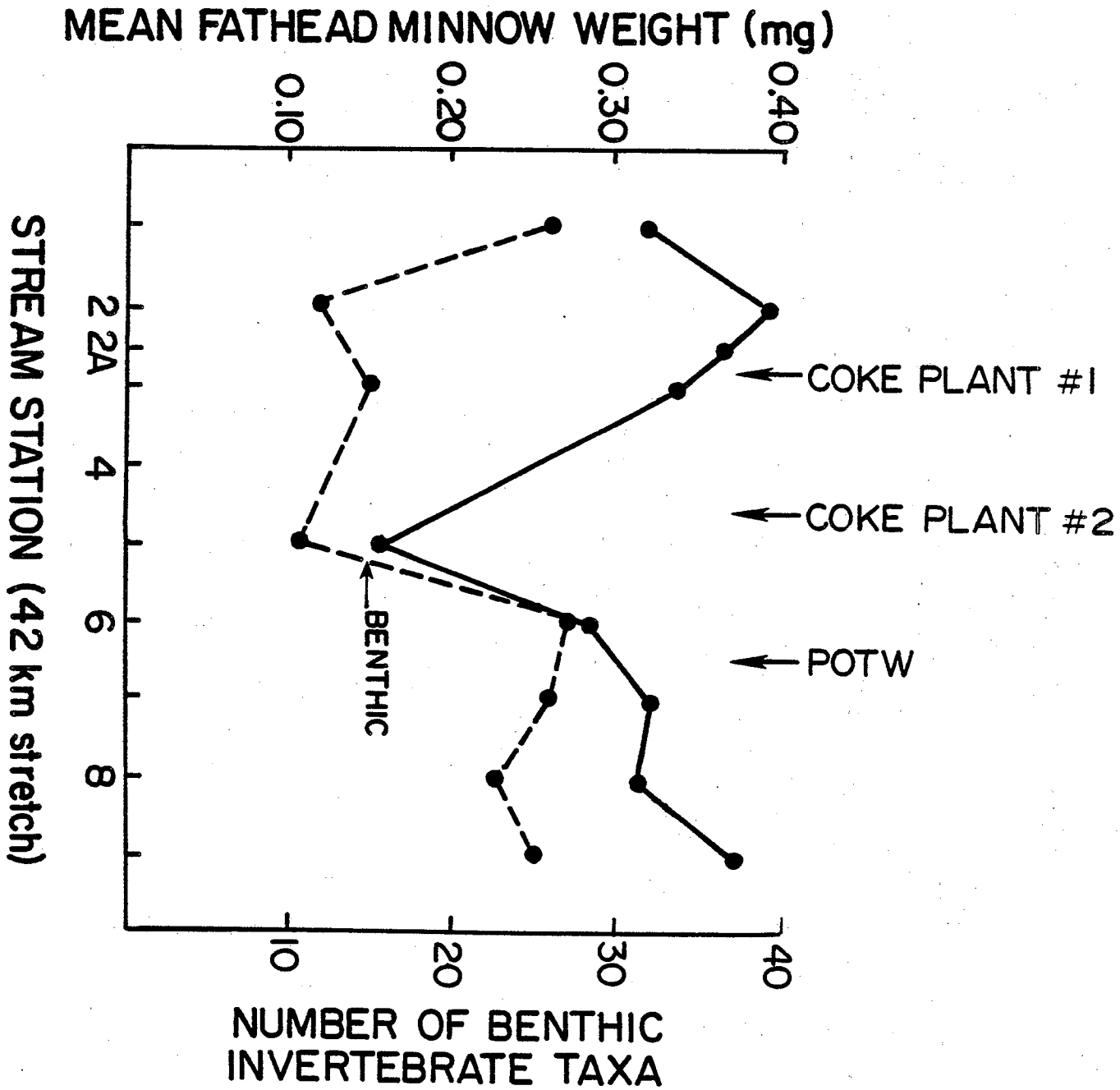
STREAM STATION (42 km stretch)
20
15
10
5
NUMBER OF FISH TAXA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2 2A 4 6 8
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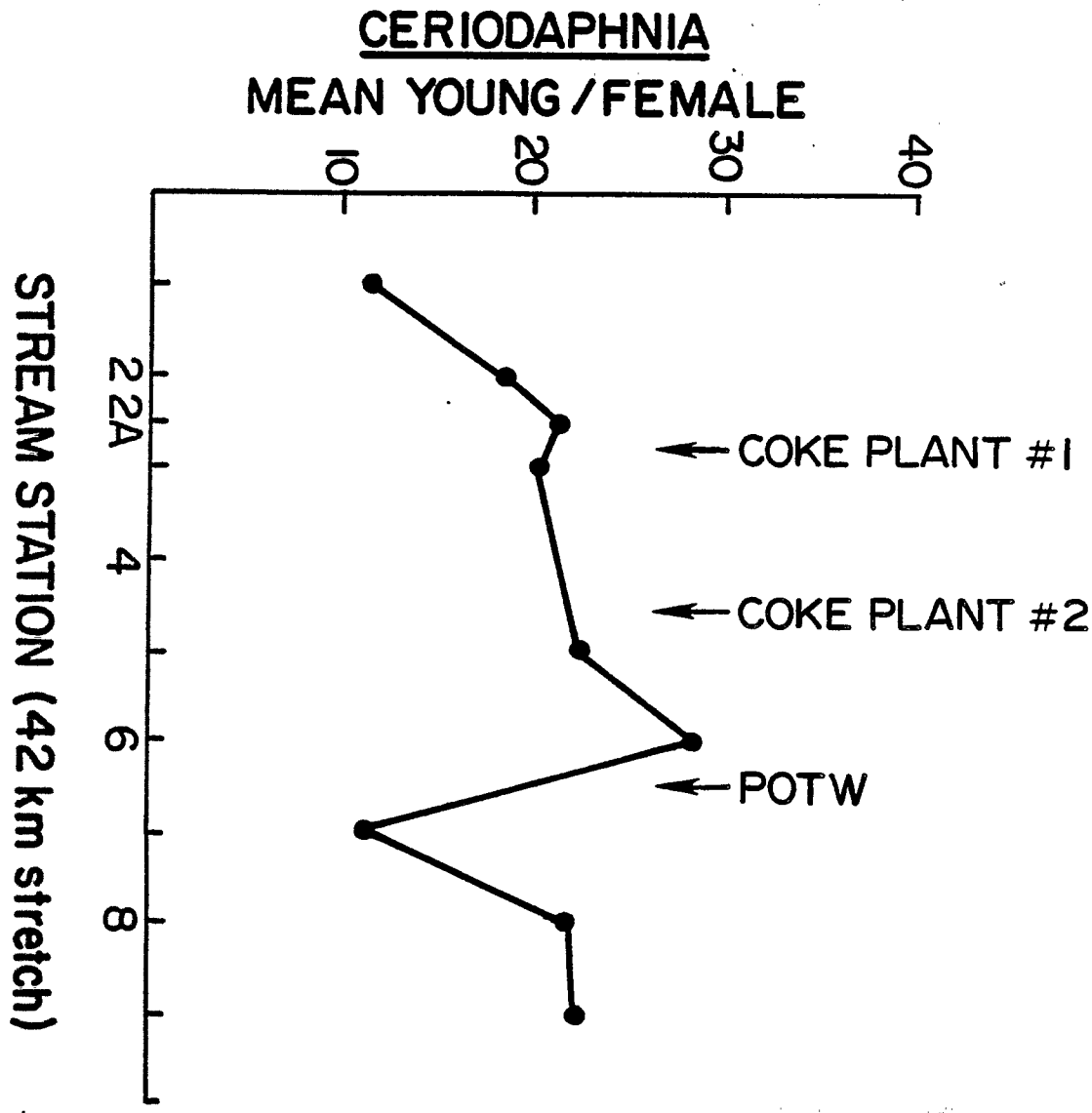
30
20
10
NUMBER OF BENTHIC INVERTEBRATE TAXA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30
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POTW
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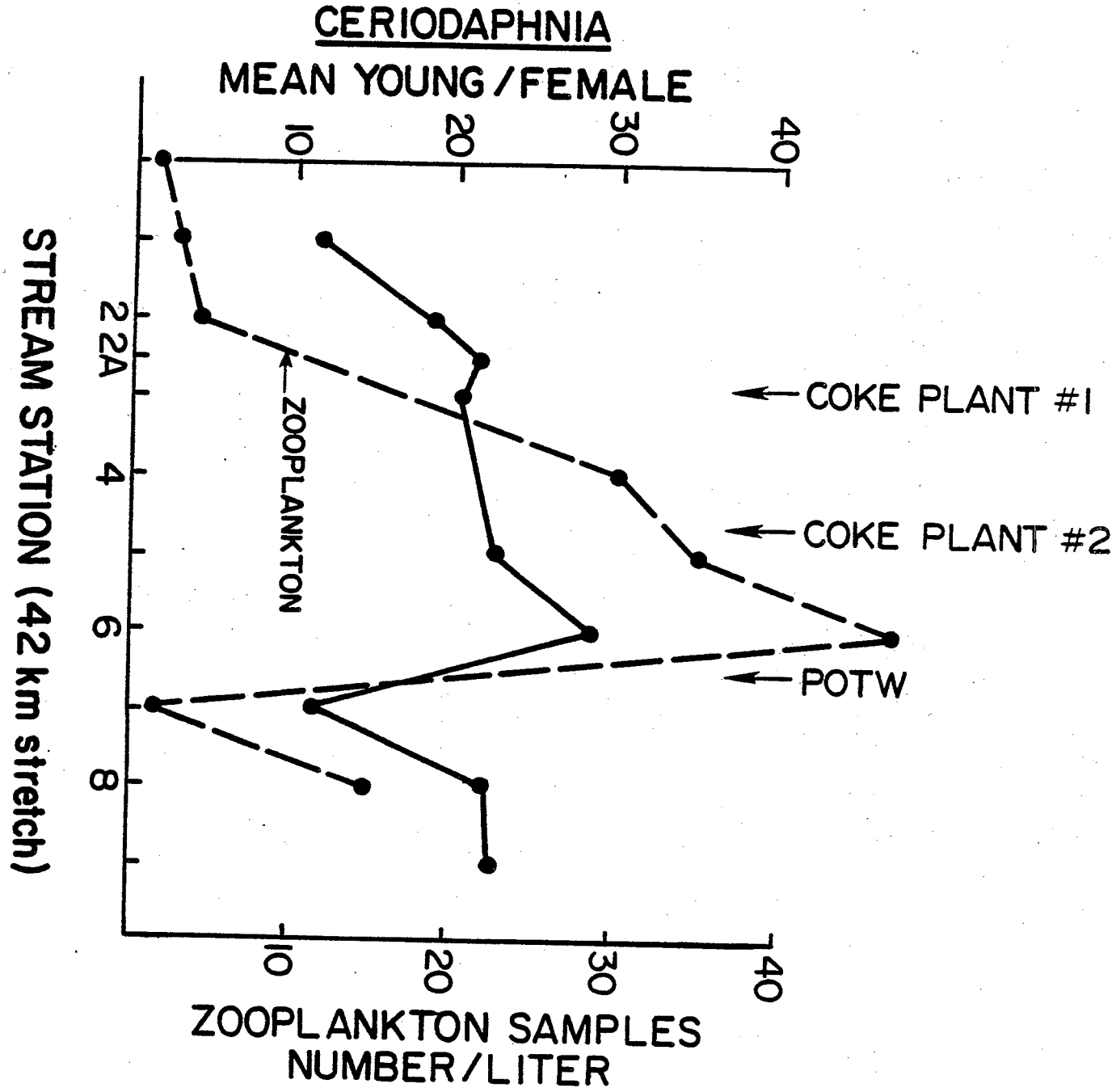
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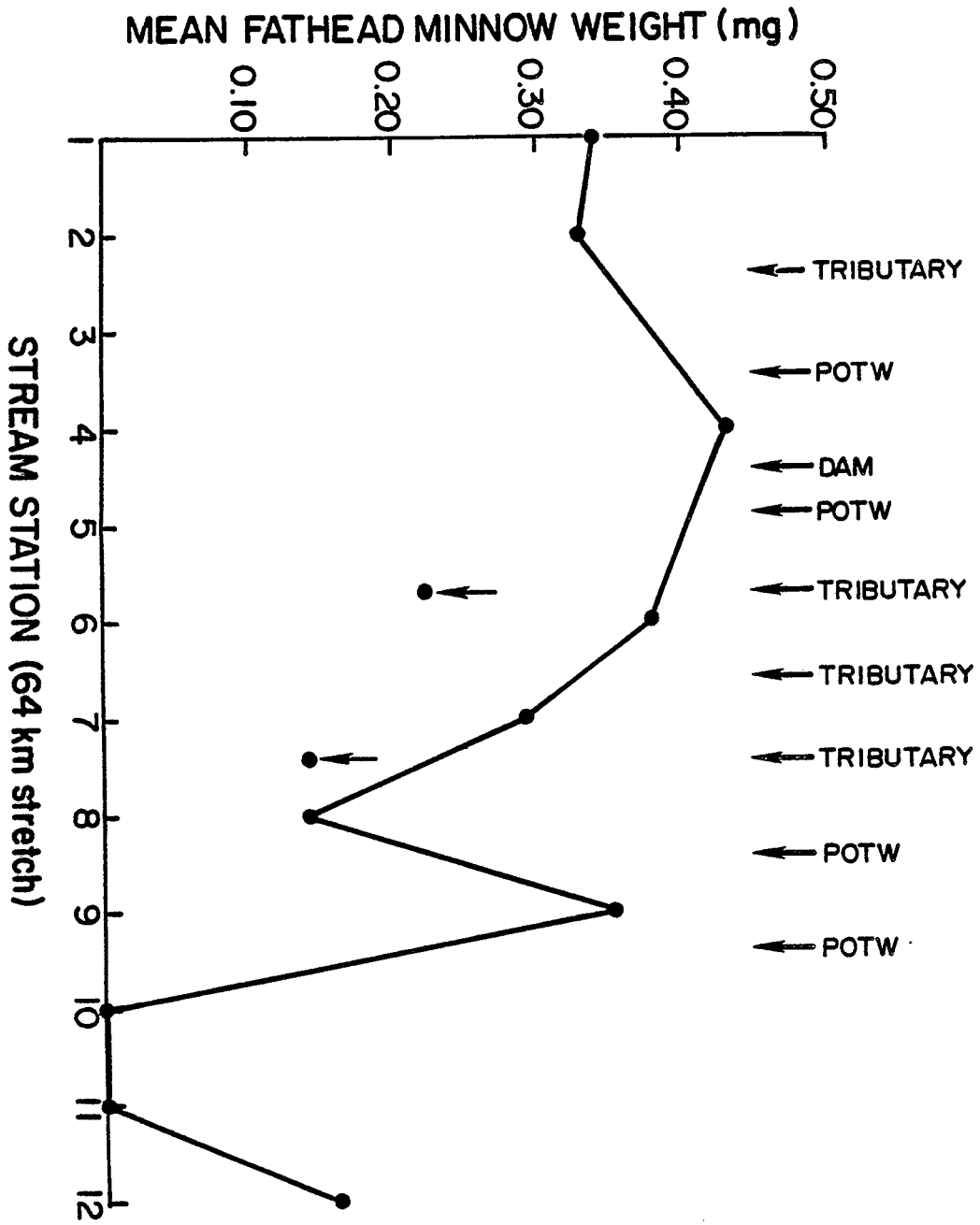
NUMBER/LITER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TRIBUTARY
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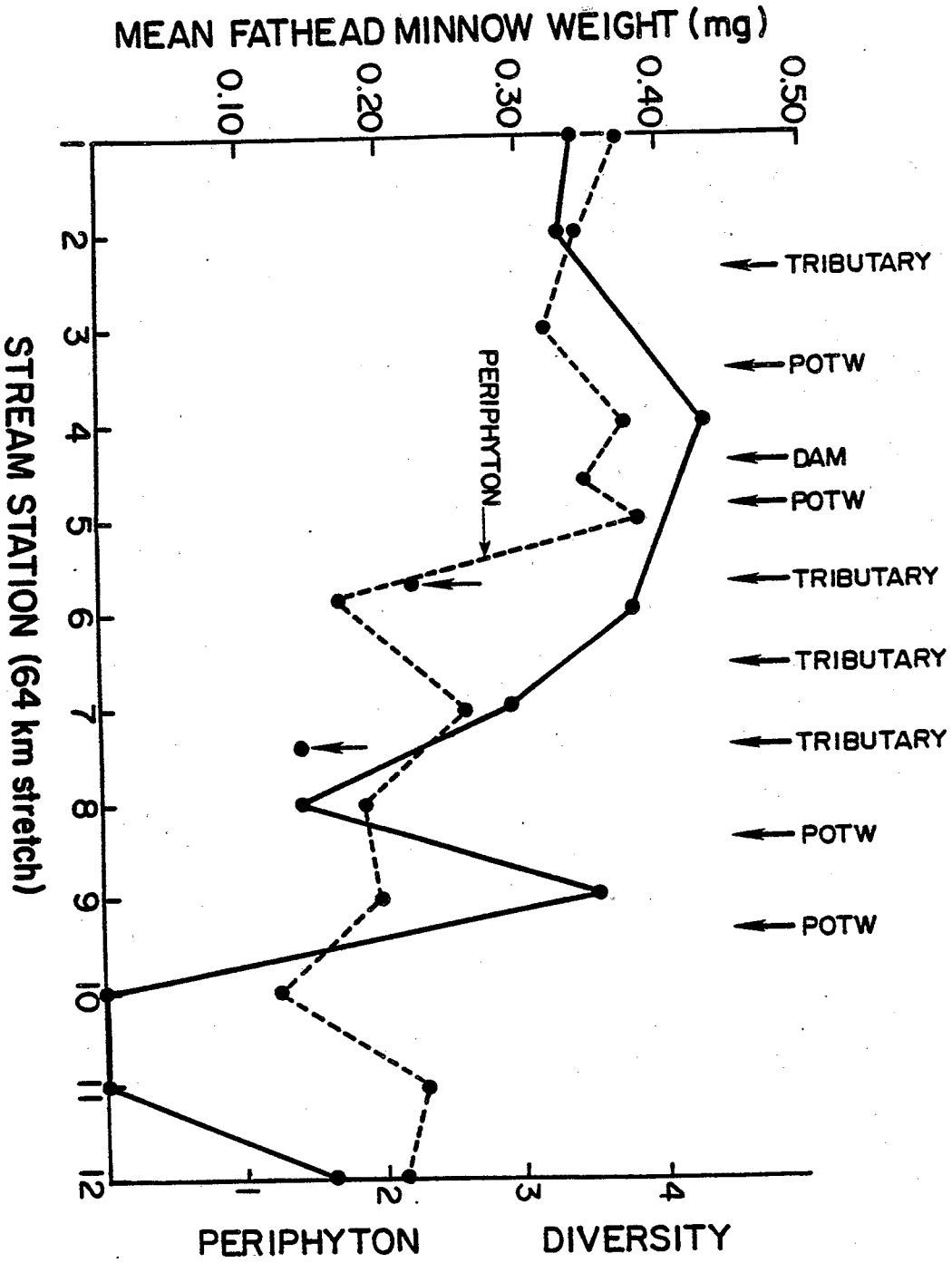
2
3
4
5
6
7
8
9
10
11
12
STREAM STATION (64 km stretch)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TRIBUTARY
TRIBUTARY
POTW
POTW
PERIPHYTON
STREAM STATION (64 km stretch)
1
2
3
4
5
6
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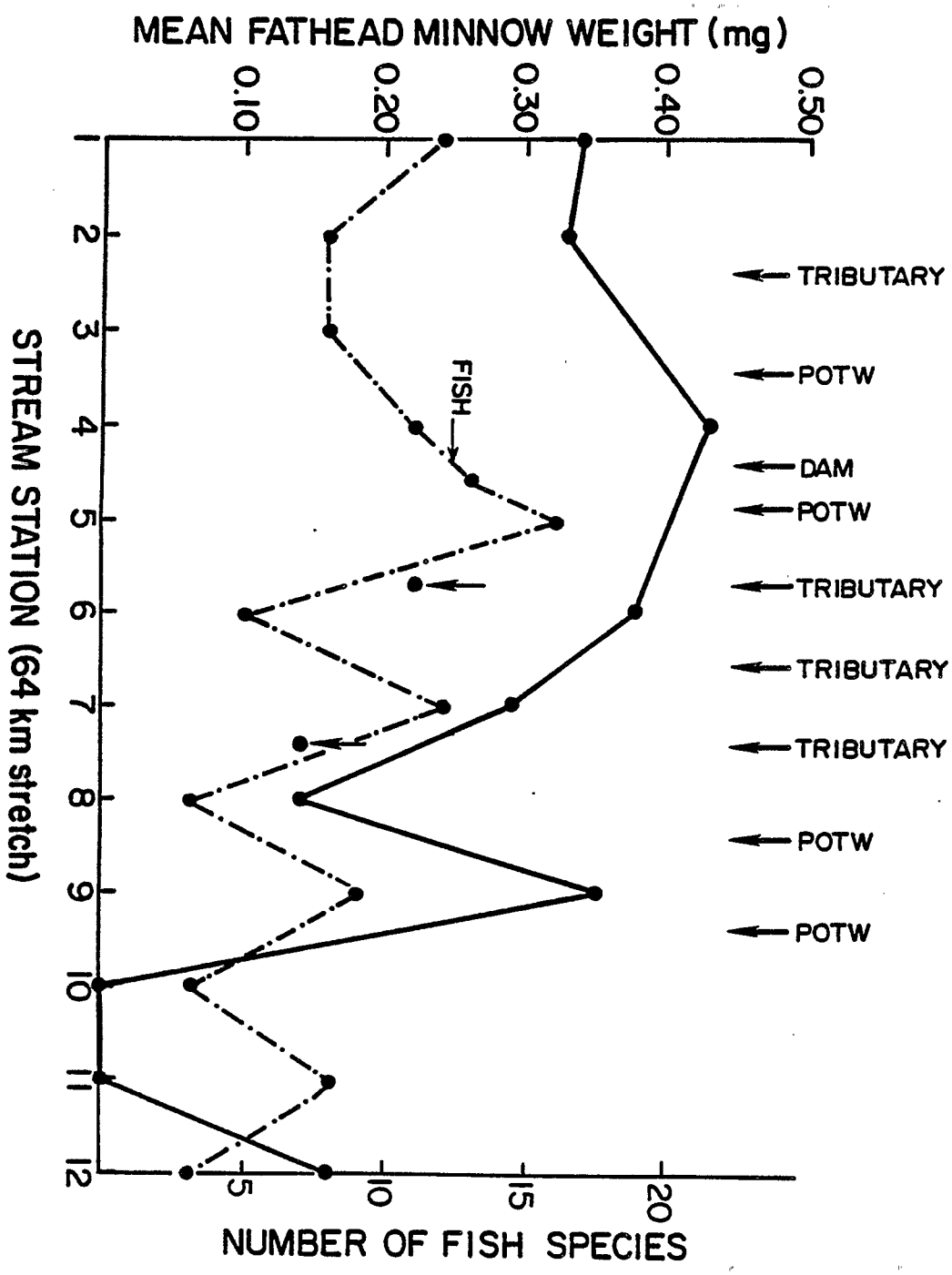
10
11
12
PERIPHYTON
DIVERSITY
1
2
3
4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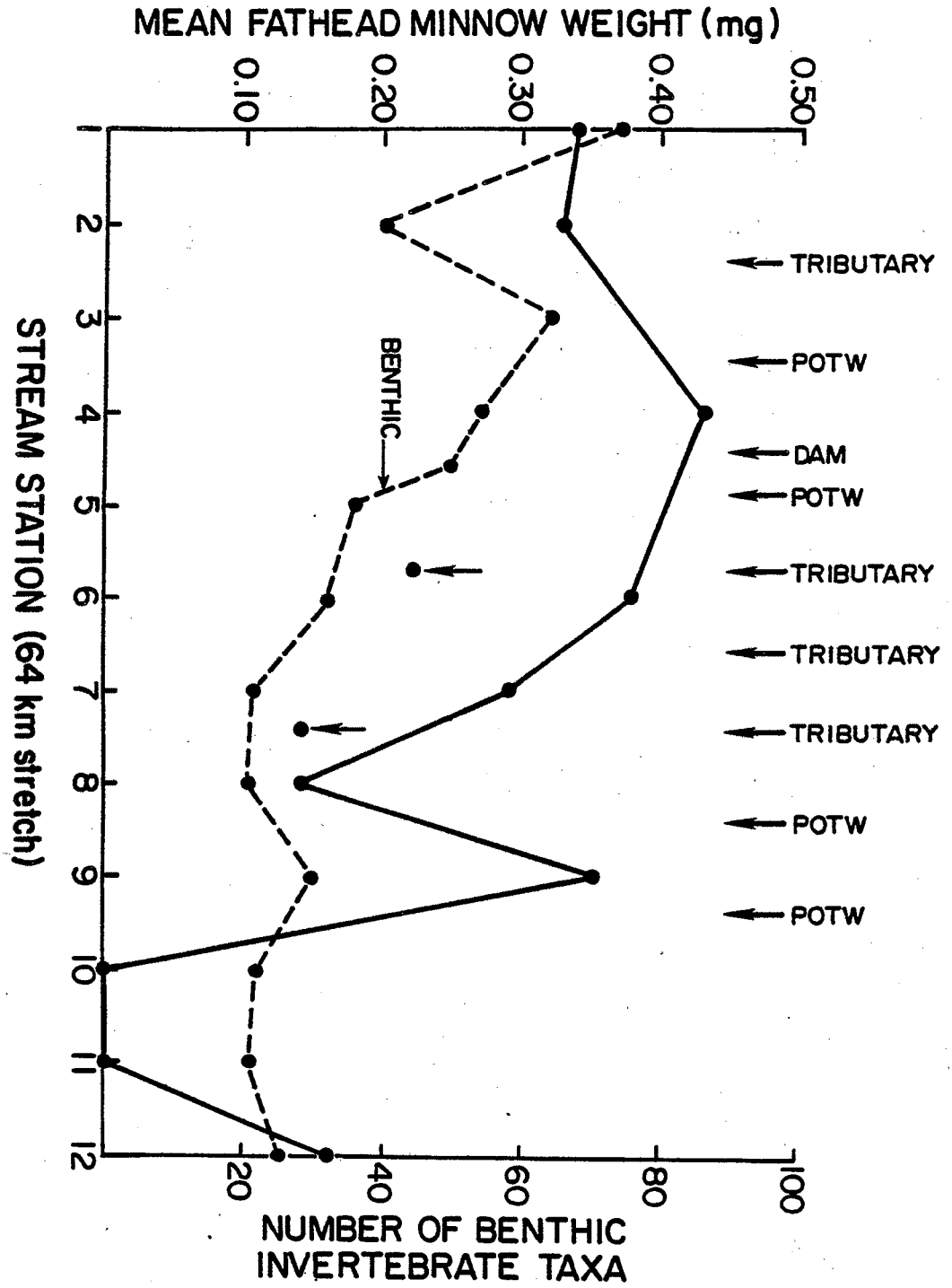
TRIBUTARY
TRIBUTARY
POTW
POTW
FISH
2
3
4
5
6
7
8
9
10
11
12
STREAM STATION (64 km stretch)
5
10
15
20
NUMBER OF FISH SPECIES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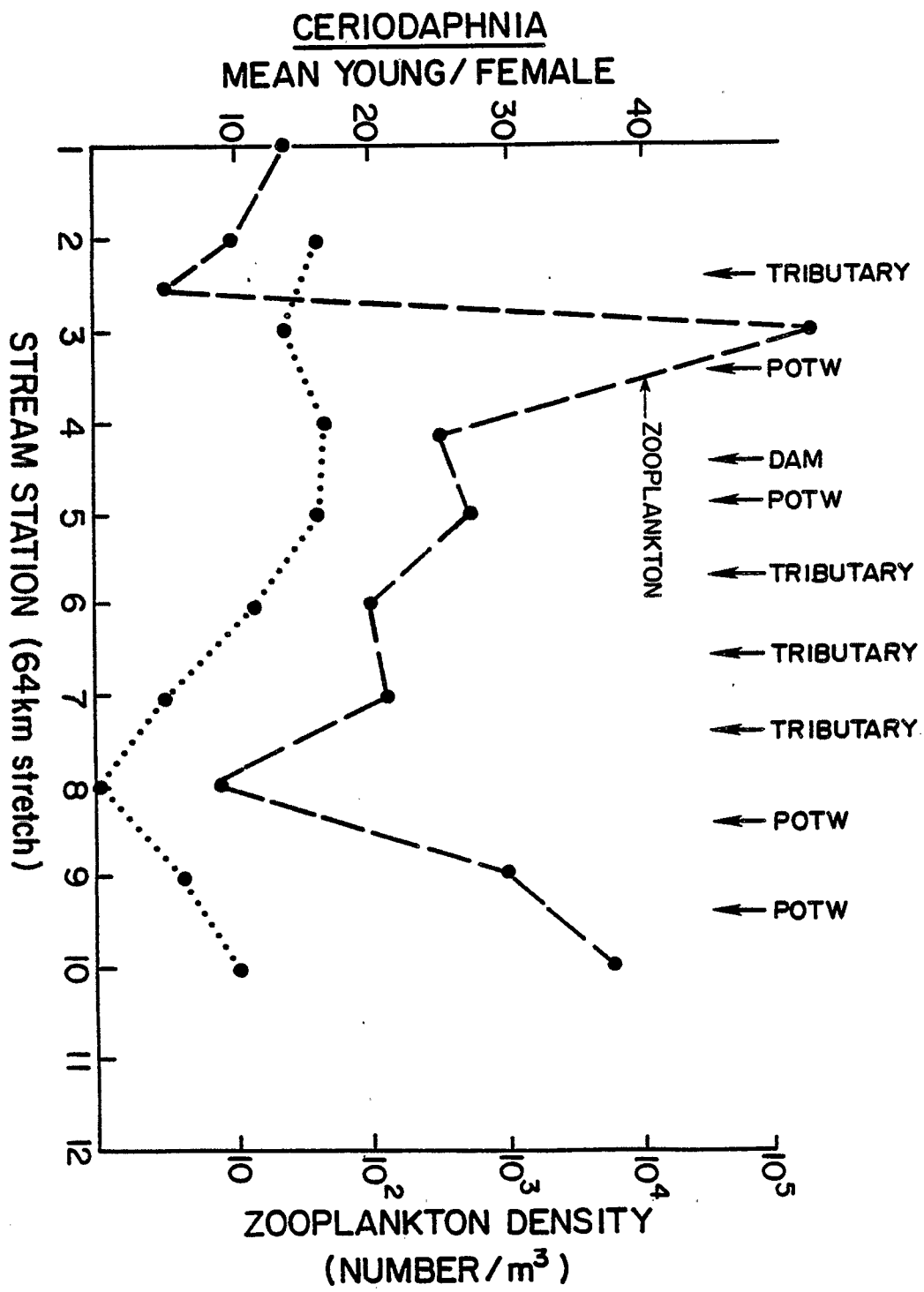
DAM
POTW
TRIBUTARY
TRIBUTARY
TRIBUTARY
POTW
POTW
BENTHIC
STREAM STATION (64 km stretch)
2
3
4
5
6
7
8
9
10
11
12
20
40
60
80
100
NUMBER OF BENTHIC
INVERTEBRATE TAXA


CERIODAPHNIA
MEAN YOUNG/FEMALE
10
20
30
40
TRIBUTARY
POTW
ZOOPLANKTON
DAM
POTW
TRIBUTARY
TRIBUTARY
TRIBUTARY
POTW
POTW
STREAM STATION (64km stretch)
1
2
3
4
5
6
7
8
9
10
11
12
10
10^2
10^3
10^4
10^5
ZOOPLANKTON DENSITY
(NUMBER/m^3)

QUESTION AND ANSWER SESSION

MR. PRESCOTT: I'm Bill Prescott. Could you amplify just a little bit what this correction for drift business was? Why and how?

MRS. NORBERG-KING: Sure. One of the feelings we have is that since the zooplankton in the stream are drifting organisms, we don't expect them to be stationary. If you collect them at Station 5, they're probably reflecting the population response of an upstream station as they move downstream with the water. What we are trying to do is predict how fast they're moving with the water and then move the response of the animals upstream to where they would have been in terms of what exposure they would have received.

MR. PRESCOTT: Thank you.

MR. HUNT: I'm Gardner Hunt with the main department of Environmental Protection. Have you had any experience with the diversity index?

MRS. NORBERG-KING: Yes, we've done a number of things with the diversity indices. Right now we present indices in our reports, but we don't feel that the biological

indices are the best measure for us. They're not giving us the actual number of taxa present in the stream. We may find that in one species there may not be a true representative of the species at that station.

I'm not the one to explain the diversity indices. We have a contractor that does all the work for our biological field work, doing the biological indices. Don Mount, for instance, is a much better person to decide what we're using about the biological indices as he does the integration chapter in our report. I do a lot of the field work, a lot of the toxicity testing, and am just starting to try making some heads or tails out of integrating the approaches. I'm sorry I don't have a better answer.

MR. TUROSKI: Vic Turoski, James River. What two states have instituted biomonitoring with respect to permitting?

MRS. NORBERG-KING: New Jersey and Virginia.

MR. TELLIARD: Any other questions? I have one, Teresa.

MRS. NORBERG-KING: Yes.

MR. TELLIARD: If I was

fortunate enough to have one of these permits with biological monitoring requirements, what would it cost me to run these...

MRS. NORBERG-KING: For a Ceriodaphnia test?

MR. TELLIARD: Yes.

MRS. NORBERG-KING: Given that you have the laboratory...or say that you would have to go after a contractor to run one of these tests, our estimates for a Ceriodaphnia test are anywhere from $500 to $1,000 to run one seven-day Ceriodaphnia test. That's extraordinarily low. The fathead minnow test probably runs up to $1,000 to $1,500. It's just a little more labor-intensive.

MR. TELLIARD: Thank you. I'd like to thank this morning's speakers--Steve, Teresa and Tom. This is our first time talking about critters and I think it was very good. Can we have a hand for them?

We're going to break now for coffee and please get back in here in 15 minutes. We've got a long day ahead of us. Thank you.

(WHEREUPON, a 15 minute break was taken.)

MR. TELLIARD: For those people who struggled in late, there's a lot of room in the front pews and they don't cost any more.

Continuing with this morning's session, we have two speakers that are going to be addressing...probably if you had a theme for a meeting, we had critters and probably LC/MS. LC/MS is a new initiative for the industrial technology division. We, of course, feel that it's only fair to stay ahead of API and CME by grabbing new analytical tools when they're not looking and running off and using them.

We have, this year, started looking seriously at LC/MS which gives us a lot more flexibility in some of our areas of concern. The folks at our Athens Laboratory, the industrial technology division's lab, has been working hard at some LC/MS work. John McGuire has been working on some analysis of some of our pharmaceutical samples and so forth. We see down the road this is going to be a valuable tool both for investigating compounds that we know we can no longer beat through a standard GC.

So, with that as a brief introduction of why we're concerned about it, in that it is a new tool and it does cover new compounds, and we want to keep

your life interesting, particularly the industries who feel somehow we've slighted them by missing certain compounds.

We'd like to start this morning's presentation and we're going to talk about LC/MS as a new and powerful analytical tool.

RICHARD F. BROWNER

GEORGIA INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMISTRY

MAGIC-LC/MS: A POWERFUL NEW TOOL FOR ENVIRONMENTAL ANALYSIS

(Dr. Browner's paper will be available in open technical literature.)

ROBERT D. VOYKSNER, PH.D.

RESEARCH TRIANGLE INSTITUTE

APPLICATIONS OF THERMOSPRAY HPLC/MS FOR MONITORING THE ENVIRONMENT

DR. VOYKSNER: Thank you, Bill. My name is Robert Voyksner and I'd like to talk about thermospray LC/MS application to environmental analysis.

In today's presentation, I'd like to talk a little bit about what thermospray is, what the interface consists of, talk about optimization and going to some applications and analysis of pesticides, dyes and mycotoxins.

First of all, I'll talk a little bit about the type of samples we like to analyze by LC/MS. The technique is still not routine and if you can do it by GCMS or GC, it's still best to do it that way. Samples can be derivitized or if you don't want to run by GC/MS, HPLC serves as a good method for separation. We use mass spec as a detector. The conventional LC detector doesn't offer the specificity or the sensitivity.

Of the combined HPLC techniques available,

thermospray is an ideal technique for a couple of reasons. First of all, the interface can accept the full HPLC flow rate, so conditions that separation chemists developed don't have to be changed and it's very easy to transfer from a normal LC detector to a mass spec detection. Secondly, there's no reverse phase solvent restrictions. If we're using thermospray ionization, we can use any reverse phase solvent. More recently, the thermospray interface is being equipped with a filament so we can do essentially water type CI and in that case...or solvent CI, excuse me. In that case, we really have no salt restrictions at all. Finally, in either ionization case, both techniques, or either ionization soft providing level of the weight information.

SLIDE 1

The schematic of interface is shown here. The HPLC effluent enters this directly heated coil (A) to form an aerosol, which we see in area F. This aerosol contains the analyte as well as some of the solvent. The aerosol droplets shield it from the heating to prevent any thermal decomposition. To ionize the compounds I'm looking at, primarily we get gas phase ionization with the

volatile buffer, in my case, ammonium acetate. So we're effectively getting ammonia CI type spectra. For more ionic compounds where ions are actually formed in solution, the integrity of the ion is maintained in this aerosol and by means of the repeller (L) and first lens, the ions are extracted through this ion exit cone (E) into the quadrupole rods. The neutral gases or solvent basically goes out into auxiliary pumping and liquid nitrate in cold trap (I).

Probably the main critical parameter in thermospray is the monitoring of temperatures or controlling the temperatures, and we monitor the temperatures of the source block (D), the aerosol (F), slightly beyond ion exit cone as well as a vaporizer (B).

SLIDE 2

First of all I'd like to talk about parameters that affect the thermospray results. In order to develop a good analytical scheme, we should have a good understanding on the factors influencing thermospray spectra and sensitivity. Most of these parameters listed do affect thermospray sensitivity and a few affect the type of spectra recorded. I'd like to go through each parameter and show you

briefly their optimization and their effects.

The first thing I'm going to talk about is the aerosol temperature. You control the aerosol temperature primarily by the vaporizer temperature and the source block temperature. But on the aerosol temperature that's being read just beyond ion exit cone, effects analyte sensitivity.

SLIDE 3

You can see that the optimal aerosol temperature for this triazine is between 120 degrees C to 130 degrees C. If you're off by 10, 20 degrees you lose a significant amount of sensitivity. Also I should point out this plot here is very dependent on solvent composition. As you increase the percent water, your optimal aerosol temperature also increases.

SLIDE 4

Next, I'd like to show you the effect of changing the percent of water, which you do in the gradient dilution type HPLC analysis, would have effect on sensitivity. You can see here, I looked at three different percentages of water in methanol. As we increase the percent water, we gain significant factors in sensitivity. If we compare the high and the low end, we gain about, close to two

orders of magnitude in sensitivity.

SLIDE 5, SLIDE 6

To compensate for this effect, we developed a scheme to add water post-column and this way, even though you're making a dilution of your sample, you can gain over a factor of four in sensitivity.

It's very simple to do. We're using a coaxial team which we can add our water or buffer post-column and mix it with your HPLC effluent going into the detector and minimize band broadening, in effect enhancing our sensitivity.

SLIDE 7

Also, the percentage of water effects slightly the type of spectra we generate. As I show you here, we do see different types of ions either (M+H)+ or (M+solvent)+ that are cluster ions, for a percentage of water. The lower percentage of water in methanol, we see more cluster and more acetate addition. In a high percentage of water, we see more pronation. The same roughly holds true for acetonitrile in water. In general, though, the change in solvent composition doesn't drastically affect the spectra and really there's no gain or loss as one goes from one extreme to another.

SLIDE 8

Another factor that plays an important role in thermospray is the selection of a buffer. For thermospray ionization to occur, a volatile buffer should be present. We evaluated five volatile buf fers: triethylamine, ammonium carbonate, bicarbonates, formates, and acetate. For this organophosphorous pesticides, you can see that ammonium acetate produced the best response. In some cases, ammonium formate was comparable to ammonium acetate, but for most of the work I've been using acetate because it gave the best response.

SLIDE 9

As for concentration level of the acetate, we found doing a plot of concentration versus (M+H)+ intensity, we see it plateaus near about .08 M and any addition really gains very little in sensitivity. For people who worry that the acetate will degrade the separation, an ammonium acetate solution can be added post-column. For example, you can add .3 M solution post-column so that your final concentration might come out to be .1 M. So actually the acetates or the buffer requirements for thermospray doesn't really affect analysis from the viewpoint of a chromatographer.

SLIDE 10

Next, I'd like to show some examples of analysis of pesticides, primarily carbonate pesticides in the soil and water by thermospray. Now that we have a handle on optimal conditions where we hope to give us the best sensitivity in thermospray, we tried to design an analysis scheme keeping these points I showed you in mind, to get the best possible results both chromatographly and by mass spectrometry.

For sample preparation for water samples, we stuck with literature-type extraction techniques. We compared two extraction techniques, sep pak and methylene chloride extractions. We found out that methylene chloride extraction came out the best with a 98 percent recovery and sep pak was reasonable with 50 percent recovery.

One advantage to the LC/MS technique would be to inject the entire volume of sample on column, which is something you can't do with GC/MS unless you dried down to a very low volume.

SLIDE 11

The conditions we used are developed for the carbonate pesticides that are shown here, Zorbax ODS (25 cm.). We're doing a gradient/multi-

gradient, solvent 50 program. You can see we're working on normal flow rates (1.2 mL/min). I have a UV detector in line for comparison purposes. Mass spec was scanning (m/z 160-600). Primarily I do most of my work in positive ion. The vaporizer, as I showed you before, is temperature dependent. We compensate for the loss or change in sensitivity of optimal temperature by programming our vaporizer, so this way we can always maintain optimal sensitivity with varying solvent composition. The vaporizer is programmed down in sequence with the gradient.

SLIDE 12

Here's an analysis or one example where you have the HPLC UV trace and I selected ion chromatograms for about five pesticides spiked into lake water. This water sample contained one part per billion of each pesticide. This is under full scan conditions. You can see here in the selected ion chromatograms, each peak was easily detected with very little noise, very little other ion interferences. If you look in the UV chromatogram, they're very difficult to pick up where these peaks would be detected.

We can go much lower. We can see per part per billion there is very little noise in most of these

channels. Due to detection limits on these types of samples, we found for most of the carbonates we could go close to about 10:1 part per tray.

SLIDE 13

Typically the thermospray spectra for these samples are very simple, consisting of an (M+H)+ or (M+NH4)+ ion, or a combination of both. This is one of the examples, propoxur, which showed in the selected ion chromatogram. Very simple spectra. Fro most of the spectra, I usually just map out the most or base peak for each of the compounds. The big disadvantage for these simple spectra is there's really no structural information gained from fragmentation so it's very difficult to make a qualitative identification of an unknown.

SLIDE 14

In summary, for the carbamates we worked in the concentration range from 100 parts per billion to 10 parts per trillion and it proved quite linear over that range. We looked at about 15 pesticides and they did show a significant variation in detection limits. For the ones that were able to go down to 10 parts per trillion, we used for determining the linear coefficient (0.99). So for about 8 or 10 that did go down to about 10

parts per trillion, we did have a linear range of about 10 to the fourth.

SLIDE 15

This method worked very well for soil analysis. A 100 parts per trillion of pesticides were spiked into soil, and as observed in the HPLC UV, it's pretty hopeless. I'm not sure you can make any sense out of that chromatogram, however, in the ion chromatograms you can pick up the pesticides quite easily, looking at the base peak for each representative pesticide. Again, most of them show very little interference. I think propoxur shows the most channel interference with some other components showing common ion interference. But in most cases, thermospray seems to be transparent to a number of biological or environmental matrix interferences.

Next, I'd like to talk a little about another example, analysis of dyes. In this example, we're looking at dyes in gasoline. Dyes in general are very difficult. There's really no mass spec methods available. Dyes are added to gasoline for a few reasons. One, they're a fingerprint for the manufacturers. If you have four service stations and there's a ditch nearby with gasoline leaking

into it, somebody could point a finger at the proper service station that is leaking. Also they use dyes to differentiate between leaded and unleaded gasoline.

Typically, TLC has been the main way to analyze dyes in gas. TLC shows that the supposedly pure dye component contains a multitude of components. What is bought from a manufacturer with a given structure is not very pure. The only thing else we know about dyes is that they're spiked into gas about the part per million level. Since the USA uses millions of gallons of gas, we are talking about kilograms of dye being used or thrown into the environment after burning.

To get an idea of what degradation of product are formed, we have to have a good handle on what the initial products are to begin with. So we try to characterize some of the dyes initially just to see what's in the parent dye to get a better handle on what will come out after burning.

SLIDE 16

An HPLC and a TIC trace for a commercial red dye is shown here. There's really not too much in the UV-- multitude of peaks, very broad peaks, indicating probably a multitude of components. In

thermospray, quite a few peaks there.

SLIDE 17

We knew the dye had this basic structure with R-group as being a methyl group. When we looked at the selected ion plots, we found that R-groups, ranging from H all the way down to C10 H21 were present and in many case, many isomers for each R-group were present. So this supposedly pure dye, or being sold as a pure dye, is a multitude of alkyl homologs of about 20 to 30 components.

SLIDE 18

Not only that, we also detected some other colored dyes in the sample. We determined the structure of this dye by purchasing the standard. I think the standard was the methyl R-group again and showed the same type of spectra as the sample. This orange pigment was found in this commercial red dye and again, a whole range of alkyl substitutes were found.

So in this one dye, we had to deal with on the upwards of 30, 40 components, making detection of a specific component very difficult. We are looking at concentrations in the lower parts per billion now, instead of in the parts per million level.

SLIDE 19

To show a real example of a gasoline extract, this was supposedly spiked with one part per million red dye. We did a sep pak silica gel extract. There is nothing in the HPLC UV trace where the dye should elute. However, we looked at HPLC selected ion chromatograms. The red pigment (m/z 381) and the orange pigment (m/z 249) were detected. You can see the two very neat peaks way above noise level as detected. This we estimated, assuming that each alkyl substitution has the same response, was about 10 to 20 parts per billion of dye in gasoline.

So in this way, if we wanted to characterize the red dye, we might choose two or three of the major components found in the red dye, measuring the area ratios as a fingerprint for a particular dye. The multitude of components in the dye make it very difficult to detect each with adequate sensitivity.

I'd like to go through one other example. This is a study of mycotoxins and this was a big issue last fall with Afghanistan and the Yellow Rain issue. I'd also like to talk about highly toxic contaminates in feeds and grains.

The methodology for these toxins is rather limited, primarily doing GC/MS techniques using sample derivisation. However, GC/MS techniques have a few major disadvantages. In EI conditions one detects low mass fragments below m/z 200. That mass range is very easy to have interferences from blood and serum samples. NCI overcomes some of this problem by providing molecular anion information. However, to get reasonable sensitivity, we have to do at least two derivatives to analyze these mycotoxins. There are extreme differences in sensitivity of the different toxins with derivatives.

On the other hand, thermospray offers some advantages. First of all, there's no sample derivisation. We can bypass that step completely so your samples don't have a short shelf-life time. Thermospray does provide molecular weight information for all components. Primarily the spectra are consistent of (M+H)+ or (M+NH4)+ ion with very few fragments. If there are fragments, they're very simple fragments like the loss of water.

The common interferences found in blood and urine are pretty well transparent to thermospray ionization, so the examples I'll show you, there's

very little backdrop problems. Again, like I said before, there's no splitting HPLC effluents, so we can inject an entire sample into HPLC and maintain good sensitivity.

SLIDE 20

For those not familiar with the structures of buffing toxins, they're shown here. Primarily T2, HT2, DAS, T2 tetrol have this basic structure that's varying R1 through R5 groups. F2 has the structure shown below. With the weight ranges, you're dealing from 296 on up to 466.

SLIDE 21

The first example I have here is a variety of toxins in the urine. We have a quick isocratic HPLC analysis. In about 10 minutes we can do a complete assay of these four or five toxins. Again, we have good sensitivity for these toxins (two nanograms injected.) You can see in the chromatogram there's a high background, making F2 a little bit more difficult to detect.

SLIDE 22

Here's another example of detecting for HT2 in serum. HPLC UV chromatogram shows the retention time of HT2. (See arrow.) Here's the actual thermospray selected ion chromatogram for the

standard and sample. The standard represents one nanogram of HT2. One can see detection limits below one nanogram for HT2 are achievable. It also shows you the advantage of specificity of the mass spectrometry over HPLC UV.

SLIDE 23

One more example, the detection of T2 in serum. The serum sample again shows very few interferences. An one nanogram standard chromatogram is also given. The mycotoxin work proved to be quite linear and about from 0.1 nanograms up to about 50 nanograms.

One other thing I should mention. Our detection limits can be reduced by about another factor of 2 or 3, by performing post-column buffer addition. We did some experiments where we added an aqueous solution post-column (0.3 mLs/min), of 100 percent water. This increased our overall percentage of water because we were operating with a high percentage of methanol in a range where thermospray sensitivity is not optimal.

SLIDE 24

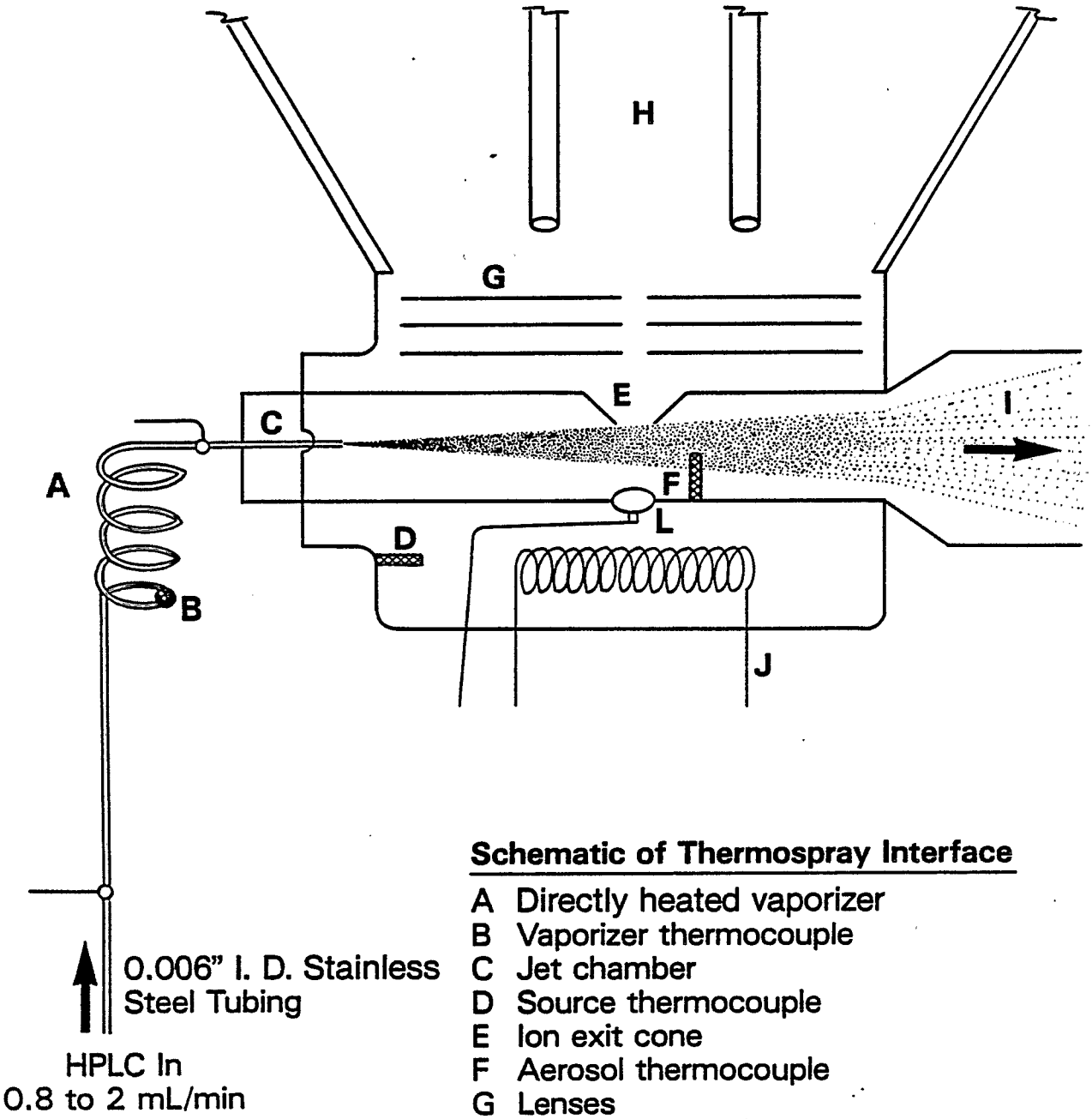
This is an older slide comparing detection limits for toxins using various methods.

Recently, we've been able to reduce thermospray sensitivity down to 0.05 to 0.01 nanograms. You can see how it compares with other techniques, both NCI, GC, EI, HPLC, UV. I believe HPLC/MS is the method of choice for analysis of toxins.

In conclusion, I think thermospray pretty well revolutionalized the technique of LC/MS. It offers a new combination of sensitivity and specificity and is very versatile for a variety of compounds. I think all these reasons have made it one of the most popular techniques for LC/MS. It's at the point now where it's just becoming to be routine for some assays.

I'd like to thank you for your attention and EPA Cincinnati, Ohio, for their support.

MR. TELLIARD: Questions?

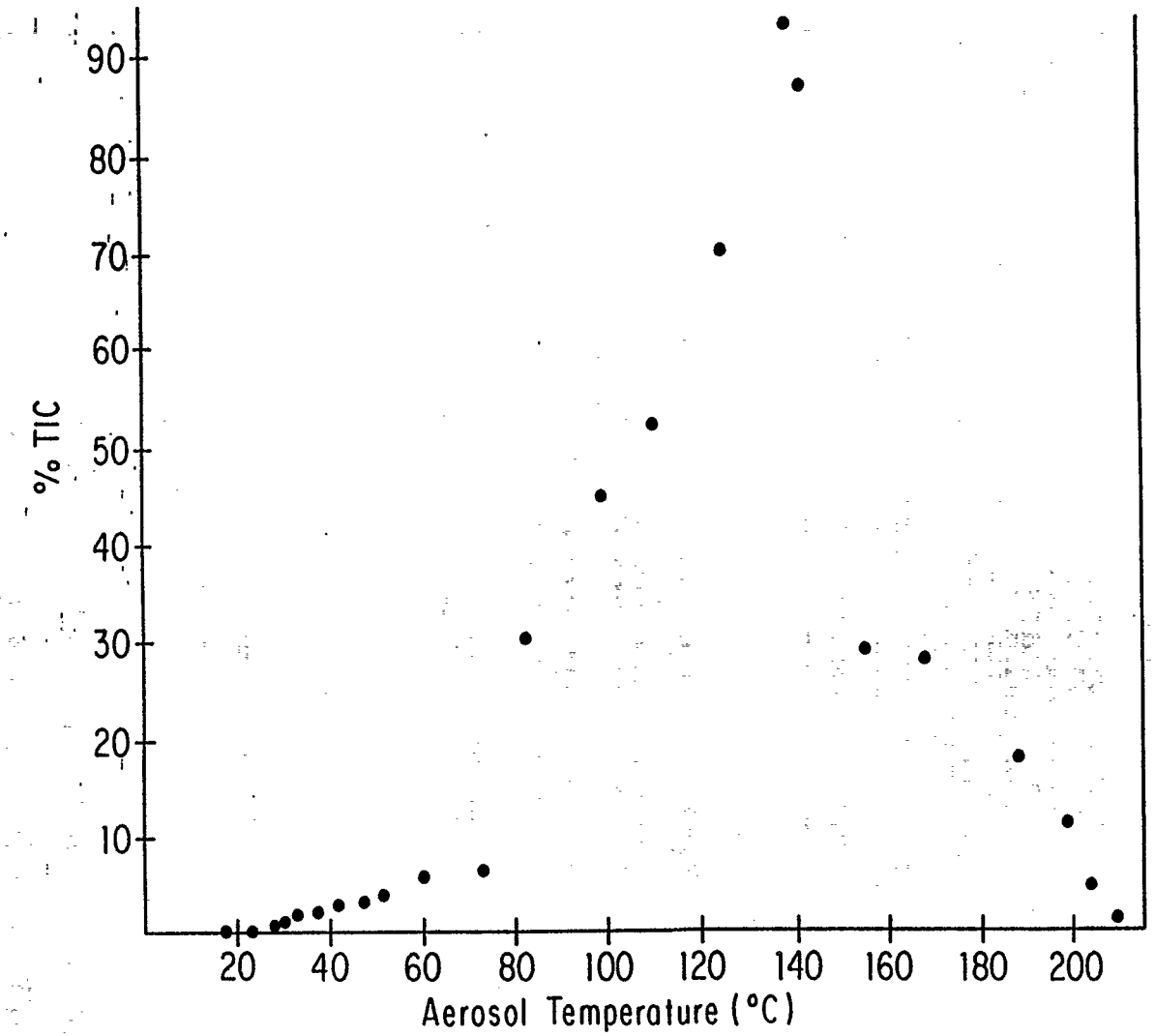


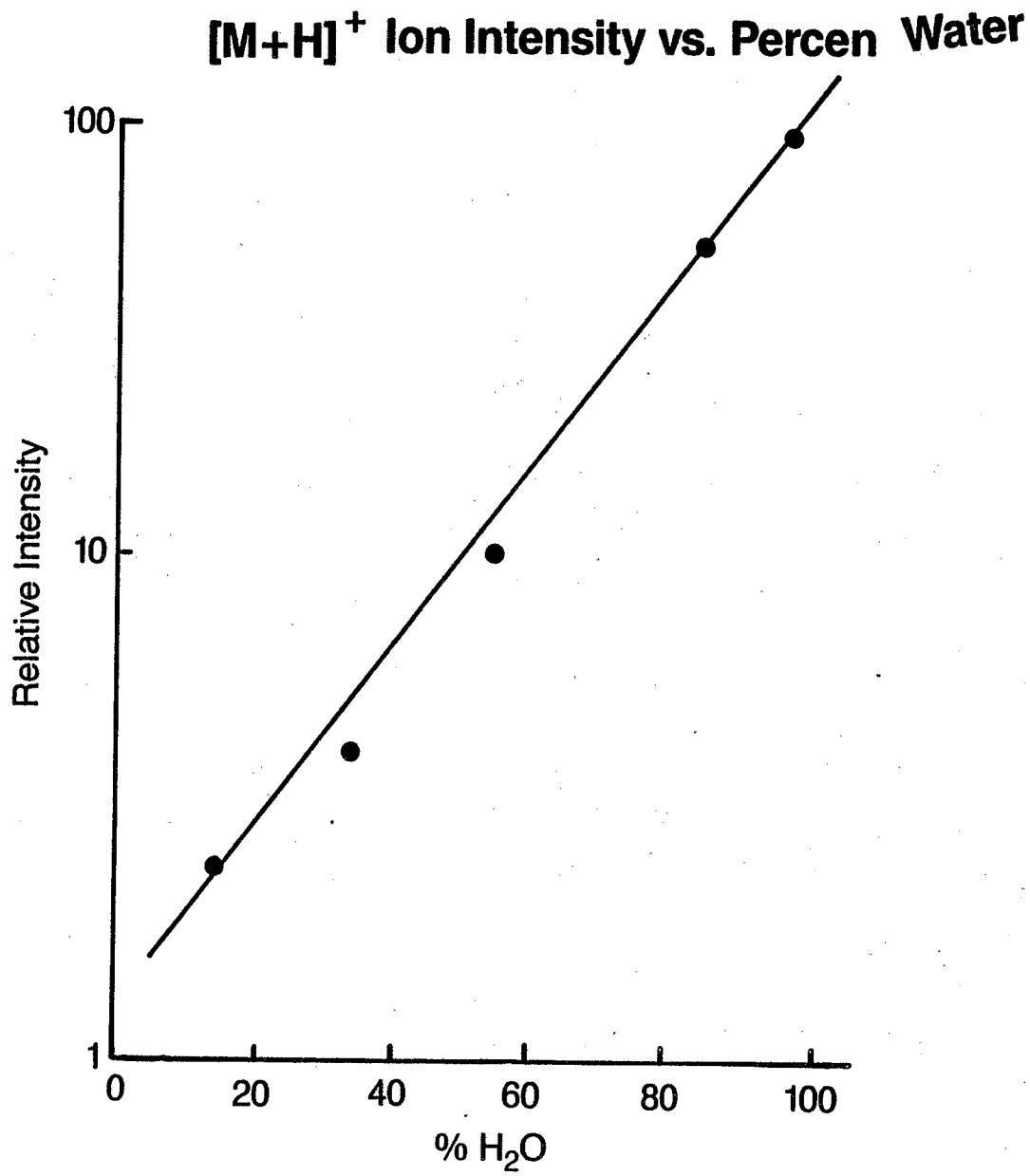
Schematic of Thermospray Interface

A Directly heated vaporizer
B Vaporizer thermocouple
C Jet chamber
D Source thermocouple
E Ion exit cone
F Aerosol thermocouple
G Lenses
H Quadrupole assembly
I Liquid nitrogen trap and forepump
J Source block heater
K Vaporizer heater
L Repeller

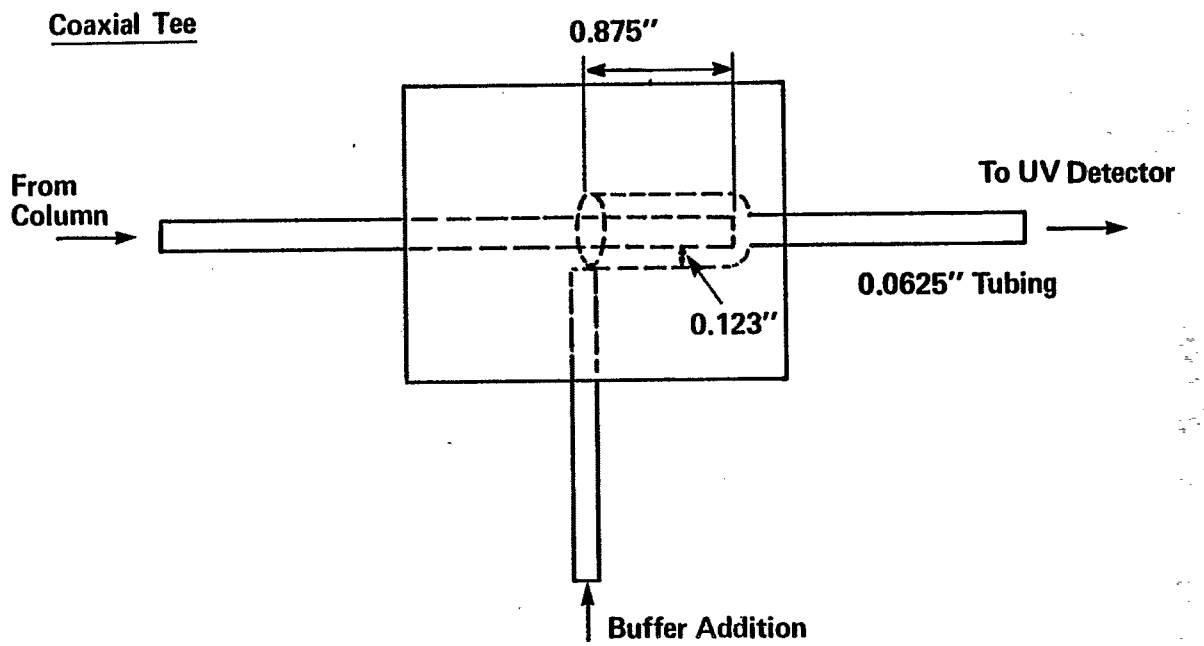
Thermospray Parameters Evaluted That May Effect Sensitivity and Spectral Characteristics

- Interface temperature
- HPLC flow rate
- Solvent choice
- Solvent composition
- Buffer choice
- Buffer concentration

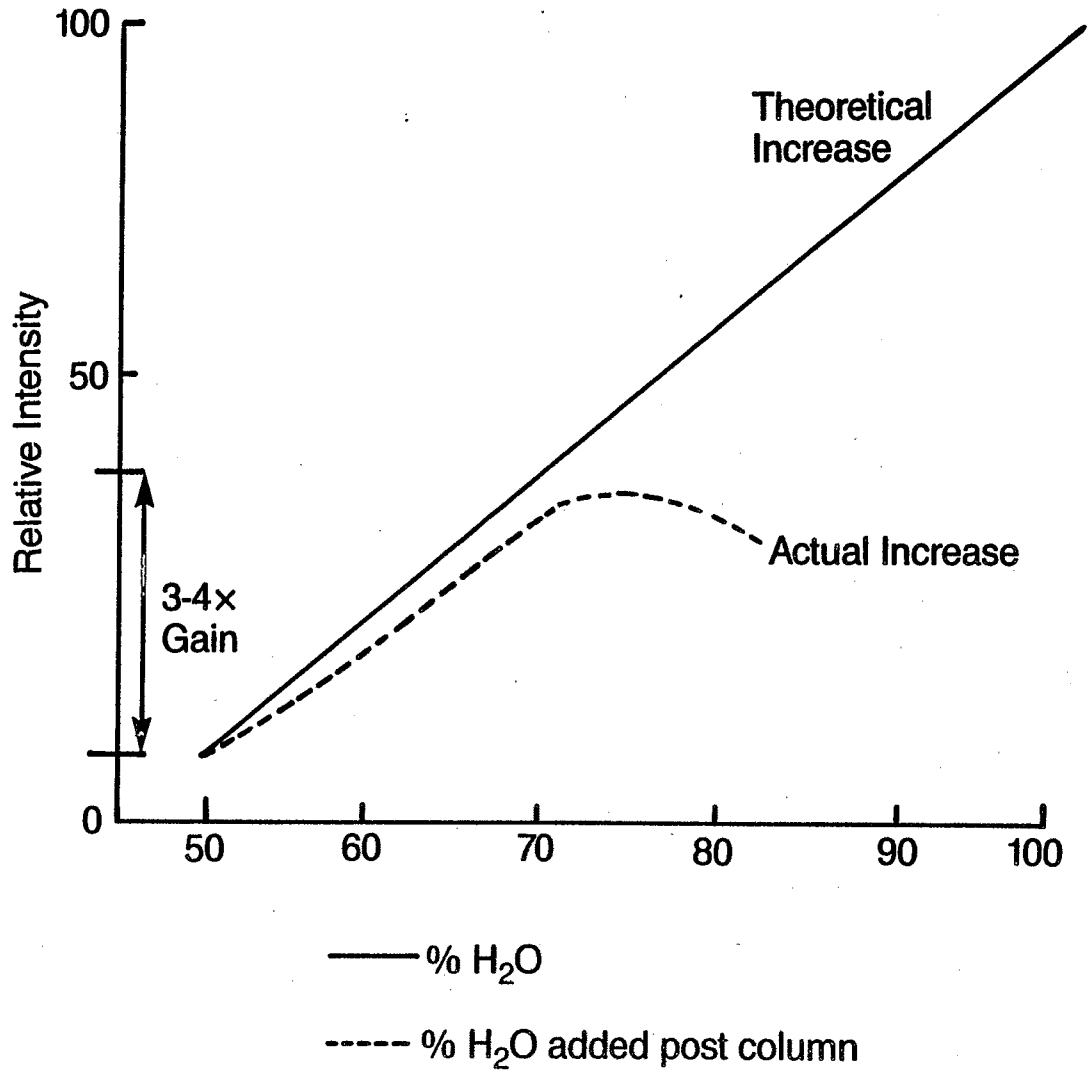

% TIC
90
80
70
60
50
40
30
20
10
20
40
60
80
100
120
140
160
180
200
Aerosol Temperature (°C)


[M+H]+ Ion Intensity vs. Percen Water
Relative Intensity
100
10
1
0
20
40
60
80
100
% H2O

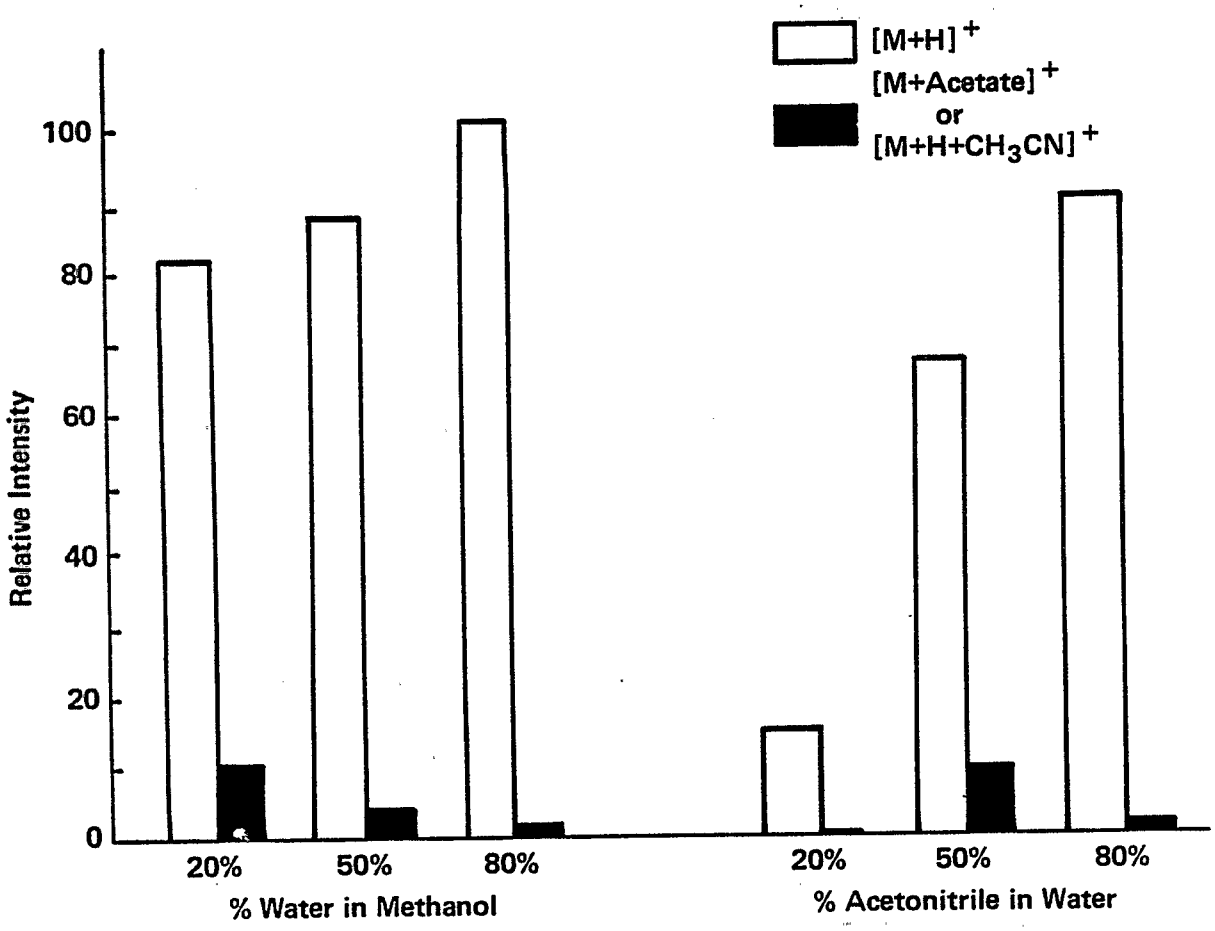
Post-column buffer addition tee



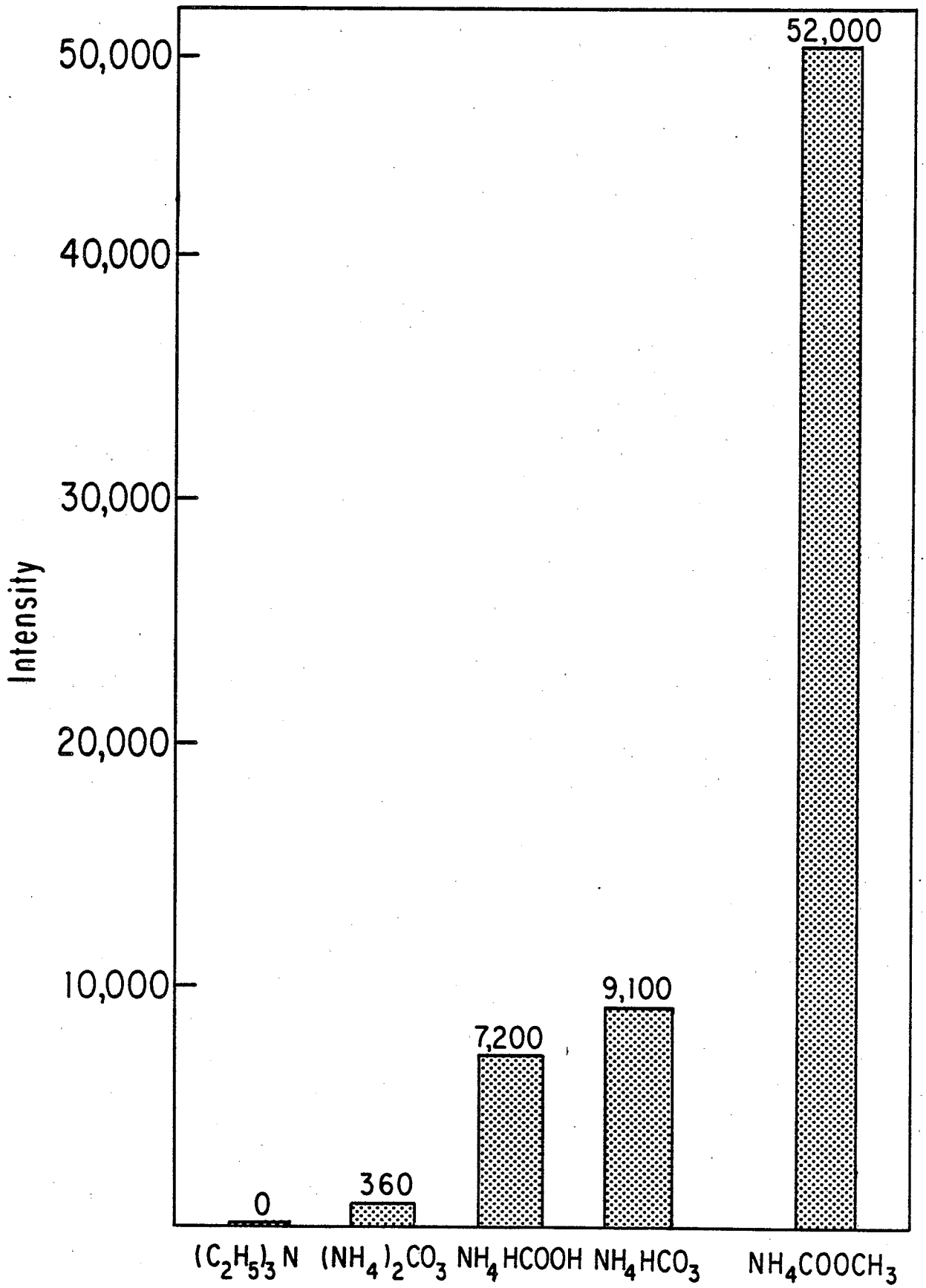
Sensitivity Increase for Post-Column Addition of Aqueous Buffer



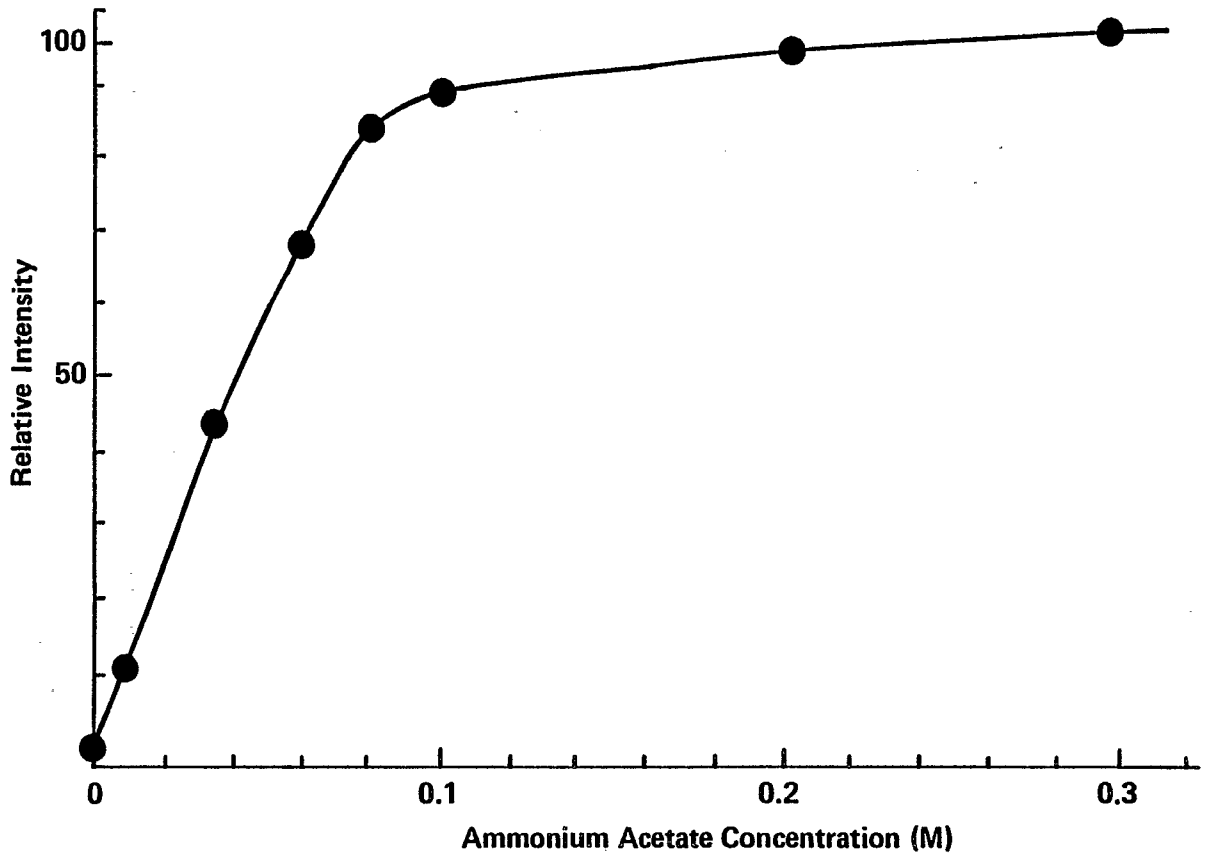
The Effect Solvent Composition Has On Thermospray Spectra



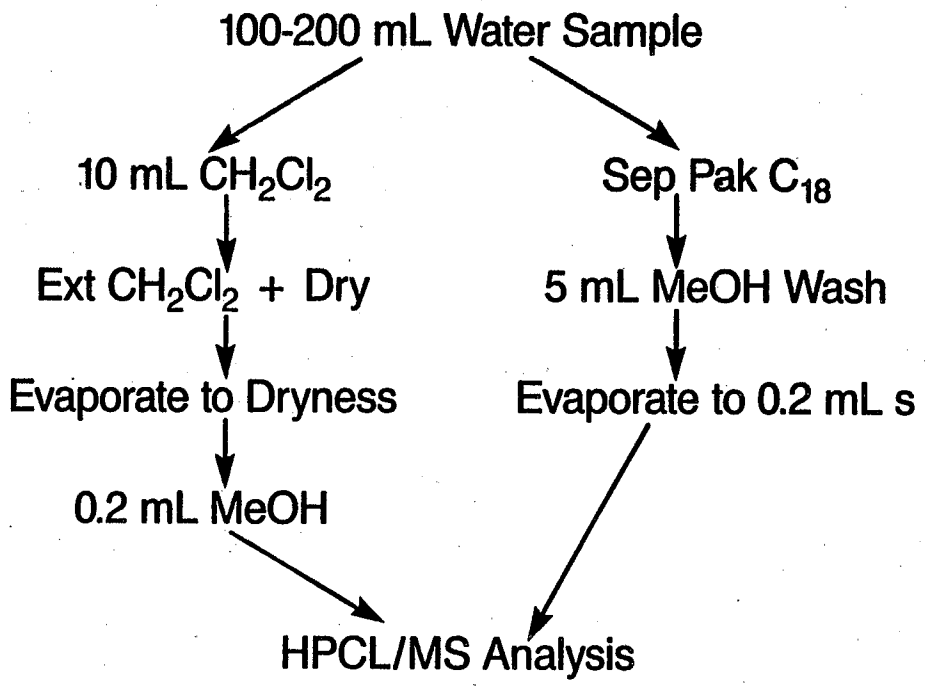
COMPARISON OF BASE PEAK INTENSITIES FOR SIMAZINE IN EACH BUFFER



The Effect of Buffer Concentration On Thermospray Signal Level



Water Sample Preparation

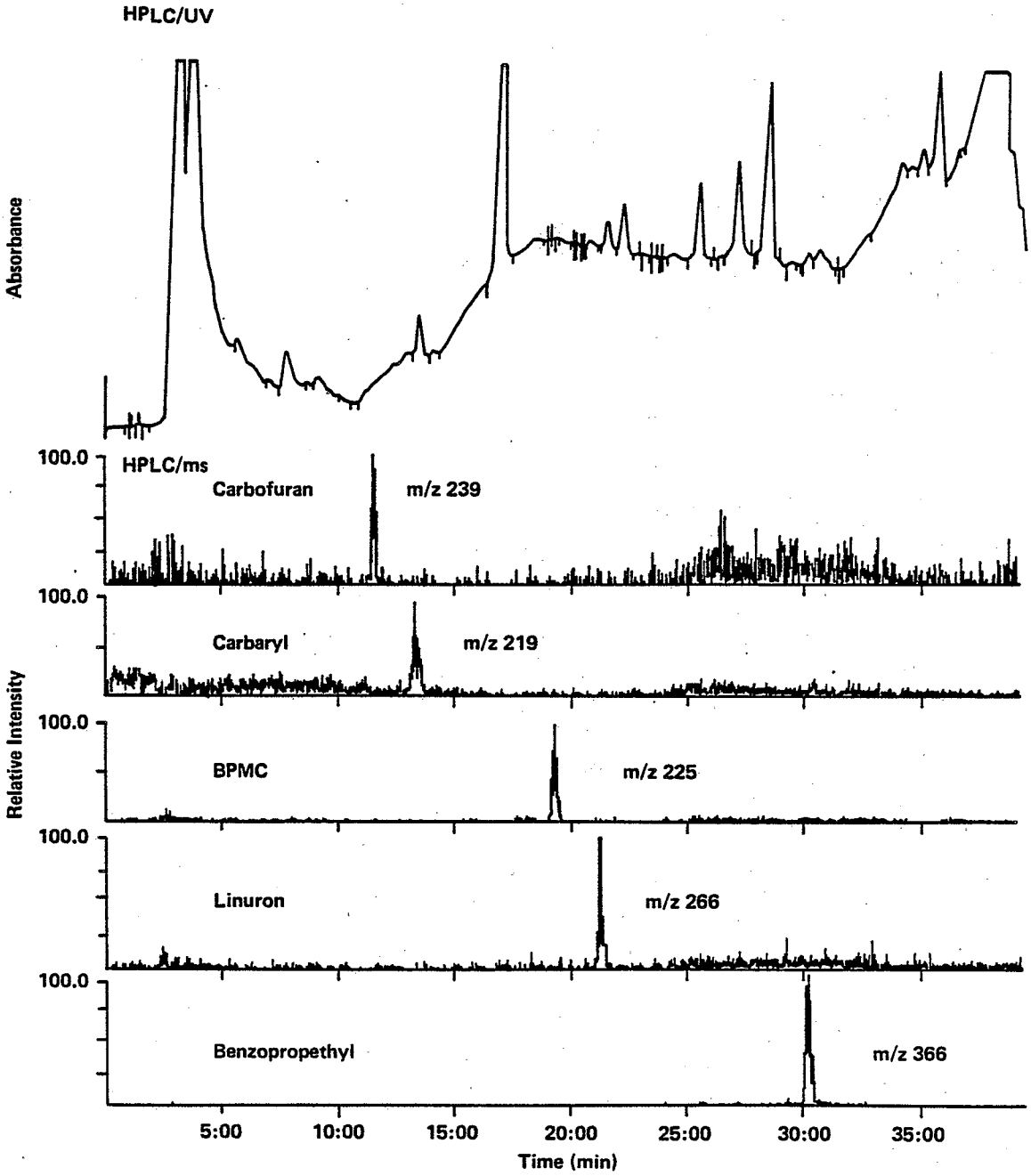


Mean % Recovery:	98%	52%

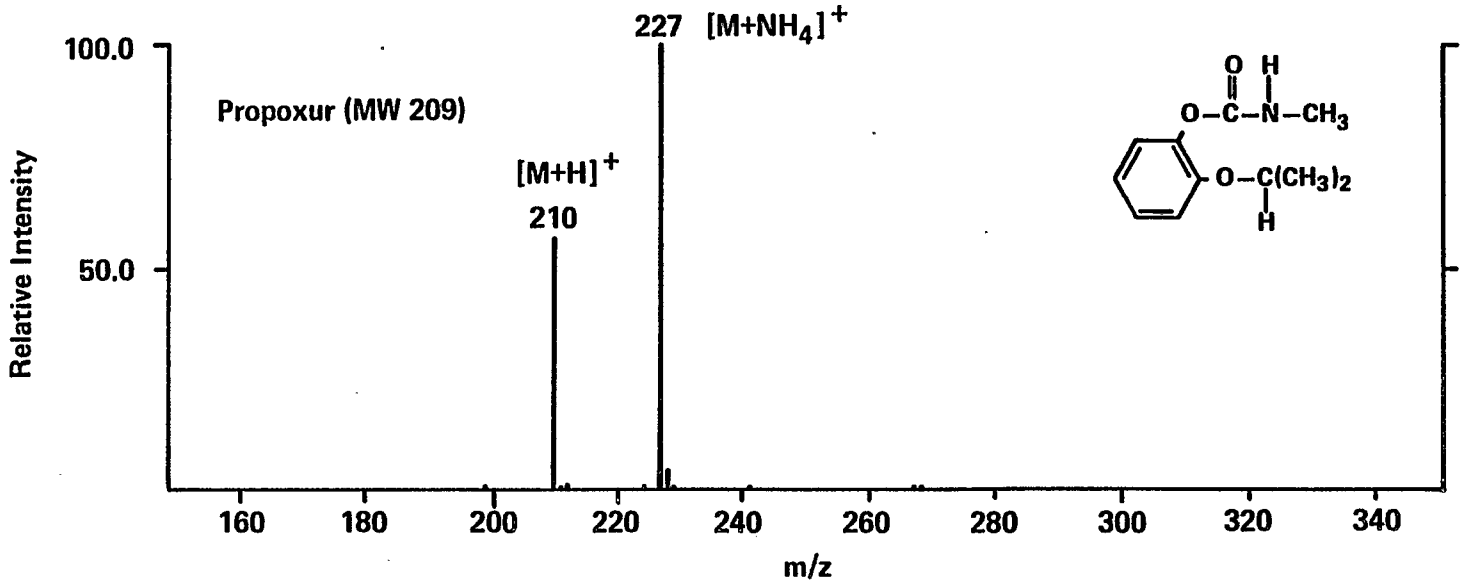
HPCL/MS Analysis

Column:	Zorbax C_{18} 25 cm × 4.6 mm
Solvents:	Methanol/water (0.1 m AA)
Gradient:	50/50 $\xrightarrow[15\ min]{}$ 70/30(5) $\xrightarrow[5\ min]{}$ 85/15(10)
Flow:	1.2 mL/min
UV:	254 nm
MS:	Scan 160–600 in 2.0 s (+ ion det.)
Thermospray Interface:	Vaporizor 106° C $\xrightarrow[25\ min]{}$ 96° C Jet 270° C

Analysis for Pesticides in Water (1 ppb)



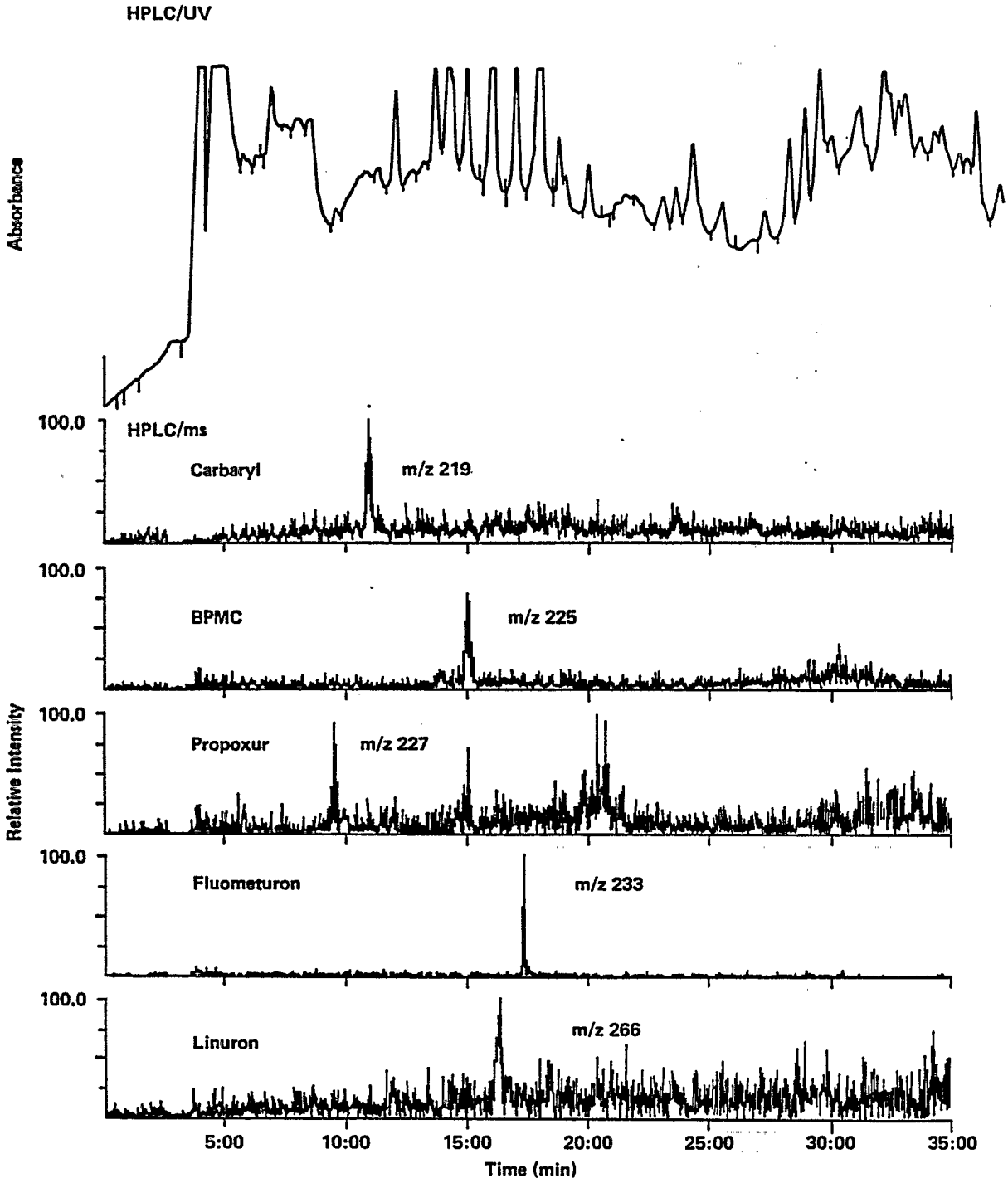
TSP Spectrum of Propoxur



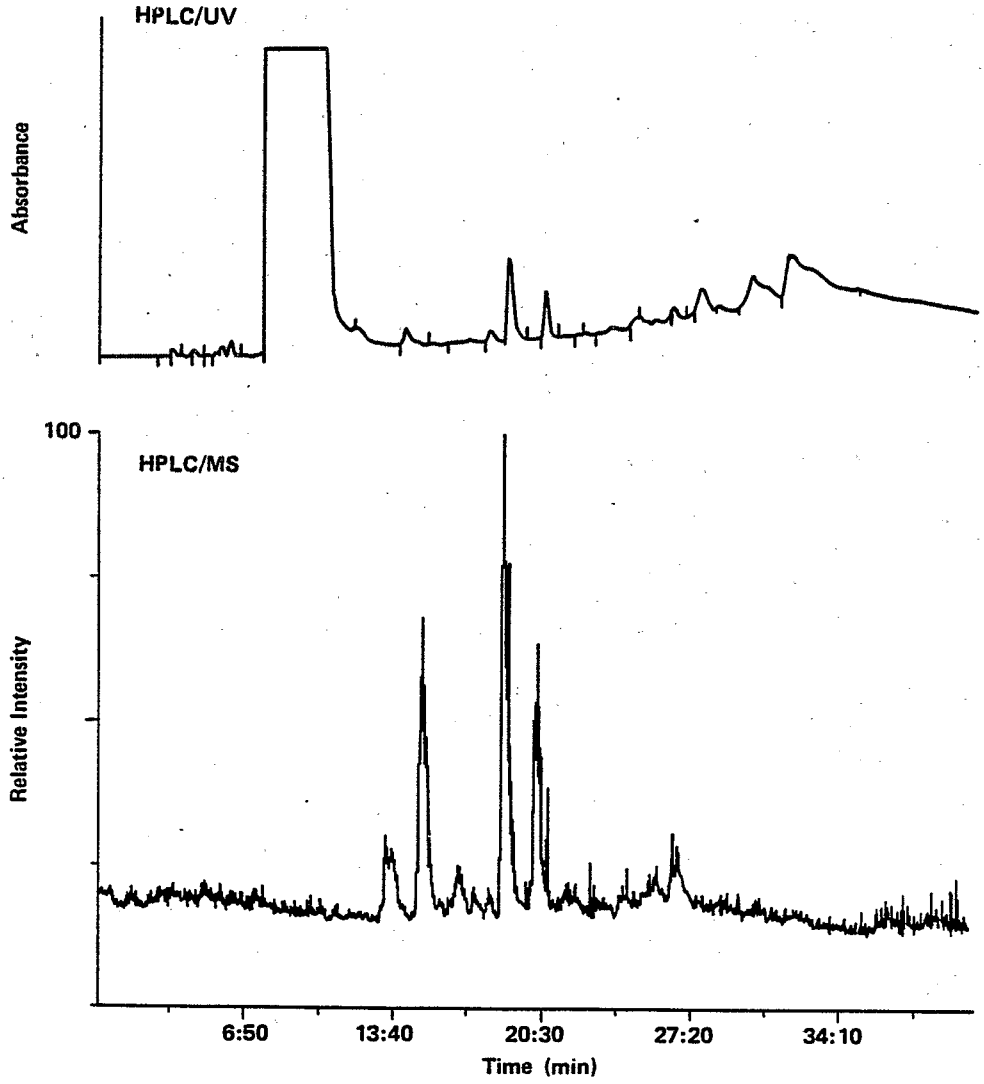
Pesticide Analysis

Concentration range:	100 ppb-10 ppt
Linear correlation coefficient:	0.99
Detection limits:	1 ppb-10 ppt

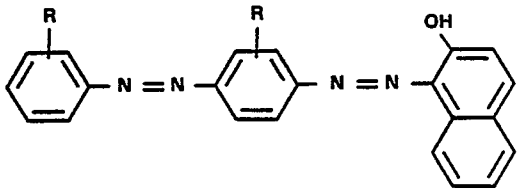
Analysis of Pesticides in Soil 100 ppt

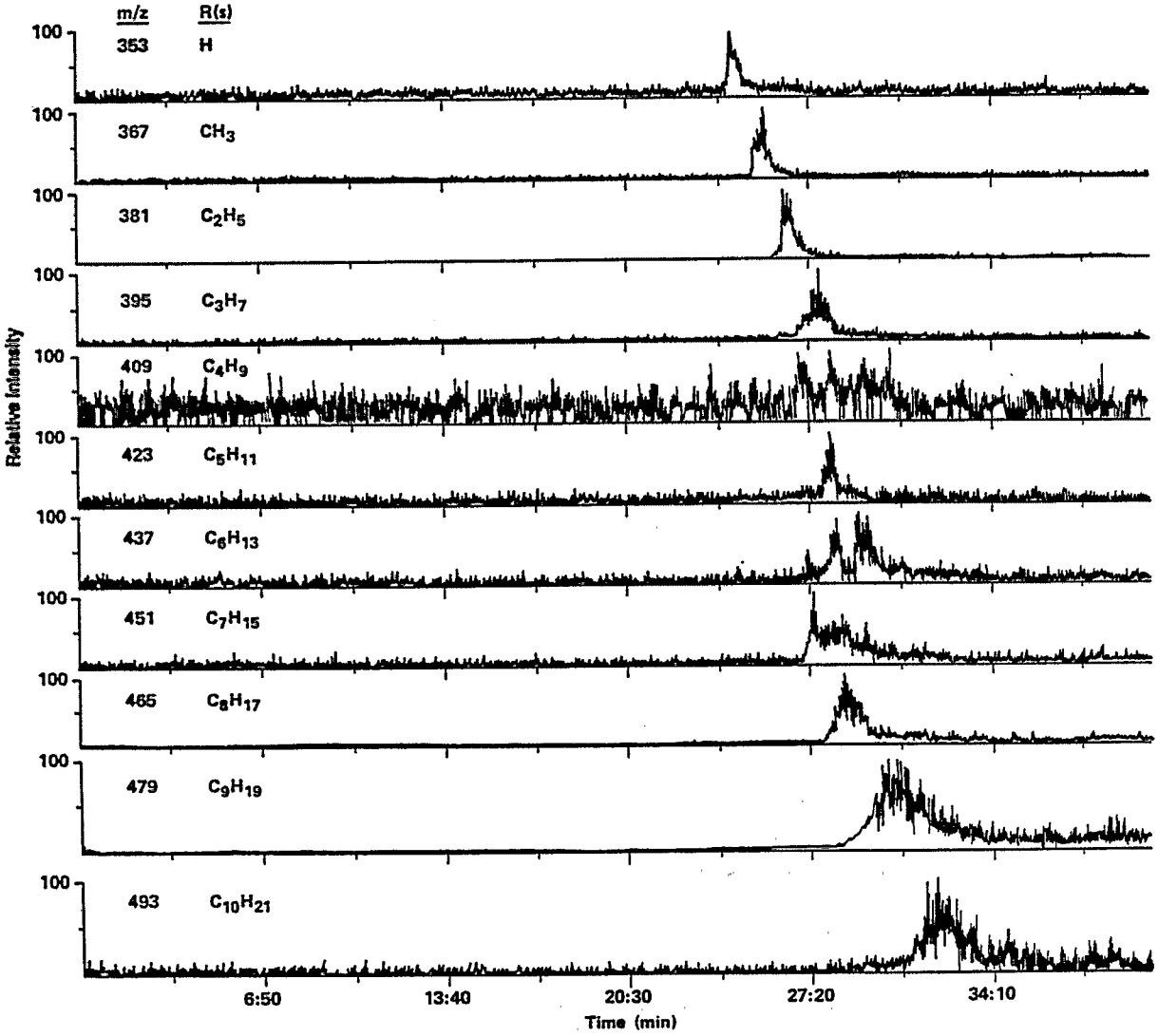


Commercial Diazo Red Dye

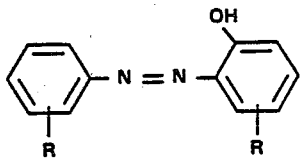


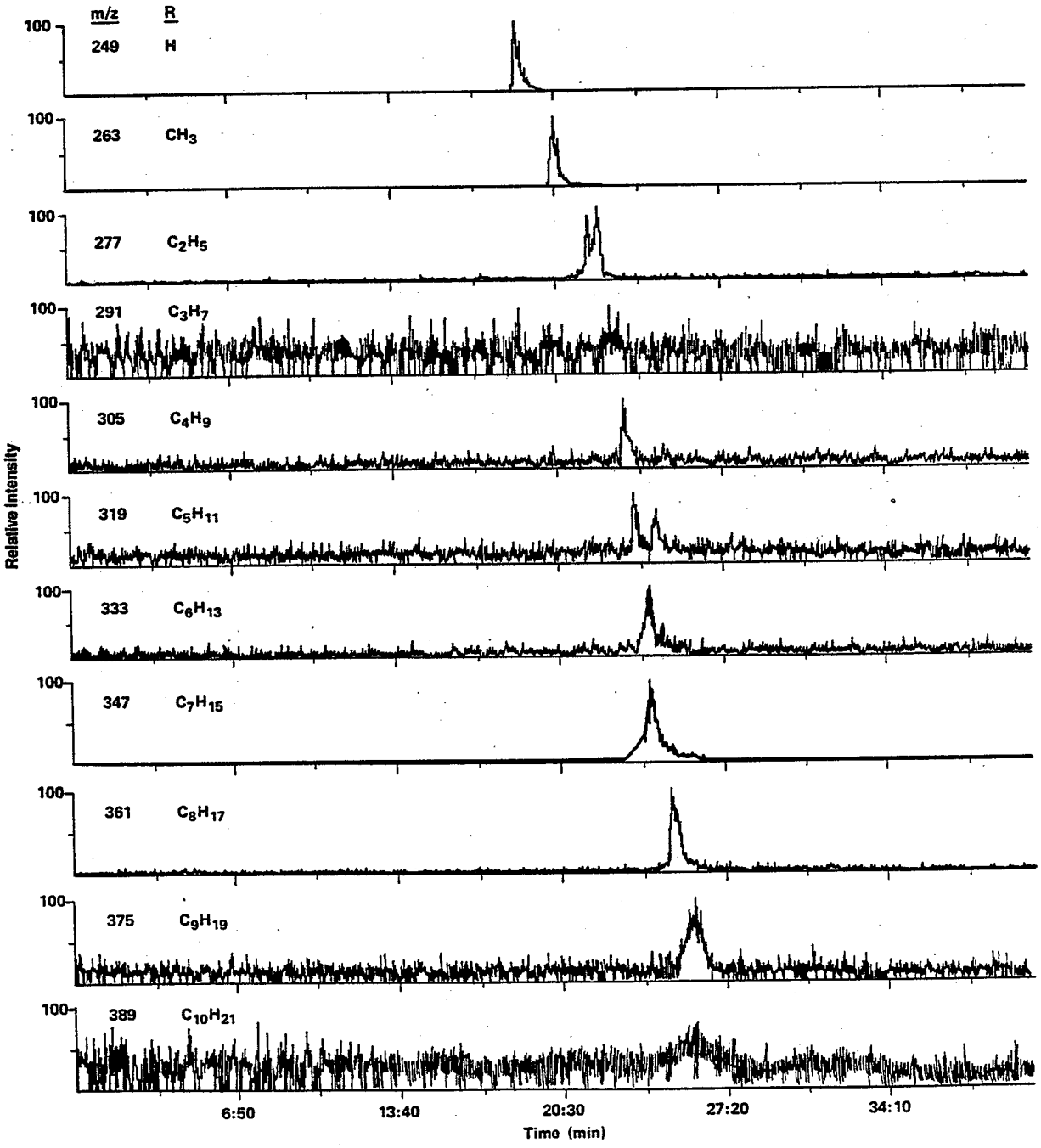
HPLC/MS Ion Chromatograms for the Various Alkyl-Substituted Azo Benzene-Azo Naphthols



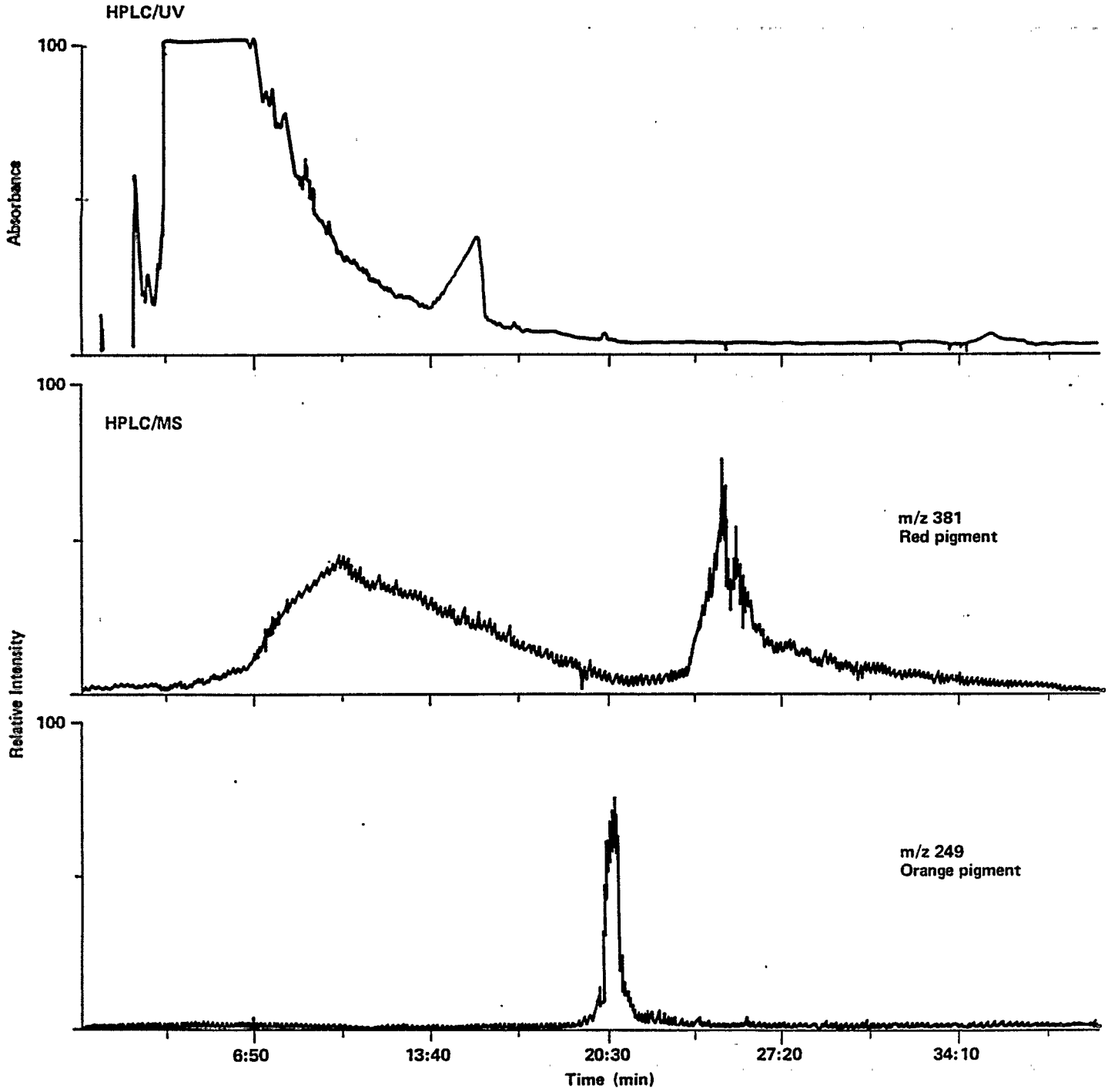


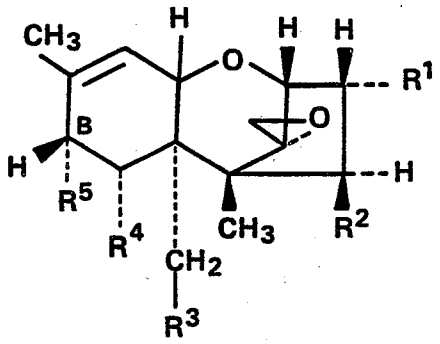
Various Alkyl-Substituted Phenyl-Azo-Naphenols (orange pigments) Found in the Red Dye



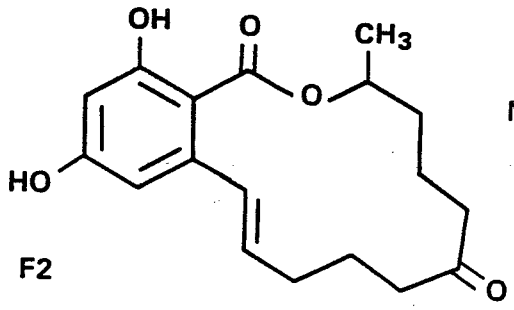


Analysis of 1 ppm of Red Dye in Gasoline





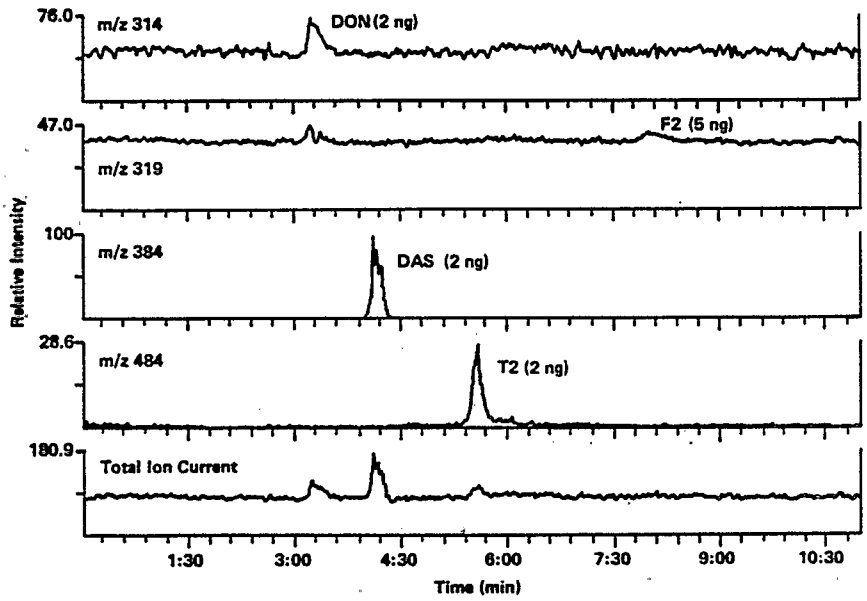
Compound	R^1	R^2	R^3	R^4	R^5	Molecular Weight
T2	OH	OAc	OAc	H	i-$C_4H_9CO_2$	466
HT2	OH	OH	OAc	H	i-$C_4H_9CO_2$	424
DAS	OH	OAc	OAc	H	H	366
(a) DON	OH	H	OH	OH	=O (a)	296
T2 Tetraol	OH	OH	OH	H	OH	298



Mol. Wt. 318

F2

Spiked Urine



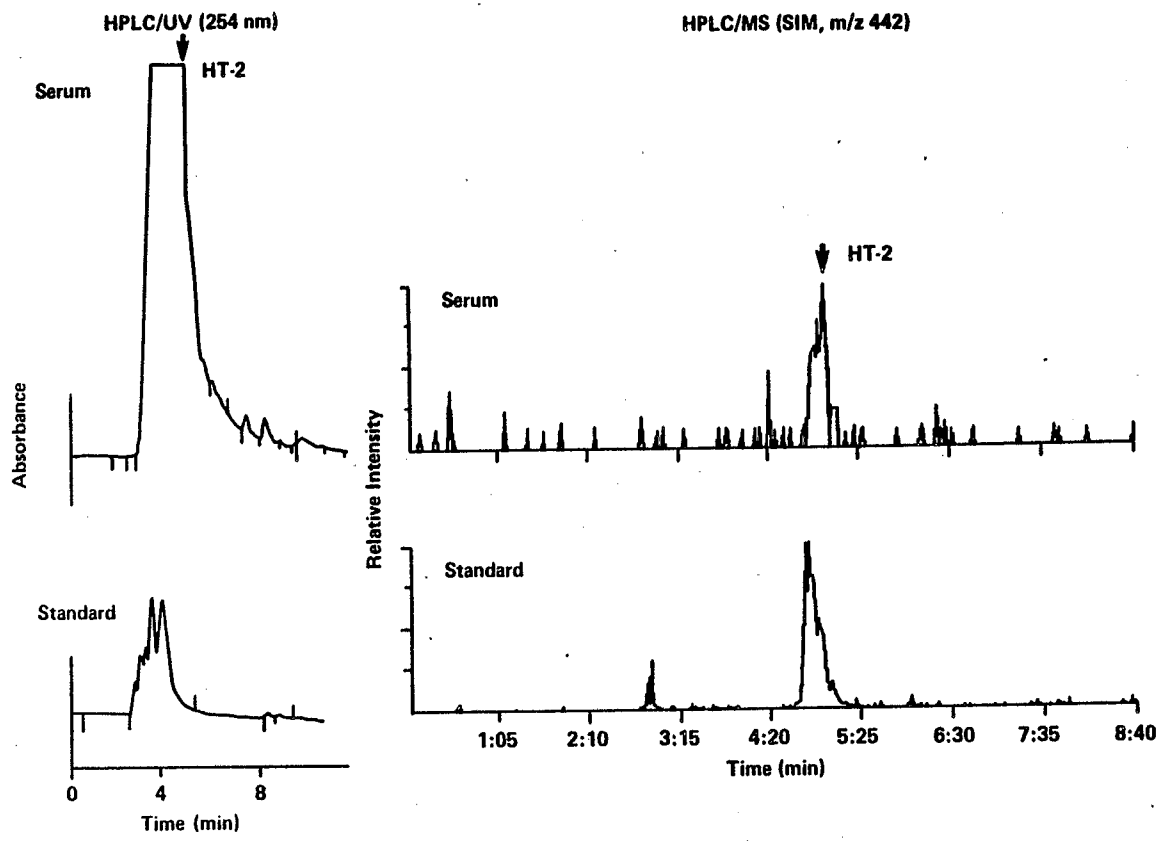


Figure 6

HPLC/UV and TSP HPLC/MS chromatograms for the analysis of HT-2 in serum and solvent.

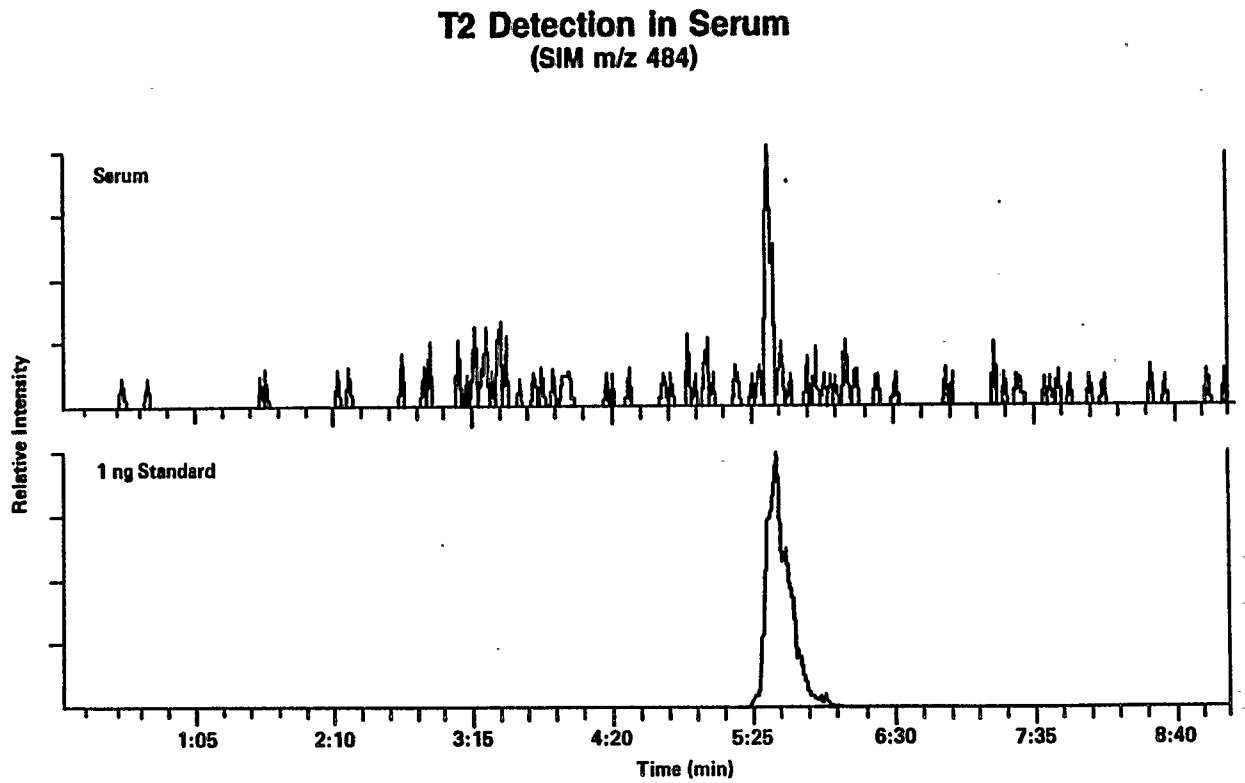


Figure 7

TSP HPLC/MS ion chromatogram for the analysis of T2 in serum and solvent.

Detection limits of mycotoxins analyzed by TSP HPLC/MS and other analytical techniques.

Compound	Detection limits (ng)				
	SIM TSP HPLC/MS	SIM NCI/GC-MS	SIM EI-GC/MS	HPLC/UV	HPLC/RI
DON	5.0	near 0.2[1)]	1 – 5[3)]	2 – 3[7)]	
F2	10.0		5 – 10[5)]	5[6)]	
DAS	1.0		1 – 5[5)]		
T2	0.5	1 – 0.2[1,5)]	5 – 50[2)]	1,000 – 10,000[3)]	1000[4)]
T2 tetraol	20.0	1 – 2[5)]			

QUESTION AND ANSWER SESSION

MRS. LAURIN: Any idea what kind of sensitivity moving belt LC/MS has for carbamates?

DR. VOYKSNER: From other comparisons, I would say it's probably not too good. I think the closest comparison is carbonate pesticides analyzed by moving belt LC/MS, was about a factor of 10 to 20, less sensitive than thermospray HPLC/MS.

MR. TELLIARD: It's lunch break. Lunch is being served next door. We're due back here at 1:30. I'd like to have you all come in for the afternoon session on time so that we can get out on the HMS Sinkfast at our due date. Thank you very much.

(WHEREUPON, a lunch break was taken.)

MR. TELLIARD: Our first speaker this afternoon is not Bruce Hidy, but Marv is going to move up and fill in. Again, continuing the discussion that we started before lunch, he'll be discussing LC/MS and its application for environmental measurement. Marv.

MARVIN VESTAL

UNIVERSITY OF HOUSTON
CHEMISTRY DEPARTMENT

RECENT ENVIRONMENTAL APPLICATIONS OF THERMOSPRAY LC/MS

MR. VESTAL: I'm afraid my title may be a little bit misleading because I had thought we might get around to actually doing some real environmental applications. We've been involved in developing the thermospray technique for about 10 years, but the definition of an academic analytical chemist probably applies to me even though I don't really consider myself one. The definition I heard is that an academic analytical chemist is one who never runs any real samples.

What I would like to do is to tell you a little bit about the thermospray technique, how and why it works, without going into all the details. I could spend a couple of hours telling you everything I know and some things I suspect, but I'm not sure that would be productive. I'll just give a little bit of an overview of how the technique works, some of the instrumentation that's involved, and then give some examples of the various

alternatives that you have available.

Bob Voyksner gave, I thought, an excellent talk on thermospray this morning and with some real examples. I'm envious of some of his results. However, you have to realize that he was doing all that on one leg. He was using the technique which is sometimes called thermospray ionization, sometimes called direct ion evaporation, in which the primary ions are produced directly by ion evaporation from charged liquid droplets.

This technique requires that you have a substantial concentration of ions in solution, on the order of .1 M/L for it to work well. That doesn't mean your sample has to be in anything like that concentration, but you have to have something that's ionized in solution, and in many cases of real liquid chromatography that is not what you have. Furthermore, if you're using something like ammonium acetate, that technique requires that your sample have a higher proton affinity, for example, in the positive ion mode than ammonia, and there are a great many environmental samples for which that is not true.

So, from the beginning in our thermospray systems, we have had an alternative method of

ionization, such as a hot filament, which all of you doing mass spectrometry are very familiar with. It's a nuisance, but it does provide a very efficient way of producing ions when you don't have them by some surprising new technique. We find that external ionization is extremely important for many applications as is the ability to do both positive and negative ions. I will try to give some examples of where one of these modes works and others do not.

First of all, I'd like to tell you a little bit about what thermospray is, and since we made up the word, I guess our definition should count. If you put the words thermo and spray together, you get something like the production of a jet of fine liquid particles by heating. That's basically what we're doing. We force the liquid through a capillary tube, typically on the order of 100 to 150 microns in internal diameter, and supply enough heat to that tube to vaporize almost all of the liquid. It is important that we not quite reach 100 percent vaporization. Out of this capillary we produce a supersonic jet of vapor which contains some entrained liquid particles or solid droplets. Since the samples that we are interested in are

generally somewhat less volatile than the solvent, these sample molecules tend to be contained in the droplets that survive this initial process.

Now, it is also true that if one has ions present in solution, then you can produce ions by ion evaporation from the charged liquid droplets or solid particles. This process is in fact quite analogous to ordinary field desorption. The difference is we don't apply any electrical field. The electrical field is self-generated simply by the charge on the particles and their small size. If you start off with particles on the order of one micron in diameter, and put something on the order of 10^5 elemental charges on it, you can calculate that the electrical field at the surface is in 10^7 volts per meter, which is the range you need for field desorption of ions. So the mechanism of this ion production, to say it very quickly and very simply, is field-assisted evaporation of ions from these charged liquid droplets.

As we discovered some years ago, if the filament burns out in your thermospray mass spectrometer, you still have ions present with no visible means of producing ions. When we first discovered this, we were quite excited about it. We're still quite

excited about it, but it's not the only way of using thermospray. The very nice thing about this ionization mechanism is it is an extremely soft ionization technique and it does not require a neutral in the gas phase as an intermediate between the sample in solution and the sample in the mass spectrometer.

That is a very important distinction because you can define a non-volatile molecule essentially as one which does not have a vapor pressure. That means it doesn't exist in the gas phase as a neutral. Basically, if a sample doesn't have any significant vapor pressure, then you're not going to be able to analyze it by conventional electron impact no matter what sort of interface or other technique you use, because that requires having molecules in the gas phase. Thermospray does, in fact, work for many kinds of molecules, for example, peptides, nucleotides and so forth, which do not have any appreciable vapor pressure; that is, they do not exist as neutral molecules in the gas phase. We're producing ions in the gas phase directly from ions in the solution and this allows a whole host of things to be done by LC/MS and by thermospray LC/MS that you couldn't do otherwise.

But thermospray LC/MS is also applicable to a whole range of molecules which are not nearly as demanding as this, which may be either neutral or ionic in solution, but are smaller, more volatile, et cetera. Those are the kinds of cases that I'm going to talk about primarily today. I will not show any slides of peptides even though that has been one of our favorite systems for studying by this technique and has turned out to be quite fruitful.

The last point on the previous slide was that thermospray is not the same thing as direct liquid introduction. This slide shows what the jet (very similar to what Rich Browner showed this morning) looks like if you don't heat the liquid. There's actually a solid jet here which is not visible because of the way the lighting was done and it breaks up into droplets by the processes that he discussed this morning.

This is not the way we do thermospray, but rather we increase the heat so that we get a supersonic jet of vapor containing droplets. When you reach the transition point, you can see a rather dramatic difference. The particle sizes are much smaller and determined by the aerodynamics now

rather than the size of the nozzle. This is at something like 50 percent vaporized. If we increase the heat still further, we get over to a jet containing fine droplets on the order of one micron or smaller in diameter. We still don't have good measurements of the actual distribution, although I think it is fairly close to a monodisperse aerosol as well.

This slide shows a schematic of a thermospray system. This is somewhat different than what Bob Voyksner showed this morning, but the principles are very similar. Many of the details are different. As he mentioned, it's very important to monitor the appropriate temperatures in order to control the degree of vaporization. The vaporizer is a capillary tube mounted into a probe which can go through, for example, the conventional solids probe inlet that is available on many mass spectrometers. The capillary is heated by passing current through the capillary. The power input to that capillary is controlled by a thermocouple attached near the entrance end. By doing that we can compensate for flow rate fluctuations by automatically adjusting the power in the feedback system to keep this temperature constant. We also monitor the temperature

at the exit of the vaporizer and downstream of the sampling point, as well as monitoring the temperature of the block itself. In fact, we use two feedback systems; one feedback system to control the power into the vaporizer, and another one which controls the heater input to the block.

If we reproduce the two temperatures and flow rates, then we can quite reliably reproduce conditions and obtain the same results from one day to the next. It now is quite routine to set this system up and run it every day, all day and all night, for that matter, if you want to.

The other thing that I want to mention is the position of the electron beam. What we normally do in all the systems that I've had anything to do with, is to supply an electron beam perpendicular to the direction of the jet in the region, well upstream from the normal position for an electron beam in an ion source.

We find that the sampling efficiencies in this ion source are very similar to what you get in a conventional chemical ionization ion source, even though we have a very large flow superimposed on this. After all, we're vaporizing up to two milliliters a minute of liquid, which in the case of

water corresponds to something like two standard liters per minute going directly into the ion source. The reason we're able to accomodate this, as Bob mentioned this morning, is we pump on the other side of this ion source off to a cold trap and a mechanical vacuum pump.

All that's required to convert a conventional mass spectrometer to a thermospray system is to replace the ion source assembly with one designed for thermospray. We bring the vaporizer probe in through a probe lock or equivalent, depending on what's available on the instrument, and we provide an additional mechanical pump and trap to pump away the excess vapor. Otherwise, conventional GC/MS systems can be used with no modification and the interfaces we've developed don't require any pertubation of the GC connections at all. The GC is not connected to the ion source when you're doing thermospray, but it's sitting there so that when you put your conventional source back in, it's ready to do GC/MS or what other kinds of measurements you want.

This slide shows a schematic diagram of the control system where we sense the temperature, feed it back to a triac control power supply and supply

current to the capillary here for the vaporization. It's all quite straightforward, and now works quite routinely.

This slide shows an ion source for a Hewlett-Packard instrument being installed. This assembly goes in place of the standard ion source and the optics are identical to the ones that are normally present on the mass spectrometer. The only part that's really changed is the source block. The control system and pumping system are in the background.

This slide shows the probe which has the vaporizer in it, going in through the standard probe lock into the ion source.

This slide shows the whole system including the control for automatic recycling of the cold trap. The vacuum pump is inside the cabinet. The refrigerator is outside. Of course, you can use dry ice or liquid nitrogen if you like to fiddle around filling traps every half an hour or so, but this system can run unattended for up to 16 hours and will recycle itself overnight while you're sleeping.

I mentioned our excitement over this new ionization technique and I'm not going to say much more, but just show one example of the reasons for getting

excited. This slide shows some results on adenosine back in the early days when we first discovered this effect. With the filament on, the total ion current shows a rather high background. Two injections of one micogram each of adenosine show a reasonable molecular ion, MH+, and significant amount of a fragment ion on top of some background at 136. We then turned off the filament and did the same injections again.

There are two things that are initially surprising about the results. First, the molecular ion leapt up by nearly an order of magnitude when the filament was turned off total area, and second, the fragment ion almost disappeared along with most of the background. Now, that's not always the case. Some people have concluded from this that there's no reason to have a filament present; in fact, it even may be a disadvantage.

Of course, everybody knows what a nuisance it is to keep a filament running, particularly in an atmosphere of water vapor and methanol and nasty things like that. But in fact, if you use a filament that's designed for hostile environments such as the thoriated irridium filaments used in non-burnout ionization gauges, this is not a serious problem.

Also, you must put enough voltage on the filament to get the ions to penetrate into this high pressure region; we typically use a kilovolt for that purpose.

The characteristics of the direct ionization, without the use of a filament, are summarized in this slide. If you have multiply charged ions in solution, you very often observe the multiply charged ions in the gas phase, particularly in the positive ion mode. We've seen up to quadruply protonated peptides. You don't see these for small ions because presumably they're too reactive in the gas phase. But we do produce abundant molecular ions, generally with little fragmentation. This can be good or bad depending on whether you're interested in structure or in getting the maximum intensity at a particular ion. Of course, the performance depends rather critically on the temperature, the capillary dimensions, flow rate and so forth. We now understand all of those factors in much more detail and can now control them rather well, but it still is rather more critical than running with the filament present.

One of the questions that always comes up concerns calibration of the mass spectrometer. With polar solvents such as water and methanol,

PFK or perfluorotributlamine do not give good spectra, so you need some other calibration compound. We have found that the standard mixtures of compounds such as polypropylene glycol and polyethylene glycol both work extremely well by thermospray and give very reproducible regular spectra for calibration. This slide shows a mixture of two cuts of polypropylene glycol. This is a mixture of 1,000 molecular weight and 2,000 molecular weight. Paul Goodley from HP provided me with this slide. It was run on one of the new HPs with the 2,000 mass range, and you can see that we have two overlapping Gaussian envelopes, one centered near 2,000 and one at about 1,000. Unfortunately, the tail of the higher mass one is off the scale of the mass spectrometer, but I'm quite sure those ions are there if you look for them.

This gives you a regular sequence of ions spaced 58 units apart. You can use any molecular weight cut of these glycols and they give a predictable spectrum which you can use to calibrate the mass spectrometer. The ions observed are all ammonium adducts to the individual molecules of the polypropylene glycol present.

The next slide shows a few examples of some

compounds which have some environmental significance and are of considerable biological importance. These were all done in the filament-off mode and are typical of the kind of spectra one sees. The MH+ ion is observed if the proton affinity of the molecule is higher than ammonia; if not, you may see the M+ ammonia. Cortisol is a good test case for a thermally labile compound because it is quite sensitive to conditions, and people who have done DLI have sometimes had difficulty getting molecular ions on this. In this spectrum obtained on 50 ng. the molecular ion is the base peak in the spectrum.

One interesting thing that you can do with an electron beam that you can't do without it, is electron capture CI, which may be a very powerful tool both in ordinary CI analysis and in thermospray. If you have compounds present that capture electrons, then electron capture can give you quite high sensitivity and specificity for these kinds of compounds. In the direct thermospray ionization, there are apparently no free electrons produced, so one does not see M- ions in thermospray by the direct ionization. Only if you have the electrons present using the filament do you actually produce these. There are many examples of cases which work

extremely well with the filament on by electron capture and don't work particularly well by some of the other ionization techniques. Chlorinated benzophenone is one example, and another is this rather large floppy sugar, avermectin, molecular weight 874, which gives the M- ion as the base peak in the spectrum, with some structurally significant fragments. The masses around the 500 region correspond to breaking off the major pieces around the central branch.

Another interesting case is the organic acids. I know Bob Voyksner and others have worked on these. They don't work well in positive ions and they don't work particularly well in negative ions filament-off, but with filament-on in the negative ions, they give quite high sensitivity. In one example, in the positive ion mode, 10 micrograms of this sample was injected and not detected. On the other hand, two nanograms was injected in the negative ion filament-on mode, and one sees a very nice response, many, many times noise. I think we could probably detect this at around the one picogram level in this case, in selected ion monitoring.

You can also do hydrocarbons, and I mention that because some people think you can't do hydro-

carbons by thermospray. You can't do hydrocarbons by thermospray if your solvent has a higher proton affinity than the sample. If you use methanol or water, you probably wouldn't do hydrocarbons anyhow for other obvious reasons, but we've done quite a bit of normal phase work recently using hexane as a solvent. So, you can do hydrocarbons, filament-on. Filament-off, you don't do hydrocarbons because they just aren't ionized.

This slide shows some of the advantages of having the filament. This is a pyrethrin pesticide mixture. The upper trace is the LC/MS total ion current trace for an injection of a mixture of five of these at the 50 ng. level. As you can see for the first three, we get quite a nice response, but these other two, you would not even know were there in the filament-off mode. On the other hand, at an order of magnitude lower concentration with the filament-on, we see all five components.

One reason that the sensitivity is low for the filament-off mode in this case is that this was a gradient from 70 percent acetonitrile to 90 percent acetonitrile. At the higher concentration of organic modifier, the direct ionization does tend to fall off rather rapidly. So when you get up to about

90 percent acetonitrile, the filament-off ionization is not working very well at all.

I just wanted to show one final example from some work that Al Yergy and Dan Liberatto are doing at NIH. Al generously loaned me these next few slides. The question comes up quite a bit, "Well, it's okay for qualitative work but can you do quantitation?" In fact, yes, I think the answer is now that you can do quantitation and you can begin to approach what you can do by GC/MS. That may be a rather bold statement because there certainly hasn't been even .001 percent as much work done as yet, so we still have some things to learn.

This particular example is a mixture of sugars. They're interested in monitoring glucose in plasma samples from patients. This is the LC/MS chromatogram of these five sugars, done by the technique that Bob mentioned this morning of using post-column addition. The separation for these sugars involves pure water. In this case they were using 0.6 mL/min. of water in the column and added 0.4 mL/min. of ammonium acetate downstream of the column in order to get ionization without the filament.

The spectrum of glucose shows mainly M+ ammonia and loss of water; a very simple spectrum. They're

interested in doing isotope dilution experiments in patients, so they wanted to determine how well they could measure the isotope ratios for something like this on column.

This slide shows their standard dilution curve. You can see the sort of scatter they have, but you have to realize this is down at the .1 percent level so that in fact, they really are doing quite well in terms of the standard curve. They are now applying this to actual measurements of glucose metabolism in patients at NIH and I'm sure will be reporting on this work before very much longer. This is a real application although not really an environmental one.

I would like to summarize by giving you an update as to where the technique stands, at least in my view. Detection limits, of course, depend a great deal on what kind of sample and what kind of conditions and even what kind of mass spectrometer, but our experience with a wide range of not too difficult samples is that we can usually detect them in the range of 1 to 10 picograms per second. I put the per second in there because, of course, in terms of the absolute amount, it depends very much on what kind of chromatography you're doing

and how wide your peaks are, but if you use some of these short columns with small particles, getting peaks a second or two wide is not all that hard. In these cases, detecting on the order of 1 to 10 picograms of a great many samples is quite feasible. Getting full spectra on one nanogram is also quite feasible for a very wide range of compounds.

The linear dynamic range is at least 10^4. We can accomodate virtually any liquid flow, but typically for the pumping systems that are available, we work in the .5 to 2 milliliters per minute range. Going to lower flows is no problem if you make some minor changes in the vaporizer, but the standard commercial systems that are available work best in this sort of flow rate range. The time constant in fact is considerably less than one second, so there really is no detectible peak broadening unless there's something seriously wrong with the way the system is being operated.

We can handle any buffer. That is, the thermospray vaporizer will vaporize anything, including non-volatile buffers in solution. However, these tend to get deposited somewhere, often in your ion source or in your pumping line or whatever, so we really don't recommend using non-volatile buffers.

The other thing they do is clutter up the mass spectrum by giving you lots of high mass cluster ions which are a nuisance. Buffers like ammonium acetate, ammonium formate, trifluorocetic acid, HCL all can be used to produce a buffer at almost any Ph you want, I think, without using things like phosphates and sodium salts. But it may take some minor changes in your chromatographic procedures. If you can't do without them, you can in fact run non-volatile buffers and pay the price by having to clean the system a little more frequently.

Thermospray can analyze any sample. When I say any, that's obviously a bit of an exaggeration because we haven't tried everything yet, but I would say that a very high percentage of the samples that we have tried do in fact work by one ionization technique or another, provided you have the right match between solvent and sample. You obviously can't do nonpolar samples of low proton affinity in polar solvents of high proton affinity if you're looking at positive ions. It's the same as chemical ionization in that respect. There's nothing mysterious about it. Everything as far as what works and what doesn't work, is understood in principle, although, of course, in practice we may not know

enough about the individual molecule to predict what's going to happen, but we can explain anything.

Finally, thermospray is gradient compatible, and now we do have an automatic control system which automatically compensates for both flow fluctuations and solvent composition and maintains the fraction vaporized constant throughout the whole range.

This slide of remaining problems is one that I made a year or two ago. Some of them are still with us, I think, although many of these have in fact been solved, either in principle or in practice. The first one I just mentioned, I think now is in fact finished. We do know how to control the vaporizer to routinely maintain the proper operating conditions. If we want to go to lower flows and maintain the same kind of performance, we do need to go to smaller diameter capillaries. In principle that's easy, in practice it presents some problems.

In particular, the smaller the diameter of the capillary, the more prone it is to plugging, particularly if you're not careful in keeping the dirt out of your system. I'm afraid we're not very careful because with the 150 micron diameter capillaries we're using, it generally is not a problem

and we are really quite sloppy in terms of worrying about this. We don't put filters in line because they plug up much faster than the vaporizers themselves plug up, so they tend to be more of a nuisance than a help.

Item #3 is something that we're still studying. We really do need to have a better understanding of the downstream vaporization process and how to maximize the performance for really non-volatile, really labile molecules. We can do it fairly routinely, but as we get to the larger things like the peptides, the sensitivity is quite a lot lower than it is for smaller molecules. Of course, you're taking a beating here in many ways, some of which you have no control over, but I feel that we still have the potential for improving that. Now we require on the order of a nanomole, for example, of a peptide of molecular weight, 1,000 or so, which of course is about a microgram, to get a really good spectrum from that molecule, while we may require three orders of magnitude less for a smaller molecule. I feel that there's still the potential for getting the sensitivity down into the picomole or even lower range for these larger molecules, but that still remains to be worked out in detail.

Thermospray is now available for magnetic analyzers. It's lagging a little behind the quadrupoles because it is somewhat more difficult, but the basic problems of dealing with the high voltage, et cetera, are all well in hand now. Kratos has a system commercially available for magnetic instruments, and systems will be commercially available, I think, for all the standard magnetic instruments very soon.

Obtaining a better fundamental understanding of the mechanism of thermospray is something I plan on spending at least the next 15 years studying. At that point I probably will retire, and I suspect there will still be some things to be done. That's the state of the art as it stands now. Thank you very much.

MR. TELLIARD: Any questions?

QUESTION AND ANSWER SESSION

MR. KROCHTA: Bill Krochta of PPG Industries. You mentioned plugging due to dirt in your system and dealing with these small orifices. How about the fact that you have a lot of salts in that system and you're constantly vaporizing them, do you have a problem during the course of a day with this?

MR. VESTAL: No, provided the vaporizer is properly controlled. That is, if one runs for an extended period of time with complete vaporization before the liquid exits from the capillary, then of course you will get plugging rather quickly if you have non-volatile salts present. However, the way that the system is normally controlled is to keep to say only 95 percent vaporized as it comes out of that capillary. The non-volatile salts then are carried away in the droplets. We have run, for example, with sodium salts of ion pairing reagents and things like this for days, in fact, until we plugged up the ion source. The vaporizer would not plug, but in fact they would sit down either in the ion source or the pumping line. I've actually filled an ion source

completely full of solid material, say five grams or so. The vaporizer was still fine, but the ion source was no longer working. In fact, you could see it happen. Everything is working and all of a sudden, the ion beam quits and the pressure in the vacuum system goes down. What happens is that you actually plugged up the ion exit from the ion source. In some cases, you couldn't even see across the ion source, the whole thing was full of solid. But you just take it out, hold it under the water faucet, and it's all water soluble so it goes away, put it back in and it runs.

MR. KROCHTA: If you don't completely vaporize, would this give you a problem when you do quantitative work unless you use internal standards?

MR. VESTAL: It can. If you really want to get quantitative performance, it is important, I think, to completely vaporize before you get to the ion sampling point. This is why I was talking about the downstream heating. It is essential that you not completely vaporize within the capillary tube itself, but it's certainly desirable for getting the best performance to complete the process between there and the sampling

orifice, and as Rich Browner mentioned this morning, that is not a trivial problem at reduced pressures in the very short residence time. We do fairly well at doing that in the present versions because we've learned in an artistic way how to accomplish that, although I have to admit there are some details about it that we don't really understand yet.

But you're right. It is important, if you really want to do the best quantitative work, to get the sample completely or essentially completely vaporized. I think that's one of the sources of variation that have been observed by some people where some samples worked and some didn't. I think, in fact, the way they were operating, that the ones that didn't work, weren't actually getting vaporized. That's partly a matter of the design of the ion source.

MR. TELLIARD: Thank you, Marv.

MR. TELLIARD: Our next speaker is John Ballard. John is going to tell you how to get two for the price of one, then you can put a thermospray on it. Everyone should have one of these. It probably costs $30, $40, I imagine. John.

JOHN M. BALLARD, PH.D.

LOCKHEED ENGINEERING AND
MANAGEMENT SERVICES COMPANY, INC.

DETERMINATION OF DYES BY THEMOSPRAY IONIZATION AND MS/MS

DR. BALLARD: Dyestuffs are of environmental interest because of their large scale production and their potentially hazardous synthetic intermediates and by-products. Just to give you some idea of the scale and complexity of the dye manufacturing industry in this country, the annual production runs at about 350 million pounds--I think that's 200,000 tons or thereabouts, at something like 87 different manufacturing locations. This results in a product line of about 1,000 commercially available products. Forty-eight to fifty Chemical processes are needed to adequately describe the chemical inputs into this industry, and the resulting dyes can be classified into 24 different structural groups according to the Colour Index, which tends to be the Bible of the dye and organic pigment industry.

The toxicity and/or carcinogenicity of dyestuffs and some of their synthetic intermediates

has been well documented. Compounds such as 2-naphthylamine, 4-aminobiphenyl, benzidine, Basic Violet 14, Solvent Yellow #2 and Citrus Red #2 have all been identified as actual or potential carcinogens, but there is some evidence that in fact metabolites of some of these compounds are the true carcinogenic agents. The EPA and the government have acted to reduce the number of colorants that can be used as additives in the food, drug and cosmetic industries in recent years, and of the compounds that are still permitted, the permissible amounts have been greatly reduced.

Because of the need for sensitive methods of detection or identification of these materials in a variety of matrices, HPLC coupled with UV, visible or spectrofluorometric detection has been widely used. Up to the present time, mass spectrometry has not been routinely applied because of the volatility requirement for generating electron impact or chemical ionization spectra. With the recent developments over the last 5 to 10 years of the desorption techniques such as californium-252 particle-induced desorption, fast atom bombardment, secondary ion mass spectrometry and field desorption, a whole new range of molecules, initially

the biomolecules e.g. carbohydrates, peptides, proteins, nucleosides, have become accessible to mass spectrometry. We have now applied thermospray ionization to the analysis of dyestuffs.

I will also mention briefly in passing, that the organic pigments, a specialized group of dyes, so far have not been accessible to us. We've obtained about a dozen of these organic pigments designed for use in plastics and textiles, and they're generally intended to be impregnated into the substrate rather than applied in solution. We've yet to find a suitable solvent that will enable us to look at these. Of the dozen or so pigments we've examined, only three could be solubilized in DMSO; they were two sulfonated azo compounds and a polychlorophthalocyanine. Having achieved solubilization, we thought we were okay, but in our hands, DMSO was not a good solvent for generating thermospray spectra, either in the positive or negative ionization modes.

As a generalization, it became apparent to us that in contrast to the normal analytical situation using standards of very high purity, for the dyestuffs we examined that situation was the exception rather than the rule. Even with the dye "standards"

we're using, it's obvious that there's a lot more in there than just the stated dye. Sometimes even the dye itself isn't present, and I'll show you an example of that.

In general terms, we do see a lot of peaks other than the protonated molecular ion and these other peaks are variously due to solvents, dispersants and other compounds which the manufacturers put in. It's very difficult to find out what these are because the industry tends to be somewhat secretive and protective. So we just have to accept these materials, which are formulations designed to meet a specific color and use application.

SLIDE 1

The first slide is just to give you some idea of the equipment we use. The most expensive item is the Finnigan TSQ instrument, which has the capability of doing MS/MS analyses. It's been modified for thermospray ionization by Marvin Vestal here, using the Vestec thermospray probe. We use a Waters 6000 pump as a delivery system just to get the sample and solvent into the mass spectrometer. We use the Rheodyne injector valve with a 10 microliter loop. At the moment, we're not doing any chromatography, but we do have a short chromatographic

column in-line between the pump and the injector to damp out pulsations of the pump which, if not removed, cause severe fluctuations in ionization in the ion source.

SLIDE 2

The operating conditions are pretty standard, i.e., a small percentage of methanol in a large amount of 0.1M ammonium acetate. We find a flow rate of about 1.3 mL per minute is optimum for us with our trapping system. As Marvin and others have mentioned, the control of various temperatures, particularly the tip and vaporizer temperatures, are critical. We have put a range up there because no two days tend to be the same, depending on which analyte we're looking at.

For mass calibration, we use one of the polyethylene glycol mixes, typically that of average molecular weight 400, which in combination with the lower mass thermospray ions gives us good calibration from about m/z 30 up to say m/z 500-600, which is adequate for our work. As "standard" conditions for the collision-activated dissociation, we start with a collision energy of 20 volts and argon is the collision gas at one millitorr pressure. The term "standard" just refers to what we do in

our laboratory. We find that those conditions usually generate good CAD spectra.

SLIDE 3

This is yet another view of a typical interface design. I think maybe my colleague lifted this from one of Marvin's publications. He may recognize it. The pump-out line at the bottom here, is at right angles to the line of flow of the solvent and in our system it goes to a large cold-finger trap which is cooled with an FTS cryo-refrigerant unit. We get a temperature of about -80 degrees C to -90 degrees C using methanol in the trap. From just doing rough measurements and calculations of what goes in and what comes out of the trap at the end of the day, we think that about 95 percent of the solvent gets caught in the trap. You may wonder where the other five percent goes, and the answer, of course, is into the mechanical pump on the other side of the trap. What comes out of the mechanical pump when you change the oil is really a hazardous waste in its own right.

SLIDE 4

This is to refresh people's knowledge of what the triple quadrupole mass spectrometer is and how we can use it. In comparison to gas-chromatography

where you separate the components of a mixture and ionize them separately, with the triple quadrupole you can ionize all constituents of a mixture simultaneously and then use the first mass filter to do a separation. Suppose you select a particular ion, it may be a molecular ion or a fragment ion, you don't know which it is before you start. You allow it to pass into the collision cell which is pressurized with an inert gas, in this case argon, at something like one millitorr. The molecular ion is fragmented and typically you generate a spectrum which is qualitatively similar to a standard EI mass spectrum and you analyze the fragments that are formed using the third quadrupole mass filter.

SLIDE 5

This is just to give you an idea of what actually comes out of the mass spectrometer in terms of printout. You see there are quite a lot of spikes in this RIC trace on the bottom. Those are due to the pulsations of the pump which were particularly bad when this was recorded. This is quite a high loading of material in there, and so you can see that there's a reasonable amount of spreading from the injector loop and through the flow line. With our configuration, we have a flow

time of about 12 seconds from the injector into the ion source.

I'm going to divide my material dealing with dyes into three areas. The first will be generalizations on what you might expect to see in the spectrum, together with detection limits that we've established for some of these materials. The second area will be characterization of two or three dye wastes. The third area will be use of the TSQ to do the structural determination of an unknown dye.

SLIDE 6

The figures here were selected from a table of about 16 dyes which we worked up; these were deliberately chosen to show respectively the best case (at top), a typical case in the middle (Disperse Orange 13), and the worst case I could find (Solvent Red 23), which has a large number of peaks other than the protonated molecular ion in the spectrum. As I say, that's a typical case, the Disperse Orange 13. You might also notice that these two dyes have the same molecular weight and obviously the same MH+ ion. The question is-What ARE all of these ions here?-and it's a good question.

From our observations of the purest dyes that

we've looked at, I would tend to say that they're not fragment ions. This is based on our observations and those of others published in the literature, that thermospray is a soft ionization process. Also, we noticed that typically the spectrum we generate from the molecular ion when we do CAD experiments, does not contain any or all of the lower mass ions that are seen in the full-scan spectrum of the dye sample.

SLIDE 7

We wanted to look at this in a quantitative way and these are results obtained with dye standards from a well known chemical supplier. These are the percent dye content as stated on the label, tabulated with the percentage of the total ion current that was actually carried by the protonated molecular ion. This slide shows that, in general, there's a reasonably good correlation. For the Disperse Orange 3, I bracketed the 100 percent simply because no information was given and so reading the manufacturer's handbook, you were led to believe that the material is of 99 percent or better purity.

SLIDE 8

This second slide is a continuation of the first. You can see here that we've gone from reasonable correlation to virtually no correlation, especially in the case of Solvent Red 23, in which there's very little of the molecular ion compared with what's supposed to be in there. Solvent Red 3 is good. For the first sample of Basic Yellow 11 we looked at, also from this well known supplier, the dye content was listed as about 60 percent. So we ran its mass spectrum. Even expecting the worst, we were a little surprised to find that there was absolutely none of the expected molecular ion in there. Experiments showed that it's not even of the correct dye class. It does have the correct color, it is yellow, but that's just about all you can say for it. So this is one of the pitfalls for the unwary. Don't believe everything you read on the label.

SLIDE 9

Each one of these seven dyes is from a different chemical class. We've had a look at detection limits and we find, like others have reported for pesticides and other compounds, that even within the same chemical class, detection limits can vary

somewhat widely, say over an order of magnitude. The detection limits were obtained by scanning the mass spectrometer from about m/z 150 to just above the molecular ion region. We weren't doing selected ion monitoring because in a real life situation, if you're scanning a sample and you don't know what's in there, you've got to scan for every possibility, you can't just go looking for one particular ion.

The amount we get in the best case, I think, is 15-20 ng. Those figures are comparable to numbers I've seen in the literature for pesticides and other compounds. The boxes that are highlighted here refer to negative ion sensitivity and you can see that the detection limit is an order of magnitude higher than for the same dye in the positive ion mode.

Another parameter the EPA uses is called the method sensitivity, and it gives you a feel for what you get out for what you put in. Essentially, it's the slope of the straight line obtained by plotting the area counts versus the weight of material. One other point I should make about this, of course taking the best case, is that if we can see 15 ng., that's on a 10 microliter

injection, then for this particular compound, Basic Green 4, we can detect it in solutions of 1.5 ng/ML. I don't care to do the mental gymnastics to convert that to ppb or ppm, but I think it's pretty reasonable.

SLIDE 10

Moving along to the analysis of waste streams from dye manufacture, we obtained some waste stream samples. These were not chemically pretreated. The only thing we did to them was to filter the solutions to make sure that we didn't get anything solid into the system. I imagine from what Marvin said, that it wouldn't destroy the LC pump, rather it would block the vaporizer. From a knowledge of the manufacturing process, you can look up the relevant books, find out what the expected intermediates are, and then you can look for them. In only one case did we find the expected compound. That's good and bad, I guess. It means that maybe the manufacturer is doing a good cleanup job and he's getting most of the impurities out, especially the dichlorobenzidine. I think most people are aware of the carcinogenic properties of these benzidine compounds.

Pigment Yellow 12. We didn't see any of the

expected intermediates in the second sample and we didn't see the same by-product that we'd found in the first sample, but we did find these acylated toluidine derivatives and again, from the literature, you can deduce that in fact this was a waste stream from Pigment Yellow 14 or another pigment, not from Pigment Yellow 12.

In some cases, again the expected intermediates are not present in the waste stream, but there are certainly some organics present. Again, if I just put the number there, it means we couldn't identify them and that is partially because with something like 1,000 chemical intermediates used in the dye industry, it is rather difficult to get hold of all of these and run standards and generate CAD spectra of all of them. We can make guesses, but we'd rather not.

Acid Red 114. We did have a sample of the authentic dye standard and it was interesting that the dye itself also contained the two by-products that we detected in the dye waste. These are condensation products formed at one stage of the synthesis. P-Toluenesulfonyl chloride reacts with phenol which is left over from a previous stage. Some of these compounds go back two or three

stages in the synthesis. If you're prepared to put a fair amount of time and a little imagination into it, you can track some of these reaction sequences backwards.

SLIDE 11

The picture here doesn't look quite as rosy. These two direct dyes were in our waste streams. You can see that, again, they don't contain any of the expected intermediates, but they do contain a large number of other by-products, solvents, et cetera. It's very difficult to work out what some of these are, even with the CAD capability.

SLIDE 12

Now, you may remember in one of the first slides, the typical and worst cases of dyes. Two of them had the same molecular weights. Well, here they are with their structures. You can see that they're very similar. If you only have one single stage of mass spectrometric analysis, and because of the soft ionization, then assuming a pure compound, all you see is 353, the MH+ ion. The MS/MS capabilities of triple quadrupole mass spectrometry allow you to differentiate these two. You can see that although they do have some ions in common at very low mass and the mid-mass range,

these two molecules are clearly differentiated by their CAD spectra which were recorded under identical conditions.

SLIDE 13

The third area that we've looked at using the thermospray and triple quadrupole combination are some structural determinations on unknown dyes. This slide shows the full scan thermospray spectrum from m/z 120 up to 400, of Basic Red 14. You'll notice the presumed molecular ion at m/z 344. There's another peak of about 40 percent abundance at 346 and also these two ions at m/z 189, 174.

SLIDE 14

This spectrum shows the daughter ions generated by CAD of the 344 ion. The important ions...well, they're all important usually, but some of the more important ions are those at 329, 314, 303, 289, 274. If you look at 344 and wonder what goes to 289, you'd probably go crazy trying to work out what the loss of 55 is. Well, it turns out that it's not 55, it's a loss of 40 and 15; 289 is the loss of 40 from 329. These ions here, 173 and 158, are also important and I'll come back to those in a minute.

SLIDE 15

The ion at 173 was tentatively identified from

its CAD spectrum by comparison with the EI spectrum which we had in an old publication from Analytical Chemistry, of a compound called Fischer's base, which has this structure. It's a trimethylindoline. It happens that this compound is a very well known intermediate. It's used a lot in the dye industry in the manufacture of cationic polymethine dyes which are used for dying polyester and other synthetic fabrics. We confirmed that this was the structure by obtaining an authentic specimen of this and running the CAD of its M+1 ion. That was identical with the previously generated CAD spectrum.

SLIDE 16

This is the daughter ion spectrum of the ion at 189. You see there's a loss of 41 to 148 and other subsequent losses. The loss of 41 and/or 40 which we observed previously in the daughters of 344, is very characteristic of a 2-cyanoethyl group, and the structure of the dye starts to become more apparent when you look at the literature and you see that they can make these polymethine cationic dyes by condensing Fischer's base with, usually, substituted aldehydes.

SLIDE 17

So if you get out your calculator and add up

the numbers, this has the right molecular weight, 188. We obtained a sample of that compound and generated its M+1 ion in thermospray interface, did the CAD and again obtained an identical CAD spectrum, therefore confirming our guess that this was the second half of the synthetic process.

SLIDE 18

So we have the two molecules there, and you do the condensation and get this quatetnized nitrogen-containing molecule. There are probably hundreds of these with different atyl moieties and some with an extended carbon-carbon linkage as well.

SLIDE 19

Going back to the 344 ion. That's it's structure, and it is the molecular cation. Now the losses of 15 and 40 are well explained by consecutive losses of methyl groups, losses of the CH2CN and/or CH3CN, and you can get all the way down to 274. Considering the 173 and 158 ions, this molecule is mass symmetric about that carbon-carbon double bond and initially it wasn't obvious to us which half retained the charge and formed the m/z 173 ion. In fact, it's the right hand side, and 158, I think, is due to loss of the methyl from the 173.

SLIDE 20

These are the daughter ions of the 346 peak that we saw associated with 344 in the material when we first looked at it. From the approximately 3:1 ratio, we wondered at first if this was an indication of one chlorine being present. However, none of the CAD spectra we looked at showed any losses corresponding to elimination of small chlorine-containing compounds and so the possibility of chlorine being present was ruled out. In fact, there are some similarities in the low mass end of the spectrum, but the high mass end is very, very different from that of the 344 CAD spectrum which again is evidence that we're not just looking at an isotopic variant. We think that the 346 is a product that's formed by reduction of the 344 ion somewhere along the line in the manufacturing process. Either the indoline ring or maybe the carbon-carbon double bond linkage is reduced.

SLIDE 21

We looked at another one of these cationic dyes of known structure. This one is Basic Orange 21 which has a molecular weight of 315. We wanted to see if we could be sure that the 173 ion which we said was derived from the right hand

side of the Basic Red 14 molecule, was in fact correctly ascribed. This is the full scan spectrum. This is one of the higher purity dyes, although I imagine that m/z 315 is probably only about 30 to 40 percent of the total ion current. We obtained CAD spectra of these ions at 180 and 198, but we can't identify them. We think they're probably solvents or dispersants that are used in these proprietary formulations. A lot of the information on these dyes is contained in the patents literature which is pretty notorious for being ambiguous and vague and sometimes misleading, so we really could not identify these.

SLIDE 22

This is the CAD spectrum of m/z 315 from Basic Orange 21. That's the molecular ion, and the ions at 300, 285, 270 are due to the expected consecutive losses of methyl groups from that molecule. 144 is the only other ion at low mass. If you do the numbers game, you'll find that it's cleavage at the carbon-carbon double bond, with a hydrogen transfer and charge retention on the right hand side of the molecule. So, we conclude that our assumption in assigning the 173 peak from Basic Red 14 is correct.

I've run out of slides, so that's more or less

it, except that I would just like to make a few conclusions. I think for the analysis of dyes, you've got to be sure that you're looking at the dye that you think you're looking at. If possible, you'd like to have pure dye standards and that's rather difficult. At the moment we're not in a position to do quantitative analyses of dye wastes. It means we've got to get our lab coats on and actually do some recrystallizations or chromatography to purify these. We have taken delivery of a brand new, nice, complicated Spectrophysics LC recently, which somebody is leaning how to use, and we eventually want to get on line with the mass spectrometer and do some chromatographic separations followed by MS/MS experiments.

In theory, the ability to use the first quadrupole of a triple quadrupole instrument for separation of ionized species means that you can ignore chromatography, but I think you probably would do that at your peril. It's better to take advantage of all the parameters and cleanups available.

That more or less ties it up. I would like to acknowledge Don Betowski of EPA, who is my co-worker on this project. Thank you.

MR. TELLIARD: Questions?

No questions. Thank you very much.

SLIDE CAPTIONS

1. Instrumentation
2. Operating Conditions
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6. Typical Thermospray Mass Spectra of Dyes
7. Dye Content vs. % Total Ion Current carried by $(M+H)^+$
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14. CAD Mass Spectrum of m/z 344 from Basic Red 14
15. Structure of Fischer's Base
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17. Structures of Synthetic Precursors of Basic Red 14
18. Synthesis of Cationic Methine Dyes
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Instrumentation

Finnigan Mat triple stage quadrupole(TSQ) Mass Spectrometer

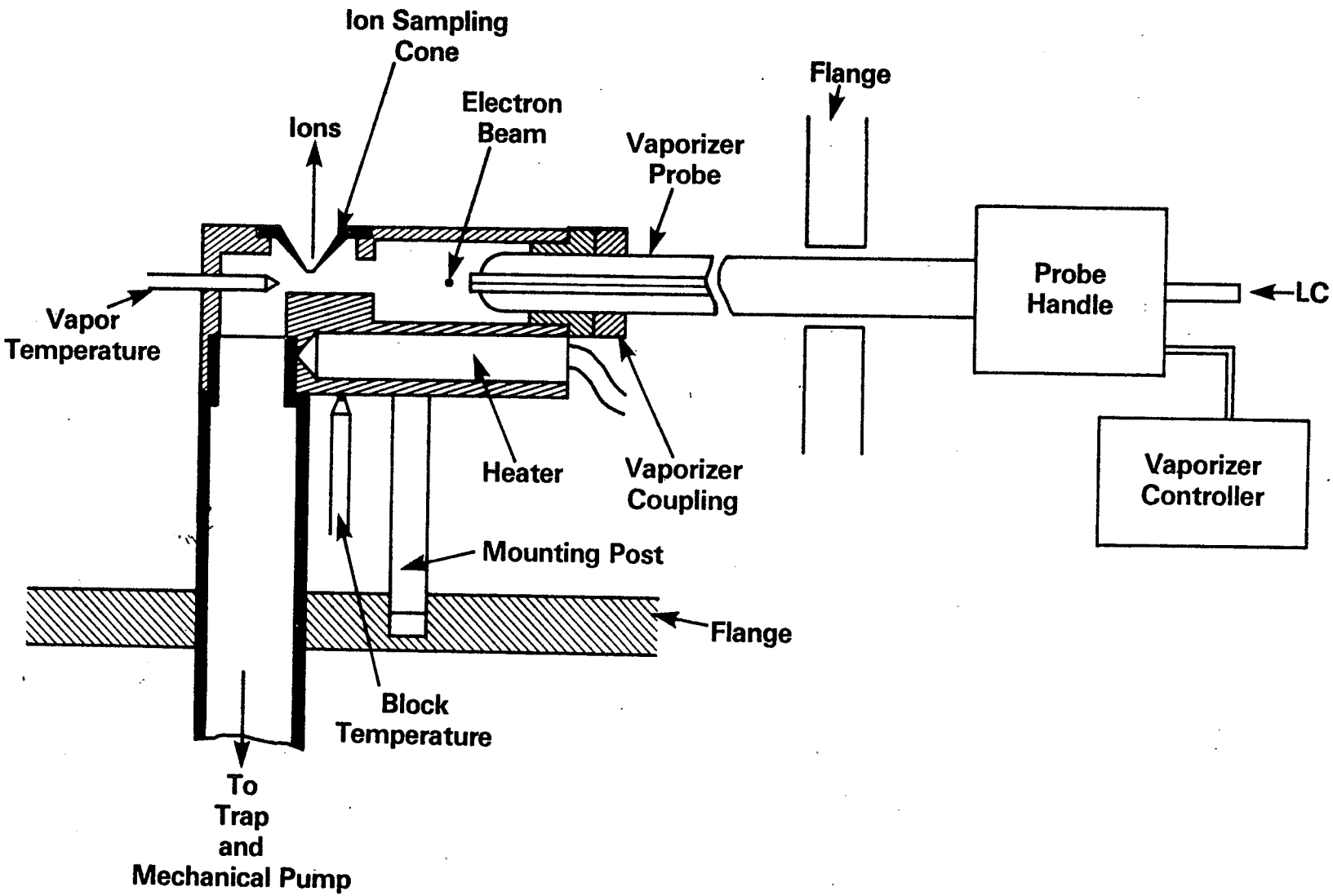
Vestec thermospray interface

Waters 6000A LC pump

Rheodyne 7125 injector valve

Operating Conditions

Buffer:	0.1M Ammonium acetate/methanol (97:3 v/v)			
Flow Rate:	1.3 ml min.$^{-1}$			
	T(vaporizer)	115-130°C	T(source)	200-230°C
	T(tip)	215-230°C	T(jet)	195-240°C
Mass Calibration:	Peg 400			
Collision Activated Dissociation (CAD) :	Energy 20eV Argon 1.0 mtorr			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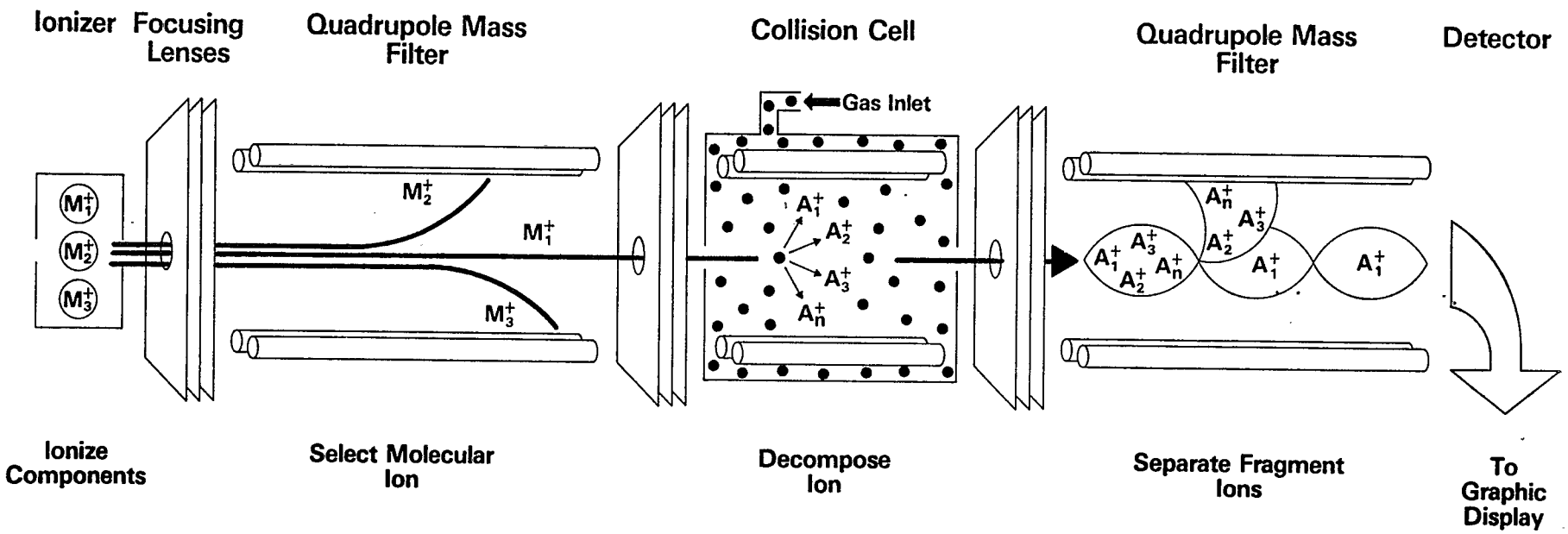
Vaporizer Probe
Flange
Probe Handle
LC
Vapor Temperature
Heater
Vaporizer Coupling
Vaporizer Controller
Mounting Post
Flange
Block Temperature
To Trap and Mechanical Pump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Focusing Lenses
Quadrupole Mass Filter
Collision Cell
Quadrupole Mass Filter
Detector
Gas Inlet
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M_2^+
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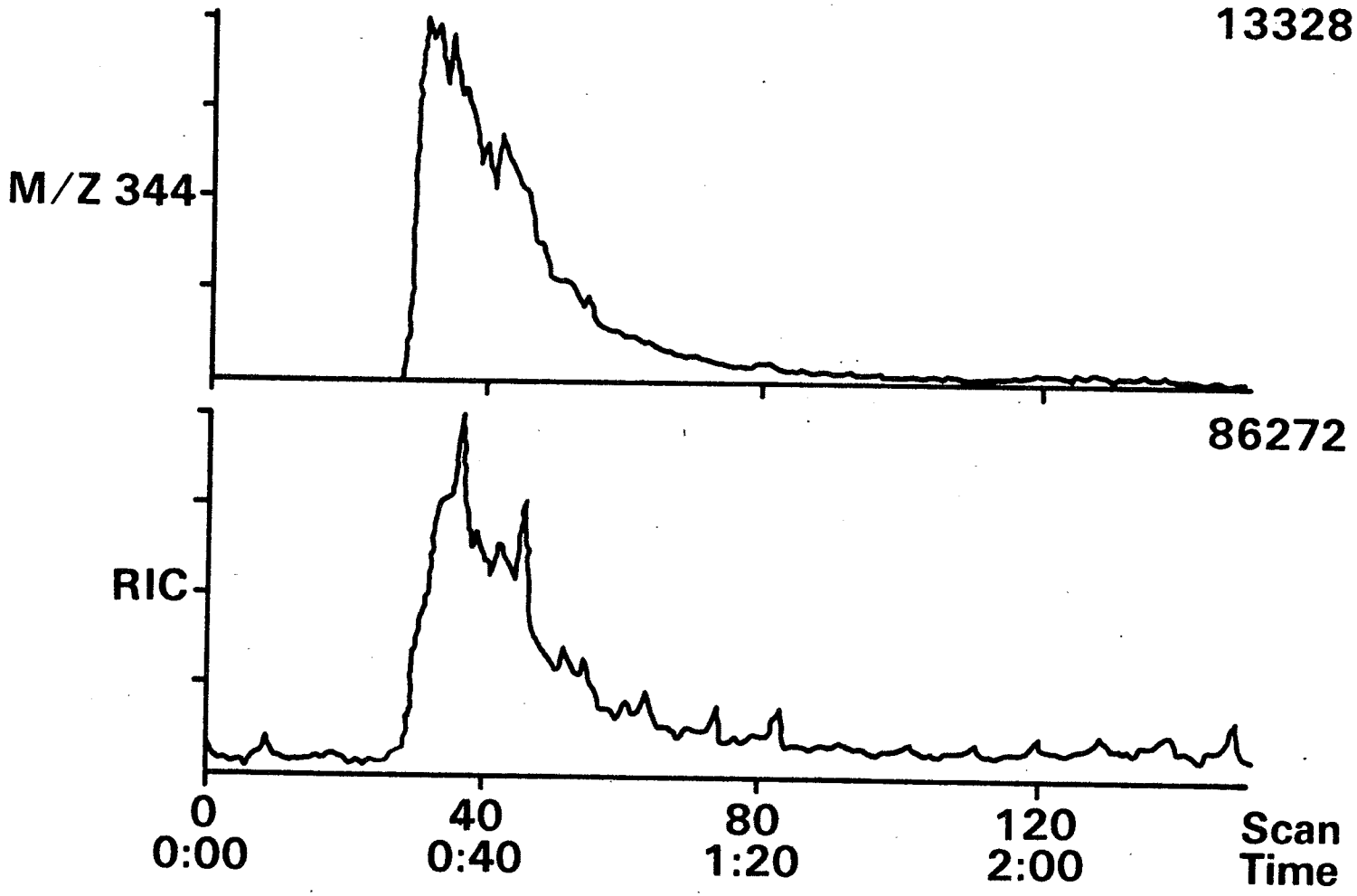
A_2^+
A_3^+
A_n^+
Ionize Components
Select Molecular Ion
Decompose Ion
Separate Fragment Ions
To Graphic Display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86272
RIC
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80
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2:00
Scan
Time

Thermospray Positive Ion Mass Spectra of Dyes

	M. WT.	Ions Observed (% RA)			
Disperse Orange 3	242	244(18)	243(100)		
Disperse Orange 13	352	354(16)	353(84)	249(17)	248(68)
		200(13)	199(100)	180(10)	163(13)
Solvent Red 23	352	354(20)	353(95)	349(52)	244(18)
		243(47)	200(16)	199(81)	198(89)
		189(24)	182(11)	181(100)	176(29)
		170(46)	160(44)	158(49)	156(14)
		152(17)	150(11)	143(12)	142(33)
		141(53)	140(11)	139(11)	136(15)
		135(47)	134(38)	133(19)	132(20)
		131(26)	128(15)	125(15)	

Dye Content vs % Total Ion Current Carried by $(M+H)^+$

	% Dye Content	%Total Ion Current $(M+H)^+$
Disperse Blue 3	ca. 20	7
Disperse Yellow 5	ca. 30	30
Disperse Orange 3	(100)	96
Disperse Orange 13	ca. 15	31
Disperse Red 13	ca. 25	33

	% Dye Content	% Total Ion Current $(M+H)^+$
Solvent Red 23	ca. 85	10
Solvent Red 19	ca. 95	27
Solvent Red 3	(100)	95
Basic Brown 1	ca. 50	11
Basic Yellow 11		
Sample A	ca. 60	0
Sample B	—	46

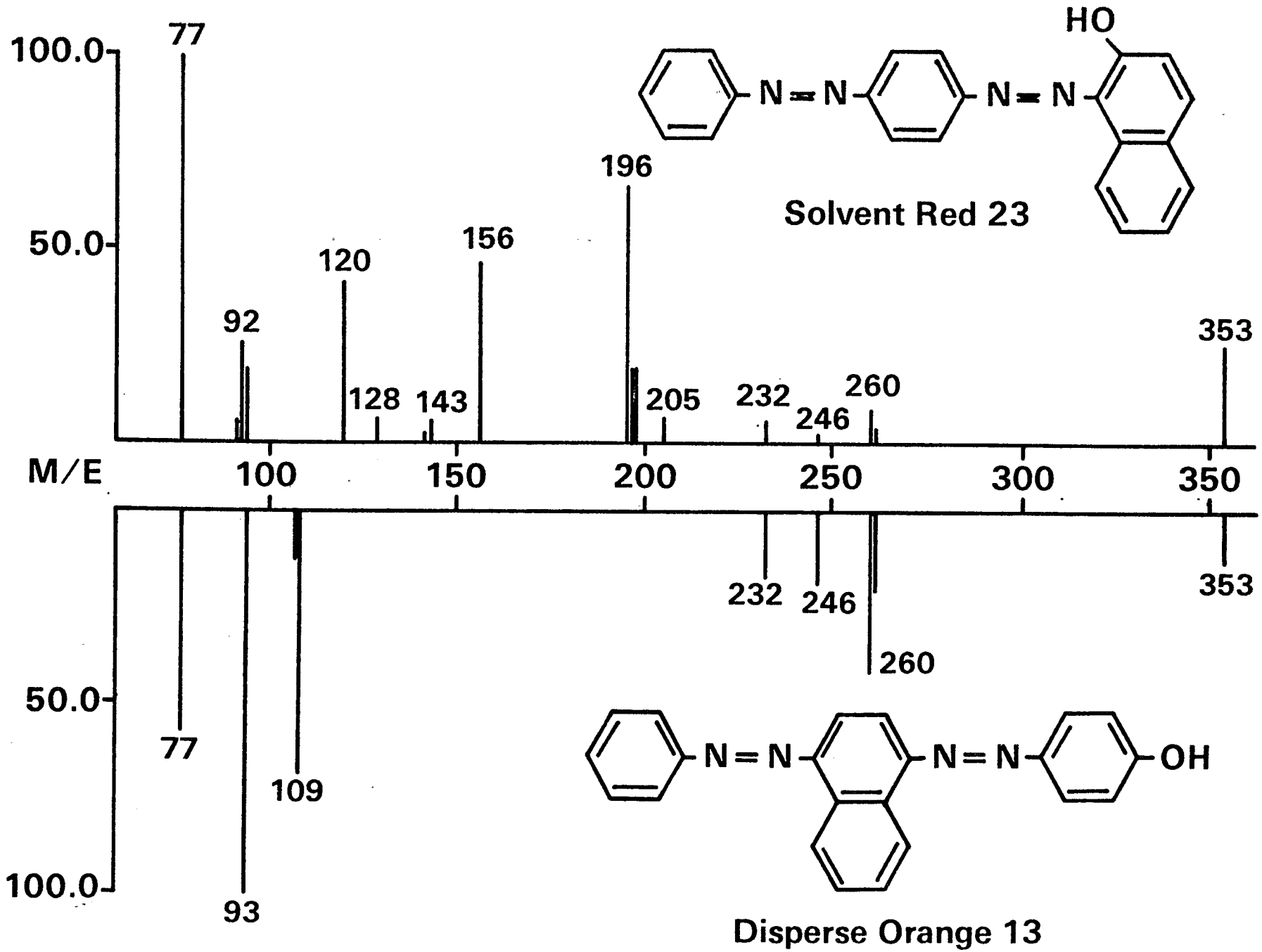
Detection Limits/Sensitivities for Dyes

Dye	Ion (m/z)	Detection limit (ng)	Method sensitivity (area counts ng^{-1})
Disperse Orange 13	353	150-200	3.5
Solvent Red 23	353	50	7.7
Fluorescent Brightener 236	390	25-50	16
Basic Violet 10	443	30-60	29
Disperse Blue 3	297	20	3.3
	267	40	30
	296[a]	1300	0.046
	266[a]	800	0.80
Basic Green 4	329	20	5
	331	15	50
Acid Orange 6	295	200	2
	293[a]	2000	0.025
	294[a]	5000	0.018

[a] Negative ion mode

Waste Stream		Intermediates	Observed	By–Products Detected
Pigment Yellow 12	(A)	Acetoacetanilide	Yes	Acetanilide
		3,3′-Dichlorobenzidine	No	
	(B)	As above	No	Acetotoluidide
			No	Acetoacetotoluidide
Pigment Red 53:1		2-Naphthol	No	5
		2-Amino-5-chloro-p-toluenesulfonic acid	No	
Acid Red 114		p-Toluenesulfonyl chloride	No	Phenyl p-toluenesulfonate p-Toluenesulfonamide
		3,3′-Dimethylbenzidine	No	
		Phenol	No	
		2-Hydroxy-3,8-naphthalene-disulfonic acid	No	

Waste Stream	Intermediates	Observed	By-Products Detected
Direct Red 80	6-Amino-3,4′-azodibenzene-sulfonic acid	No	8
	6-N-Acetylamino-1-hydroxy-3-naphthalenesulfonic acid	No	
	OR		
	6,6′-Ureylenebis-1-naphth-ol-3-sulfonic acid	No	
Direct Blue 80	3,3′-Dihydroxybenzidine	No	10
	3-Hydroxy-2,7-naphthalene-disulfonic acid	No	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77
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128
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196
205
232
246
260
353
M/E
100
150
200
250
300
350
HO
N=N
N=N
Solvent Red 23
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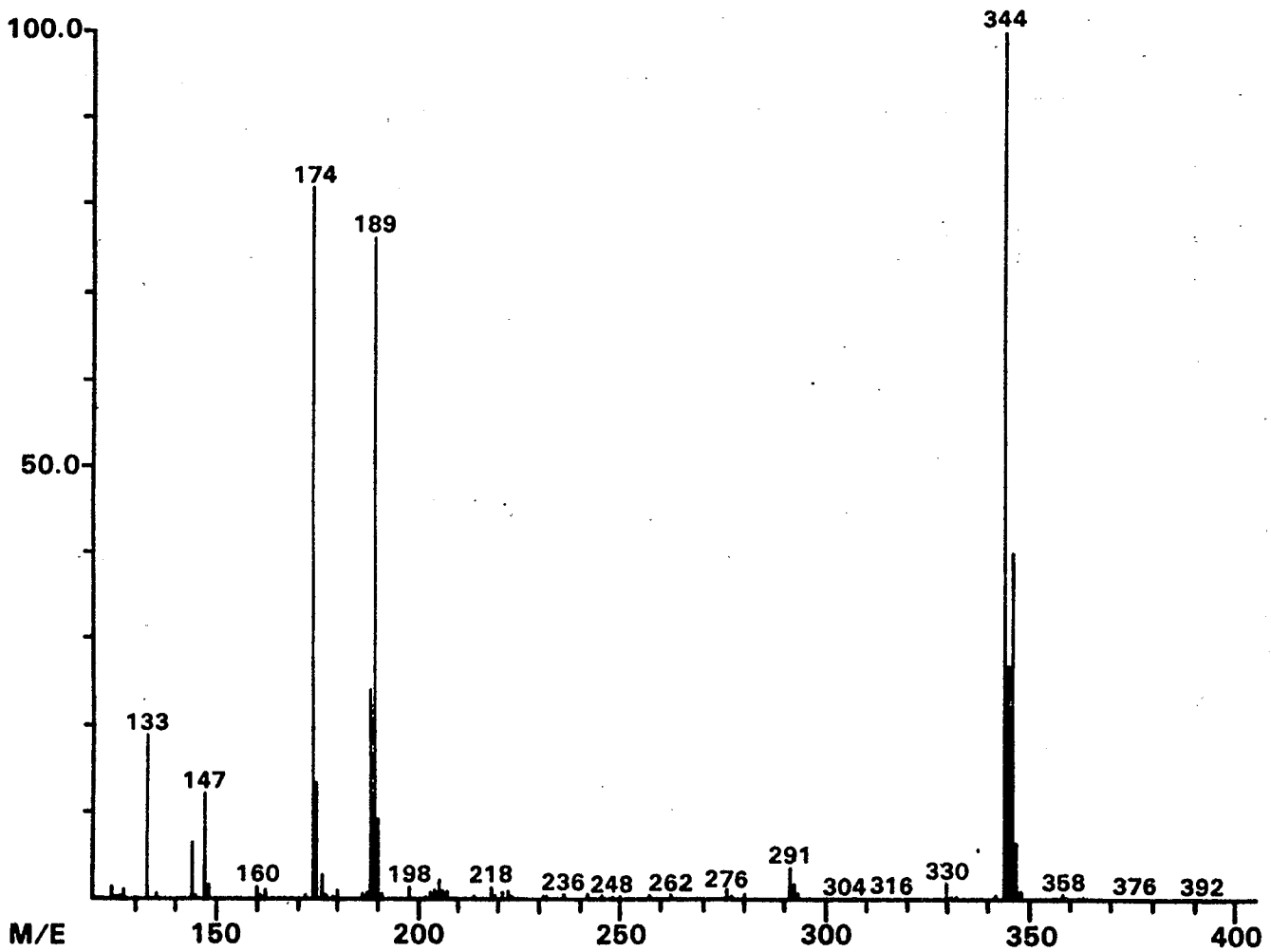
100.0
77
93
109
232
246
260
353
N=N
N=N
OH
Disperse Orange 13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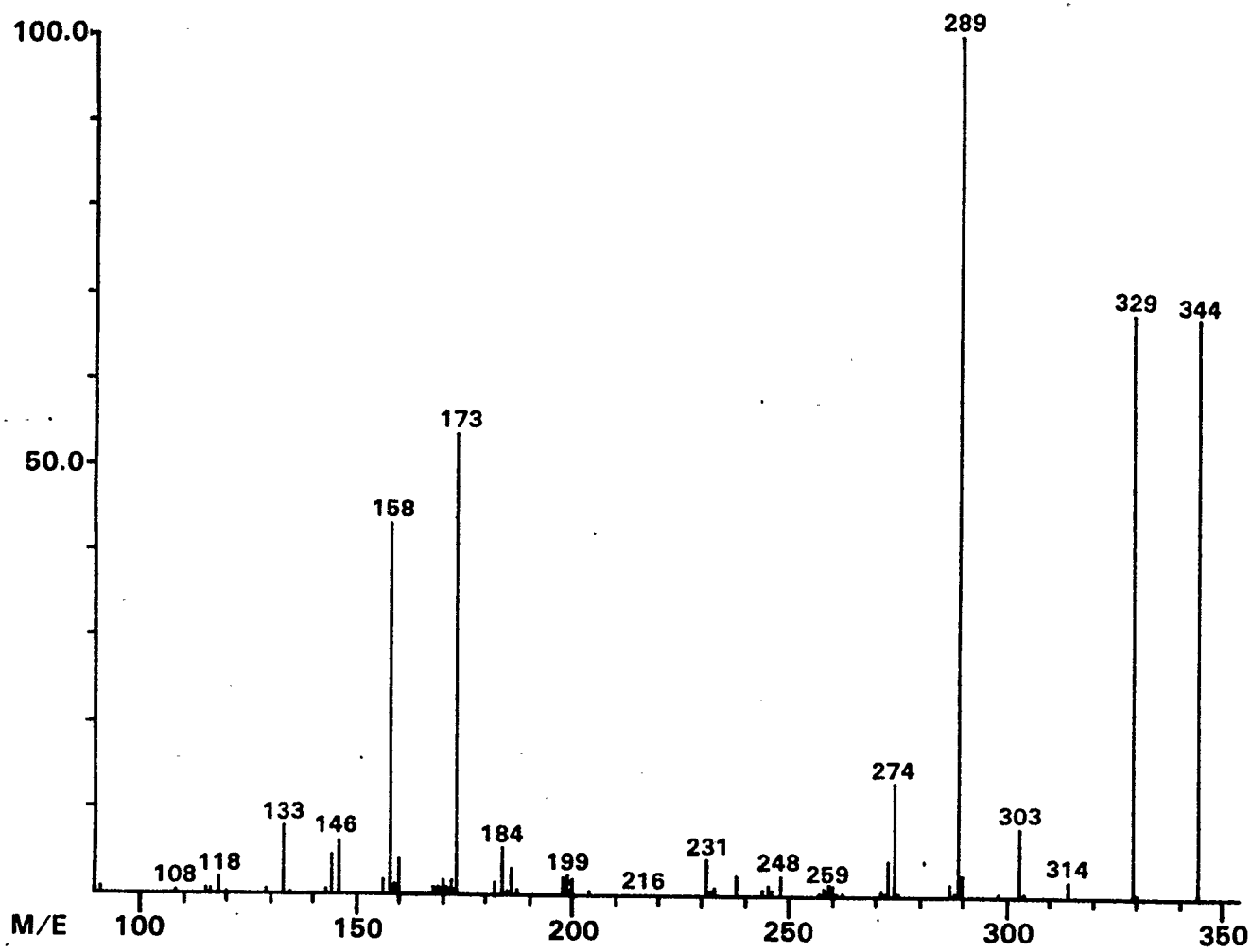
358
376
392

Figure 1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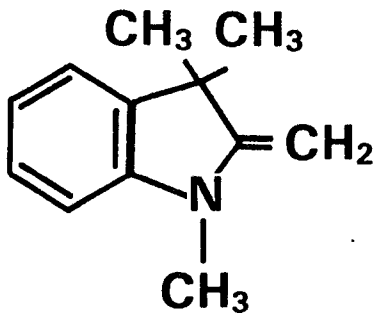
344

Figure 3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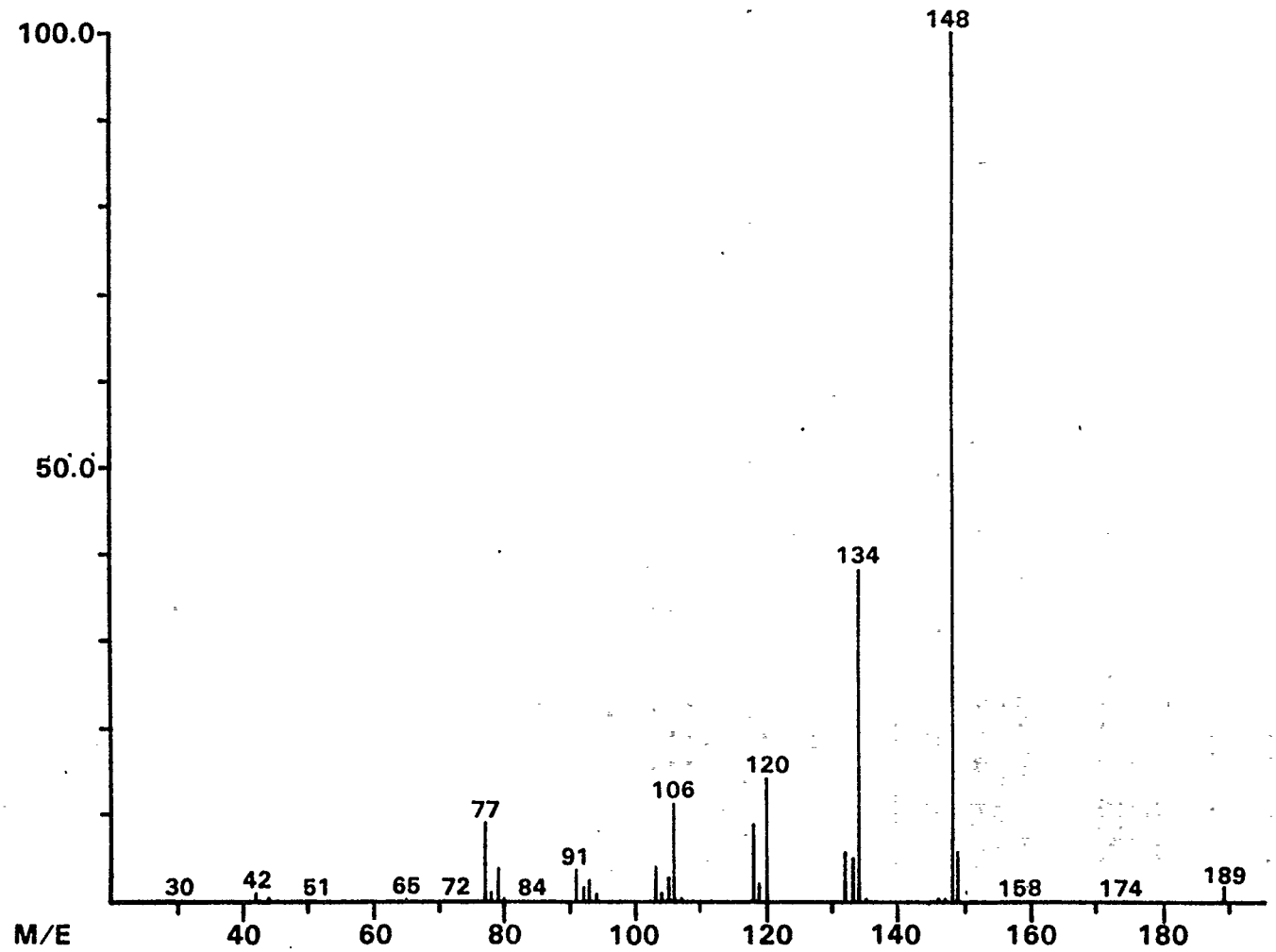
CH2
N
CH3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M/E
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60
80
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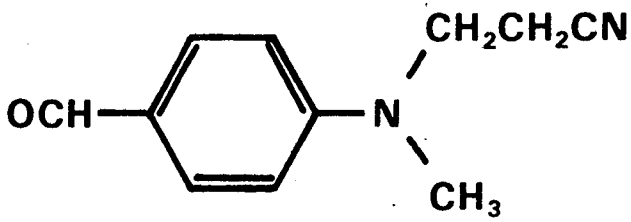
140
160
180

Figure 2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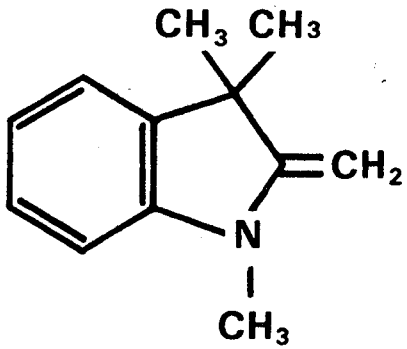
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CH2
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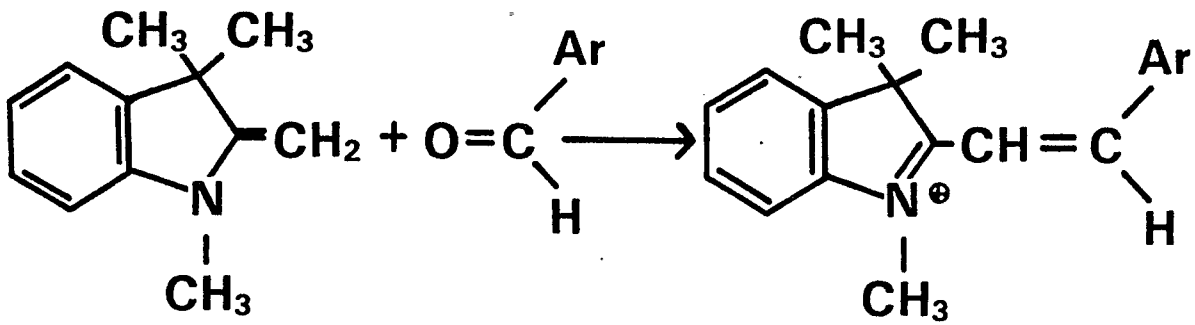
CH3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CH3
+ O=C
Ar
H
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Ar
H
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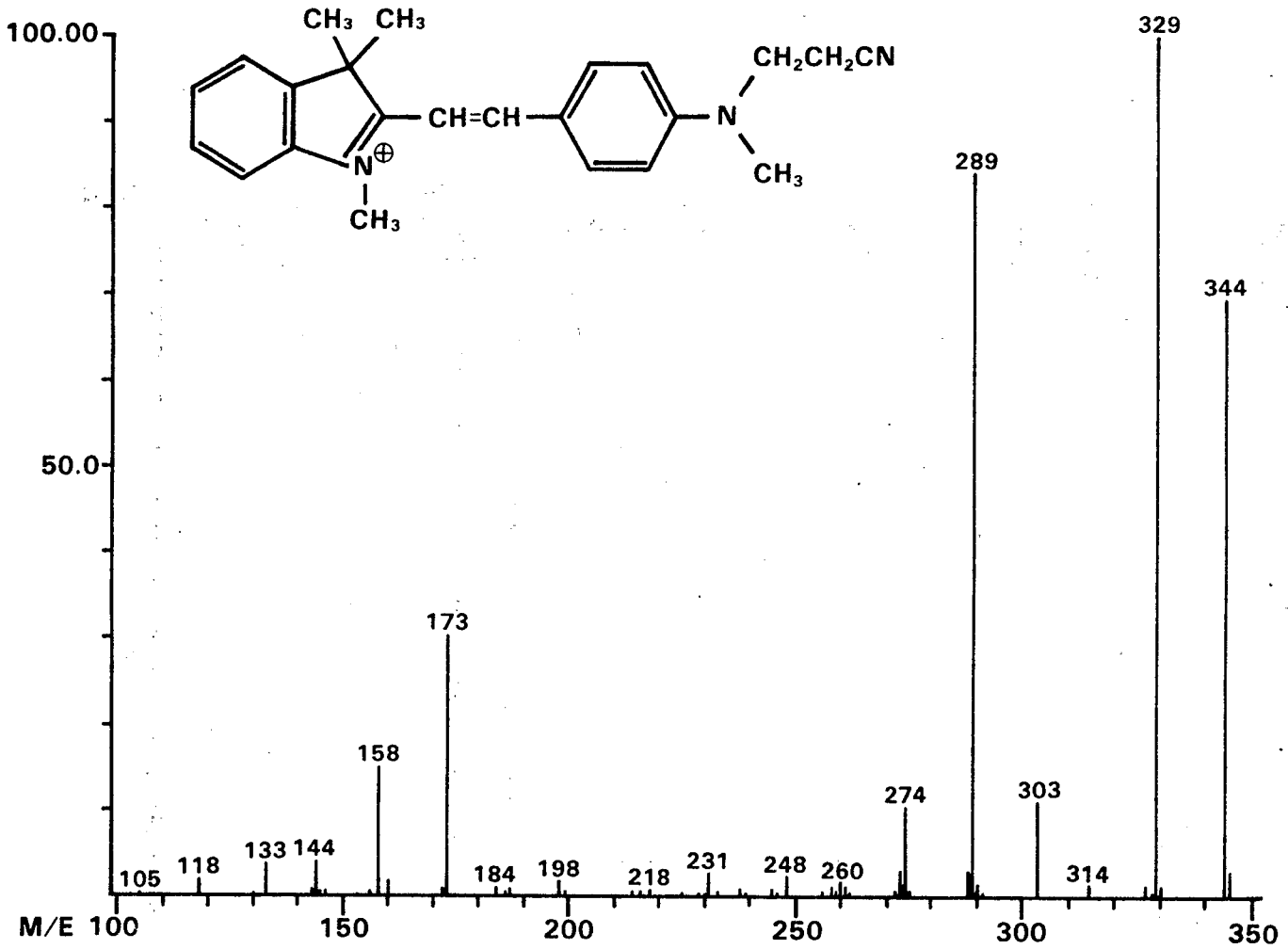
CH3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M/E 100
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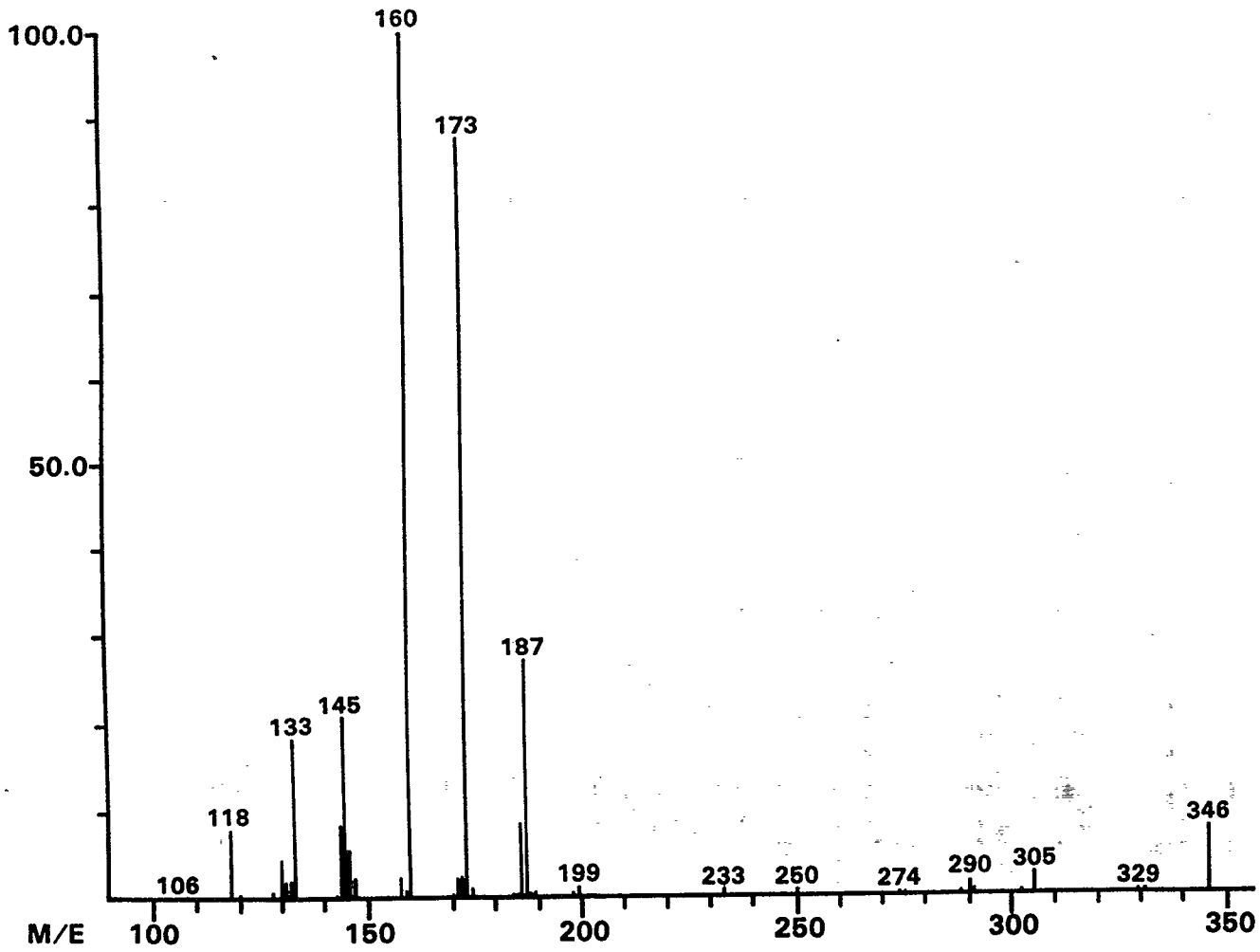
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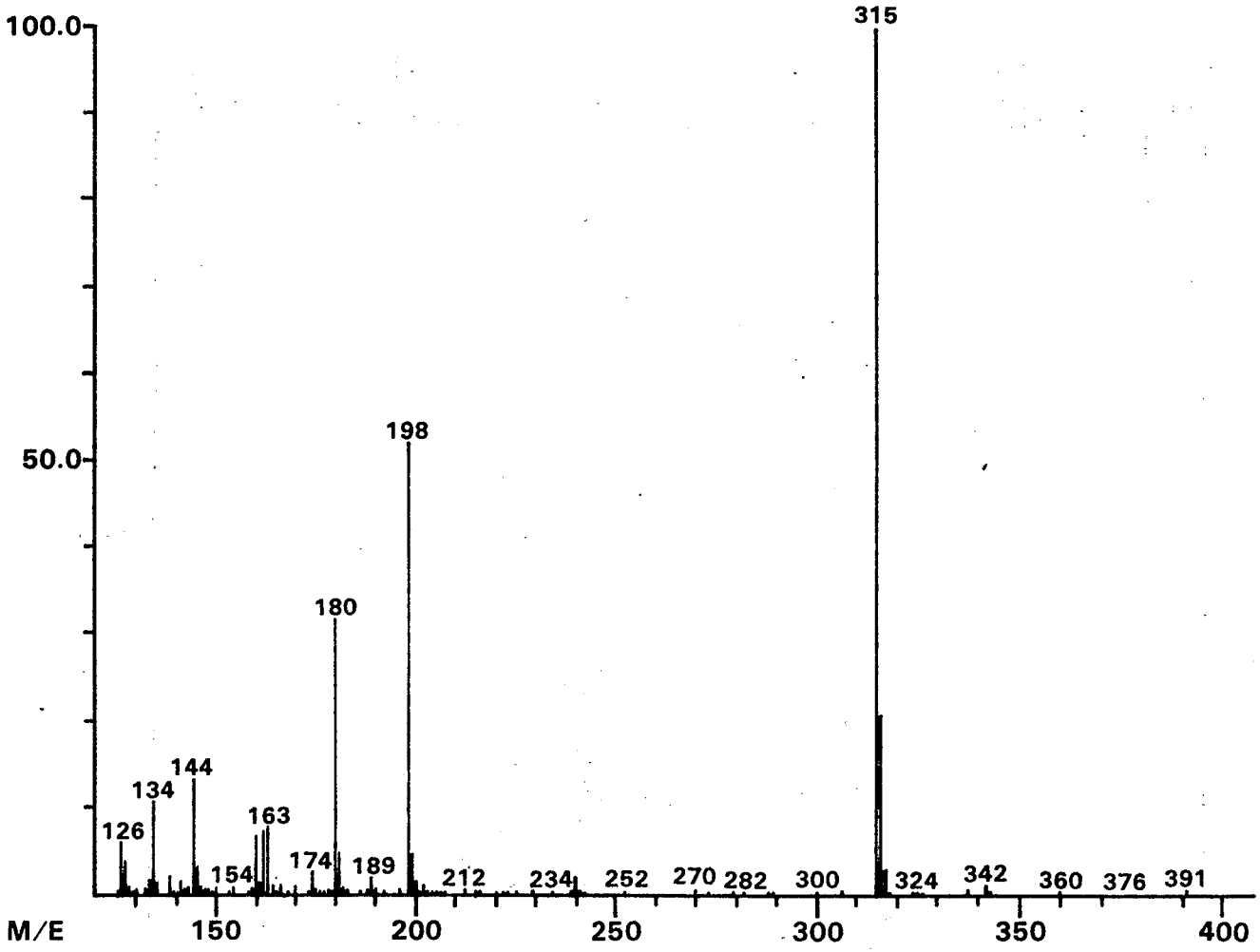
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376
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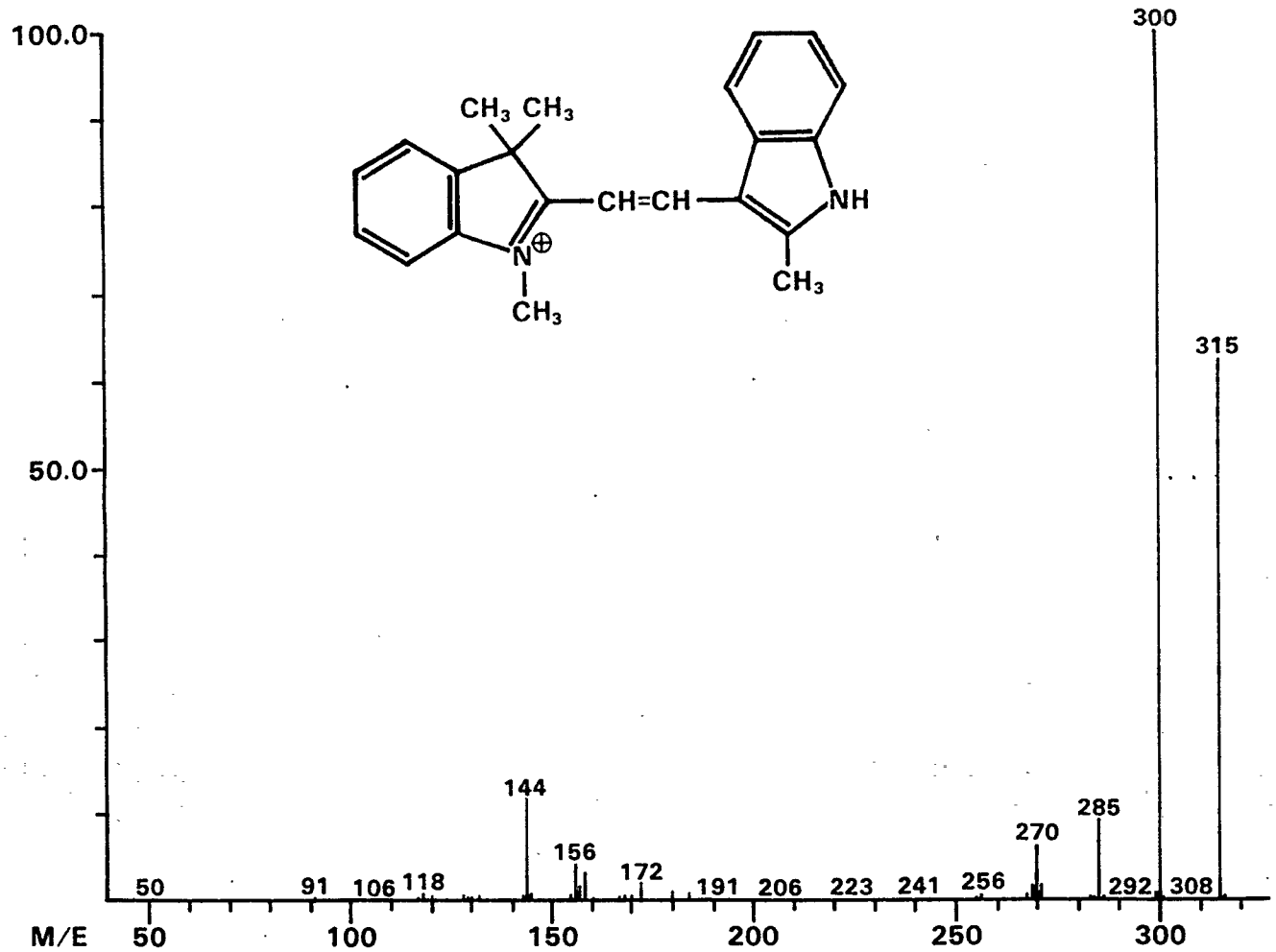
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M/E
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CH=CH
NH
N⊕
CH3
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300
315
144
156
172
285
270
50
91
106
118
191
206
223
241
256
292
308

MR. TELLIARD: Our next speaker is Walt Shackelford from our Athens laboratory. He is going to talk about problem solving and since he's from R&D, this ought to be novel. Walter.

WALTER M. SHACKELFORD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY-ATHENS

PROBLEM SOLVING WITH MASS SPECTROMETRY AND FTIR

MR. SHACKELFORD: Bill, I'm glad you put us back in R&D. You had us in your division just a few minutes ago, and a few long minutes ago, I'll tell you.

What I'd like to talk about today is essentially an extension of work that we've carried on in Athens for the past several years. It has to do primarily with understanding or characterizing industrial effluents. This sort of work has gone on in Athens for at least the last 12 years. Initially, industrial effluents were studied one plant at a time, and work was done with kraft paper mills, organophosphorus pesticide manufacturing and in textile dye works.

With the advent of the consent decree, industrial wastewater studies took on a much different profile in that a nationwide study was undertaken to look at a specific group of compounds in 21 industrial classes plus the publicly-owned

treatment works.

Our contribution to that in Athens has been, first of all, to make use of our experience in earlier work and help determine simply through data that we had in data base management systems, which compounds among the priority pollutants were worthy of study in resolving the original court list of compound classes. Since then, we have aided the Effluent Guidelines Division by matching spectra in reference libraries to EGD contractor's GC/mass spec analyses, to determine what compounds other than priority pollutants might be in industrial wastewaters that at some later time might become important.

The third part of this study has been to try to determine some of those compounds that were not determined by spectrum matching and to perhaps see the problems or design a protocol for identifying unknown compounds in some sort of efficient manner. I'm sure that if we checked in the audience, everyone would have a good idea about how to get an identification of a compound for which there was no standard nor any reference spectrum, and probably you'd come up with something very similar to what we came up with. If you look in the

literature, that's exactly what you'll see. However, in making this efficient, we seemed to have had to learn by trial and error.

Before I go any further, I would like to acknowledge that some of the data that will be presented here was acquired at Research Triangle Institute. Dr. Joan Bursey was the project officer. The GC high resolution mass spec work was done by Mike Harvey, at that time at Harvey Laboratories. I would also like to acknowledge the influence of Leo Azarraga at our laboratory, who first made a good interface for a light pipe, making GC/FTIR a possibility; and John McGuire and Don Betowski who off and on worked on one of the unknowns I will show you, for several years.

SLIDE 1

This is an overall picture of the frequency of occurrence of the most frequently occurring 50 compounds that we saw in our computer survey of the screening phase of the Effluent Guidelines work. As you notice here, the priority pollutants are certainly some of the most frequently occurring compounds. However, if you'll look again, you'll see--and I'll do this for you--that only 12 out of the first 50 compounds are priority pollutants.

This has something to say about the need, perhaps, for surveying and characterizing industrial effluents beyond just looking at priority pollutants. I don't think it's any surprise to anyone that's done any analysis that priority pollutants aren't the whole story.

SLIDE 2

This second slide gives you an indication of just how many possible compounds are there that we don't identify. In our data base management system, we logged approximately 3,000 spectra that we believe we observed five times or more within a tenth of a retention time unit. What this means is, at least as far as we're concerned, there are a lot more compounds that do not match our spectrum library than do. Of these, we chose some 55 for further study to try to identify them.

SLIDE 3

This brings us to the subject of the talk: problem solving with GC/MS and GC/FTIR. I recall here at this group in the last eight years we've had only one talk that concerned itself with GC/FTIR. I believe it was done by Jim Brasch of Battelle.

As we've seen from the earlier slides,

characterization of any sample is going to require identification of unknowns. Next, if spectrum-matching fails, barring any secondary information, we're going to have to do manual interpretation. The gist of this talk will be that the use of alternate instrumental techniques may lower your overall cost and may even increase your success rate. There are no promises here, but that seems like a reasonable tact to take.

SLIDE 4

To give you some of the limitations of spectrum-matching, in a study that was done by Bill Budde and Steve Heller of EPA several years ago, it was found that if you matched the compounds known to be manufactured in industry with the compounds in the EPA/NIH reference spectrum library, you'd find less than a 20 percent overlap. This means that there are a lot of compounds manufactured that we do not have reference spectra for. Also, if you total up the gas phase infrared spectra that are in libraries, you probably will not reach the 10,000 level. One thing to be said for the present collection of gas phase infrared spectra is that they are quite pertinent to water analyses. They were chosen for that reason.

Finally, and this is an important point as far as we're concerned, if you don't have some sort of corroborative data, your confidence levels for everything but the best spectrum match is going to be very poor.

SLIDE 5

I'd like to illustrate with this slide. This is some work we did in evaluating the efficiency of spectrum-matching. The bottom curve here is data that we took from the literature. It came out of Fred McLafferty's laboratory at Cornell. The X axis here is the match quality increasing, and along the Y axis, the number of hits that were confirmed. As you can see, at low levels of match quality, we have low levels of confidence and if we have a high degree of match quality, we're going to see something on the order of 80 percent confidence in our match.

The two upper traces, however, involve data that we collected in Athens. This data all has retention time corroboration. Not only did the spectrum match, but the retention data matched with that of our data base management system. As you see, even down for very low levels of K, we're talking about something on the order of 60 percent

confidence we could have in a match.

This point is even further confirmed when you see the diversion of these two curves. The bottom curve is for all compounds and the top curve is that of the compounds except carboxylic acids. When we first plotted the data and saw the downward curve, we felt like there was something wrong.

It turns out that our retention data for carboxylic acids of chain length greater than 14--this is all packed column data--was so imprecise, that we really were only guessing as to whether or not a carboxylic acid was within a two carbon length. The mass spectra of all these are fairly close. When we take the carboxylic acids out, we're left with essentially the same sort of confidence at the high end of matching parameters as the earlier study in McLafferty's lab.

If our spectrum-matching program hasn't helped us, the next thing we probably will try is spectrum interpretation. The only problem is that in most laboratories, this is going to be very expensive because it requires time from your most expert people. I realize that in some industries, especially where there's a lot of synthesis being done,

it probably is cost effective to keep one or more people in the laboratory who do interpretation regularly. In most of our laboratories doing water analysis, however, we're so busy trying to get the samples through that we really can't spare any of this expert help to sit down and interpret spectra. Again, unless there's some confirming evidence, the confidence levels even for a spectrum interpretation, are going to be fairly low.

The solution to this cost problem, to us at least, seems to target for identification only those compounds that are deemed to be high priority. Another thing is to build a data base management system of identifications for later reference. Our experience has been that a local library or a historical library, comes into play very, very often in determining the identification of compounds.

Another solution is to acquire data from more than one independent technique. This may be, in the long run, cheaper than having a person on board to do interpretation. Our experience was that we could contract out high resolution mass spec runs, for instance, much cheaper than we could buy a high resolution mass spectrometer for ourselves.

The criterion we use to determine whether or not a compound is of high priority is frequency of occurrence. Again, you're not going to know that if you don't keep your local data base and record compounds that you have identified. The second criterion would be high concentration. This is a type situation that would cause most perturbation to the environment. Finally, does the compound have an adverse effect on the system. This is the case where a compound may only occur once and there might not be much of it, but it may cause easily identified problems in the environment. In this case, you've got to be there with an identification.

At the present time, we have been working with the Industrial Technology Division on a case of an industrial effluent that had an abnormally high COD but yet the effluent was passing all of the priority pollutant standards. This involves a series of compounds of very high concentration but found only in one effluent. They're not occurring with great frequency. They are in high concentration and they certainly have an adverse affect on the COD from that particular industrial effluent. That work, incidentally, will be reported on later.

The criteria for compounds that we chose for study all had a frequency of occurrence greater than five. This was an arbitrary cutoff but one that we felt, from our experience, gave us enough retention data corroboration that we felt like we indeed had a compound present. The apparent concentration had to be greater than 50 parts per billion. The apparent concentration was determined by comparing a response of the unknown with the internal standard. Now, since we didn't have a standard for the unknowns, the apparent concentrations could be off by as much as a factor of 100 from the actual concentration. You can even look at the priority pollutant phenols and see that much variation in response factor.

Finally, as far as effects on an environmental system, since these compounds were found in industrial effluents, their treatability is in question.

The additional techniques that we believe to be important to get a determination were high resolution GC/high resolution mass spec to develop a possible chemical formula; chemical ionization mass spec in both positive and negative ion mode for molecular weight confirmation, when that might be needed; and finally, GC/FTIR for functional

group determination.

SLIDE 6

This is a flow chart that we worked out for identification of unknowns. The first step is to rerun the sample extract using fused silica capillary column GC/MS and check again for spectrum match. In the case that we might have had two closely eluting compounds that in none of the samples that we looked at were ever resolved, we would see the sum of those two spectra consistently. So, if we could separate them by a fused silica capillary column, then of course we could separate them into their components rather than trying to waste our time determining a mixture.

If indeed we did find a spectrum match due to our greater resolution, we then checked with our data base management system to see if we have found anything like that before. If we have, and if it matches the characteristics of something that is already in our data base, we then look for the standard to try to confirm the identification. That did not happen but twice in the ones we looked at. If there's no spectrum match or if the data base management system does not bring up a match for that compound, we then have to decide on

additional data needed.

Some of the inputs to this are whether one has a good idea if the molecular ion is present in the sample for that particular compound, how closely related are compounds that we found in the spectrum matching program, and finally, do we think that we really need to go the expense of high resolution mass spec to try to determine that molecular formula. If indeed the case is that we need all of them, then we get all that data, try to propose a structure, and get a standard.

SLIDE 7

Our confirmation criteria was that the retention with a co-injected standard must be identical on the capillary column and the mass spectrum identical with that of the standard. Also, if one had FTIR available, you would certainly want to get spectral confirmation there as well.

SLIDE 8

This is the first example of one compound that was identified. This is a good example of why not to trust your spectrum matching program or data base unless you have some confirming information along with it. If you take a look at this spectrum, we have a peak at 199 and then a loss of 15 and a

loss of 44. Also we have a big peak at 91, indicating that perhaps we have alkyl group on a phenyl ring. We did not get a match in the reference library, although the library indicated that there were compounds of similar spectrum that were alkylphenylsulfonamides.

SLIDE 9

The first thing to do was check and see if we indeed had the correct molecular ion. We ran isobutane CI for this and found the pseudo-molecular molecular ion at 200. Thus, we felt like we had the correct molecular weight. Then comparing the actual spectra that are listed in the EPA/NIH library, we found that the two sulfonamides which are actually isomeric with this compound, had very poor spectra. As a matter of fact, they only had six peaks in the whole spectrum. So the matching program could not generate a good answer. Nevertheless, when we checked the retention time of one of those compounds we were able to get as a standard, it was incorrect. We believed this to be a different isomer from those listed in the reference.

SLIDE 10

The second unknown that I'd like to go through

that illustrates some of our points is this compound. It had a molecular weight of 164, which was confirmed by chemical ionization. This compound was not only seen in the effluent guidelines study but was seen in an effluent study some three or four years before that. In that study, we got GC/FTIR data and made what we thought to be a reasonable determination of the structure. It turns out, after getting high resolution data, that we were completely wrong in the earlier study.

SLIDE 11

The next step, after determining our highest mass was indeed the molecular weight, was to get high resolution data. Of course, the computer will calculate every possible formula that it can, given the elements. The more elements you give it, the more possibilities it can come up with. But if you look at this one closely and have the benefit of examining the spectrum closely, you'll see that these two that have sulfur in them are probably incorrect because we don't observe any sulfur isotopic peaks. These two that both contain fluorine were later eliminated with the FTIR data. This one with phosphorus is fine, but you can't

find an analogue to it in any of the daughter ions down at 149 or 131. It turns out that our proposed molecular formula for that is C10H12O2. We took our infrared data, compared it to the infrared library and there was no exact match for it. Nevertheless, a close match, or one that seemed to be close to us, was a dihydrobenzofuran.

SLIDE 12, SLIDE 13

Take a look at the actual FTIR spectrum of the unknown. You can see similarities in the region at and in the region at. The region has some extra alkyl character that we felt belonged in an ethyl group which, as you look at the mass spectrum, you see the loss of an M -29. This peak, at about 3,600 wavenumbers, is due to the OH stretch, but the OH, to give a sharp peak like this in the gas phase, cannot be intramolecularly bonded. That is why we have decided the oxygen would be on the opposite side of the ring from the hydroxyl group.

We were unable to find a standard to confirm this compound. In a later edition of the NIH/EPA spectrum library, there is an isomer of this compound. It is a dimethyl isomer rather than being the ethyl, however, and it has the hydroxyl and

the oxygen in the furan ring adjacent. We believe this to be incompatible with the infrared spectrum showing the free hydroxyl group.

SLIDE 14

Here's a summary of some selected analysis results and to give you some idea of what I feel is the power of the added information from FTIR. The only unknown in this group that we were able to get infrared data on is one of two that we were able to assign a structure to. The high resolution MS data gave us the ability to come up a reasonable formula, but only with the FTIR were we able to assign a good structure.

As you can see from that table, we didn't get FTIR data on every unknown compound. One of the problems with FTIR is that only a small reference library is available. The lack of sensitivity, though, in our case, seemed to be our biggest problem. The way we were able to get around that in the few cases where we were successful with FTIR, was to use the Unicon sample concentrator as a front end for the GCIR. This way we were able to inject up to 50 microliter injections and thus increase by a factor of 10 to 50, the amount of injected material.

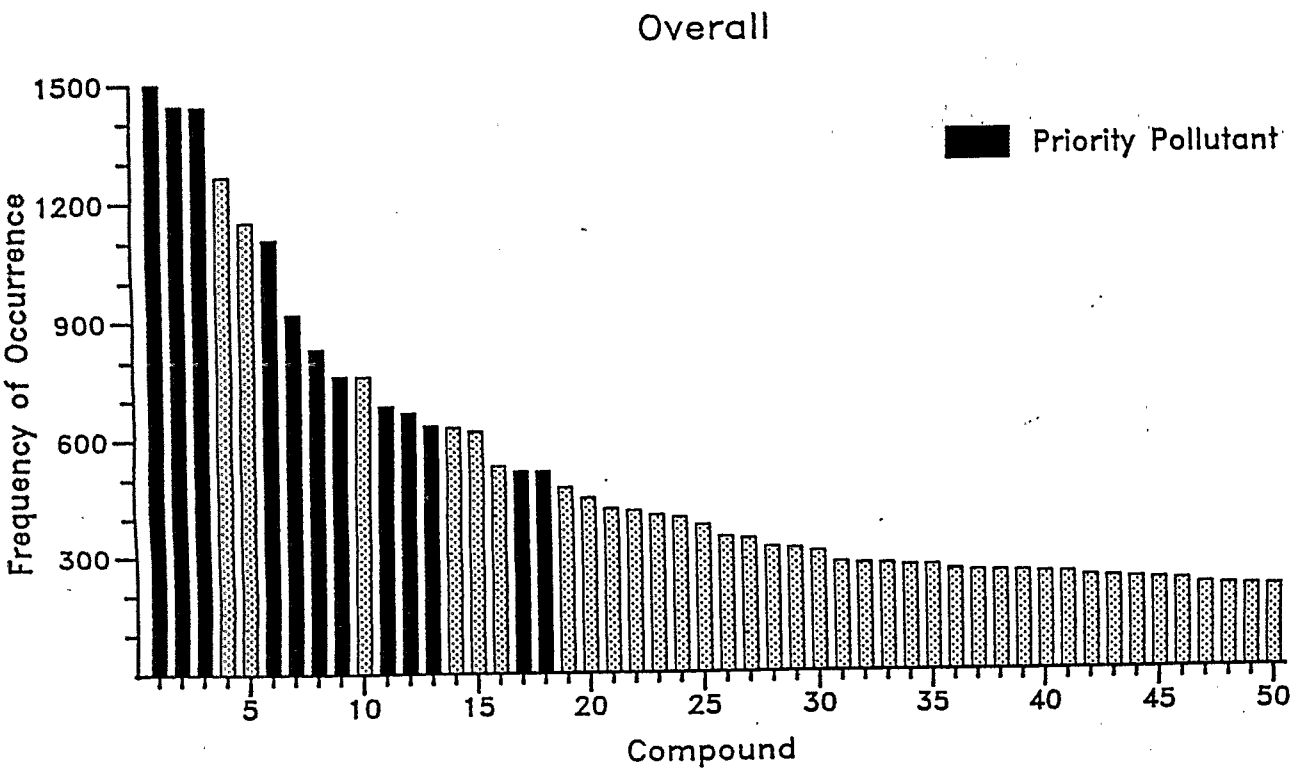
SLIDE 15

There are quite a few interesting references in the recent literature combining GC/mass spec with GC/FTIR and also some applications that I offer to you here. Interestingly enough, in all the recent applications of FTIR, you'll always see it either combined with GC/mass spec in the same instrument or at least the data combined with independently taken GC/mass spec data. The reason for this is that the two forms of data are so complementary that summed together, they form a much bigger total information package than taken separately.

Wilkens was really the first person to interface a GC/mass spec/FTIR system. He was at Nebraska at that time. He has since gone to UC Riverside and has built a GC FT/MS FTIR system. Richard Crawford at Lawrence Livermore had a paper in which he did identify in the same type scheme we're talking about here, some unknown compounds using GC/mass spec and GC/FTIR. Gurka and Betowski did some work with packed column GC/mass spec, packed column GC/FTIR and then later, Shafer and coworkers at Battelle did the same samples using capillary columns. In that work they found FTIR

to be superior to mass spec in identifying compounds. However, the results are somewhat skewed since they were looking at alkyl benzenes in which IR, of course, is much stronger confirmatory tool than mass spec would be. Finally, the report from which some of this data is taken, Joan Bursey of RTI in the EPA report dated 1984.

MR. TELLIARD: Questions?


Overall
Priority Pollutant
Frequency of Occurrence
1500
1200
900
600
300
5
10
15
20
25
30
35
40
45
50
Compound

UNKNOWN COMPOUNDS

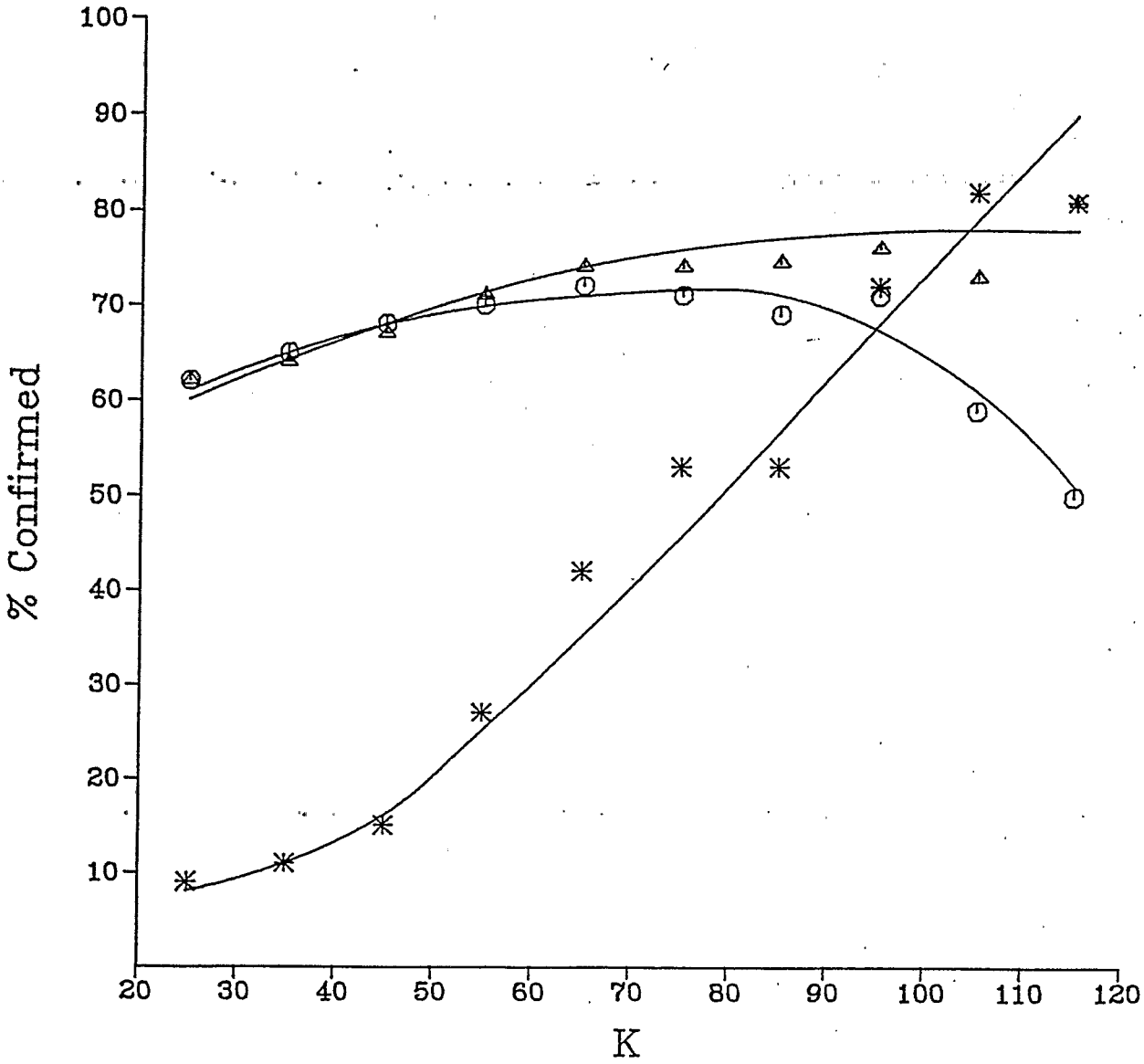
- ~ 3000 SPECTRA SEEN 5 OR MORE TIMES WITHIN ±0.1 RRT UNIT
- 55 CHOSEN FOR FURTHER STUDY

PROBLEM SOLVING WITH GC/MS AND GC/FTIR

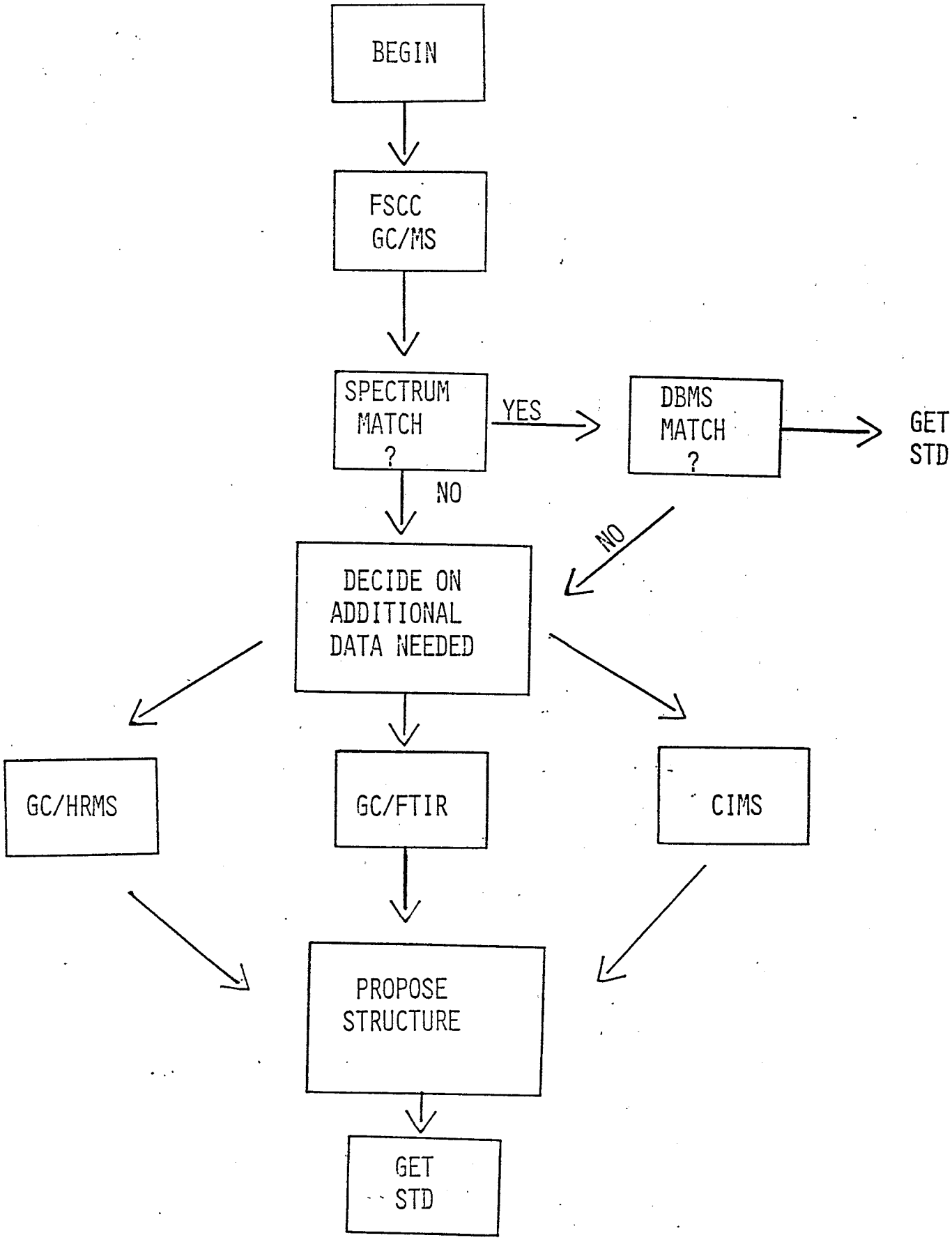
- 0 CHARACTERIZATION OF A SAMPLE REQUIRES THE IDENTIFICATION UNKNOWN COMPOUNDS.
- 0 IF SPECTRUM MATCHING FAILS, MANUAL INTERPRETATION IS REQUIRED.
- 0 THE USE OF ALTERNATE INSTRUMENTAL TECHNIQUES MAY LOWER OVERALL COSTS AND MAY INCREASE SUCCESS RATE.

SPECTRUM MATCHING LIMITATIONS

- 0 LESS THAN 20% OF MANUFACTURED COMPOUNDS ARE IN COLLECTIONS OF REFERENCE MASS SPECTRA (BUDDE AND HELLER).
- 0 ONLY ABOUT 10000 COMPOUNDS ARE IN COLLECTIONS OF GAS-PHASE IR SPECTRA.
- 0 WITHOUT CORROBORATIVE DATA, CONFIDENCE LEVELS FOR ALL BUT THE BEST SPECTRUM MATCHES IS POOR.



O - All compounds
Δ - Carboxylic acids deleted
* - Data of Atwater (16)


BEGIN
FSCC GC/MS
SPECTRUM MATCH ?
YES
DBMS MATCH ?
GET STD
NO
NO
DECIDE ON ADDITIONAL DATA NEEDED
GC/HRMS
GC/FTIR
CIMS
PROPOSE STRUCTURE
GET STD

CONFIRMATION CRITERIA

1. RETENTION WITH CO-INJECTED STANDARD IDENTICAL ON CAPILLARY COLUMN

2. MASS SPECTRUM IDENTICAL WITH STANDARD

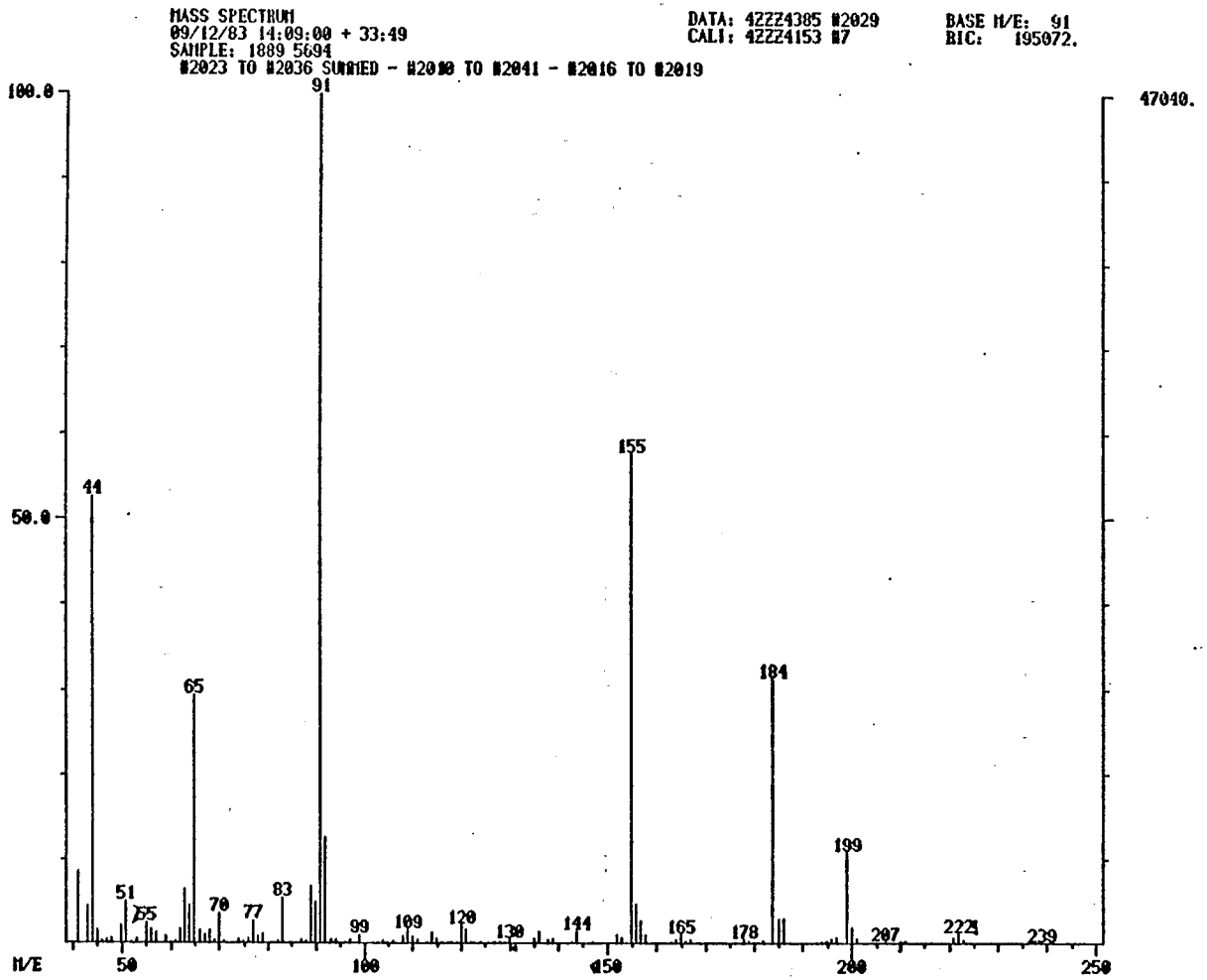


Figure B-84. Mass spectrum of EPA compound of interest, extract 5694 (base/neutral), organic chemicals industry, one isomer, scan #2029.

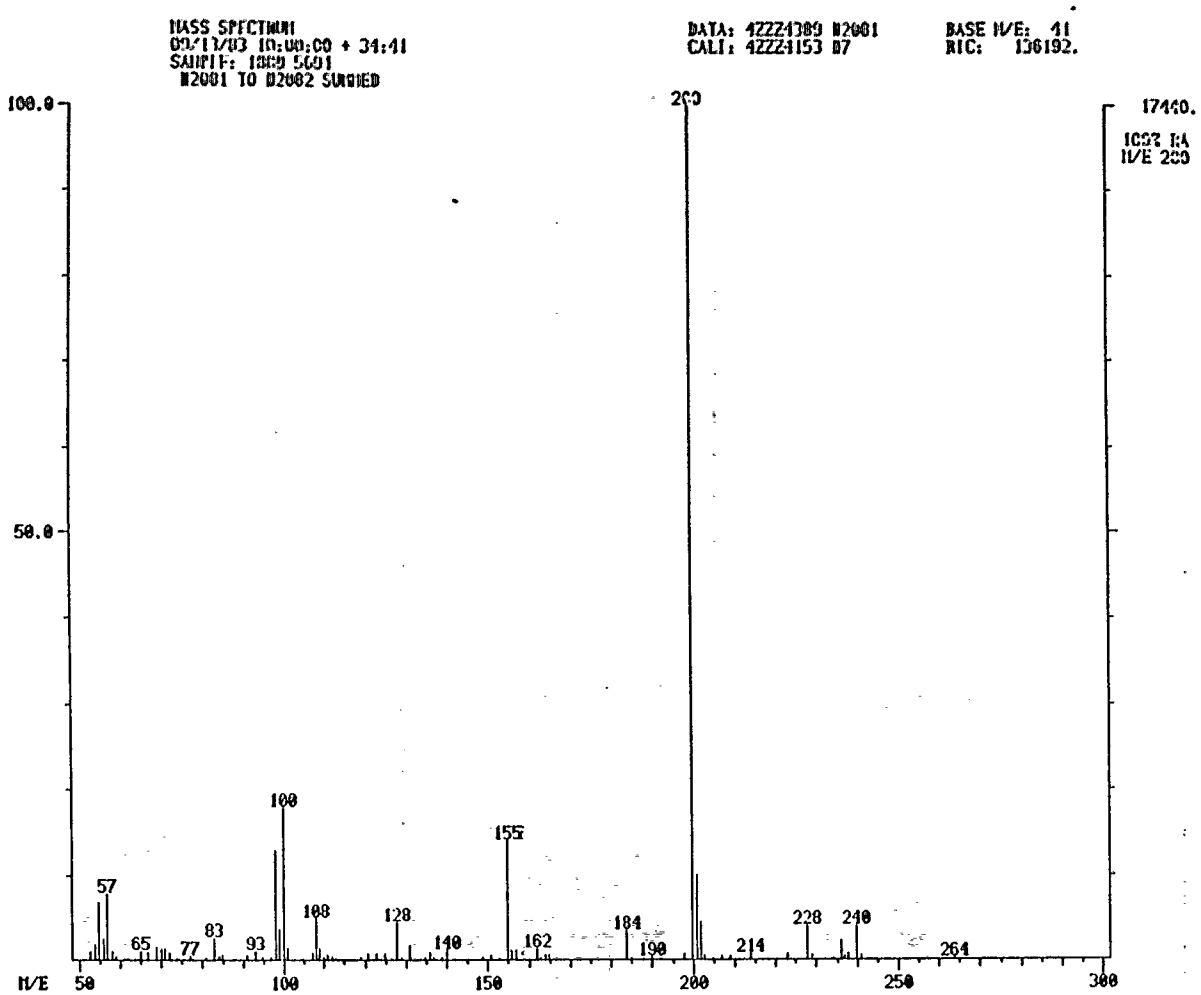


Figure B-85. Representative isobutane chemical ionization mass spectrum of EPA compound of interest, scan #2081.

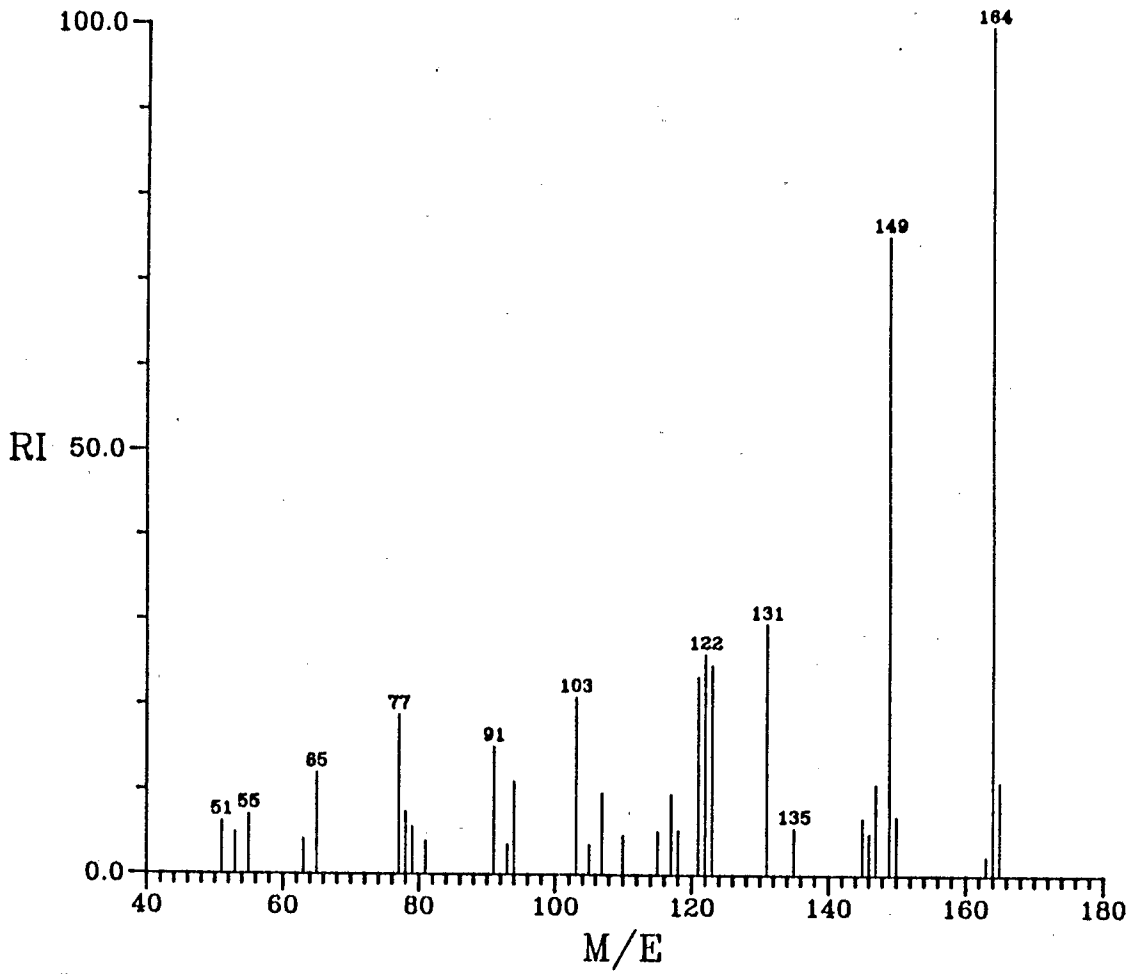


Figure 5.159. Mass spectrum of EPA compound of interest, extract 5855 (acid), pesticide manufacturing industry, scan #2400.

Figure 5.160. Accurate mass measurement data, extract 5855 (acid), pesticides manufacturing industry.

High Resolution Mass Data

M/E	C	H	^{16}O	F	^{32}S	P	MMU	OBS.MASS
164	10	12	2	0	0	0	−4.8	164.0885
	7	13	3	1	0	0	−3.6	
	8	11	0	3	0	0	−7.2	
	7	16	2	0	1	0	−1.4	
	7	17	2	0	0	1	8.1	
	7	17	0	0	1	1	−9.6	
149	9	9	2	0	0	0	3.9	149.0563
	6	10	3	1	0	0	5.1	
	7	8	0	3	0	0	1.5	
	6	13	2	0	1	0	7.3	
	4	12	0	3	1	0	4.9	
	9	10	0	0	0	1	−4.3	
	6	11	1	1	0	1	−3.2	
	6	14	0	0	1	1	−0.9	
	3	15	1	1	1	1	0.2	
131	9	7	1	0	0	0	1.1	131.0486
	6	8	2	1	0	0	2.2	
	4	7	0	4	0	0	−0.2	
	6	11	1	0	1	0	4.5	
	3	12	2	1	1	0	5.6	
	6	9	0	1	0	1	−6.0	
	3	10	1	2	0	1	−4.9	
	3	13	0	1	1	1	−2.6	

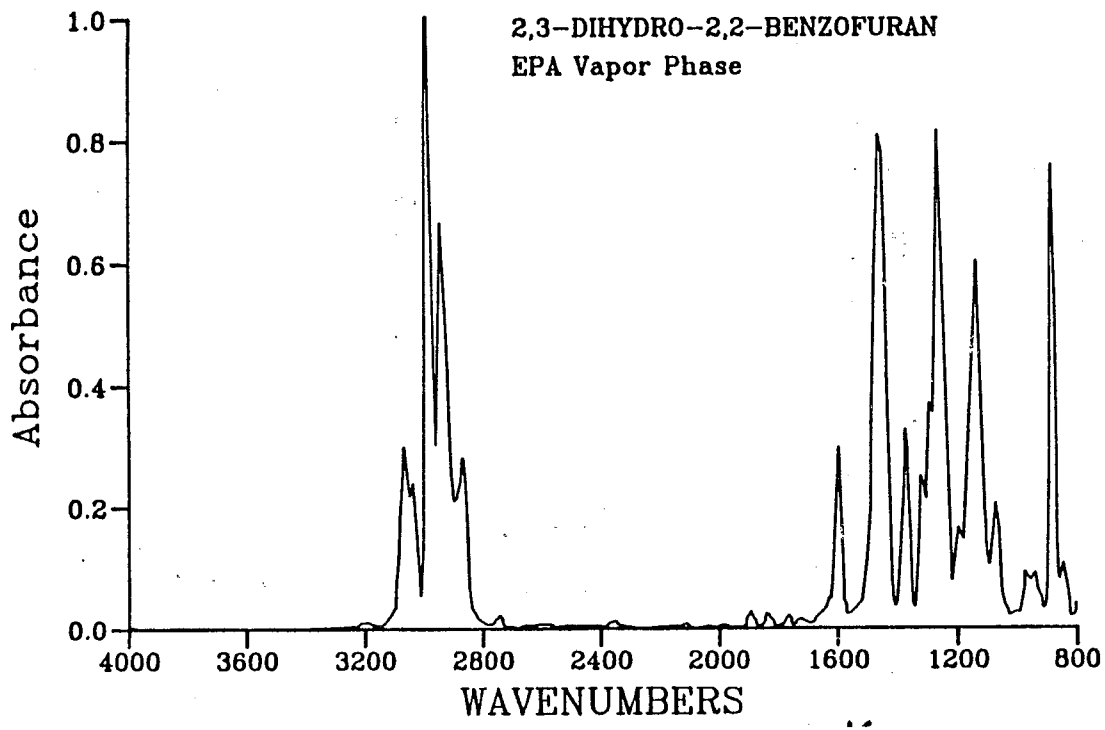


Figure 5.163. Standard EPA Vapor Phase IR Spectrum of 2,3,-dihydro-2,2-benzofuran.

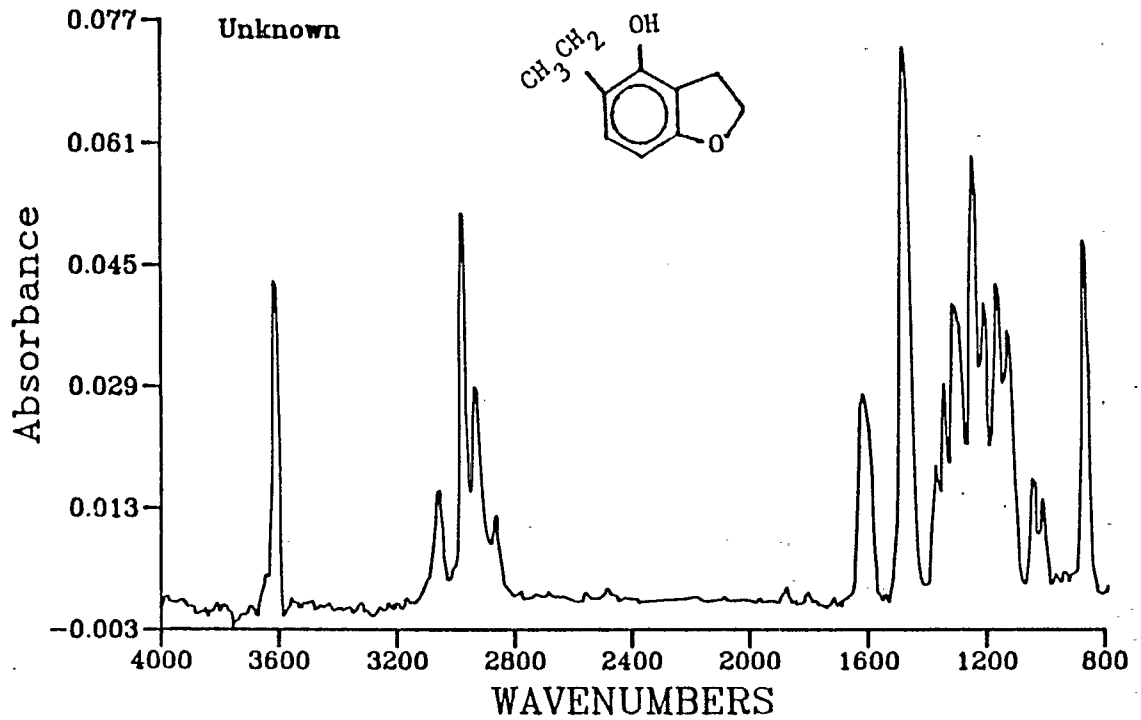
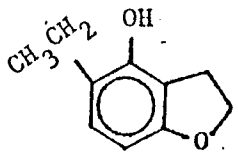


Figure 5.161. Unacon/capillary GC/FT-IR analysis of EPA compound of interest, extract 5855 (acid), pesticides manufacturing industry.

Table 2. Summary of Selected Analysis Results

Extract # (Fraction)	Industrial Category	Number of Occurrences	Postulated Formula(s)	Postulated Structure	Instrumental Techniques Used Successfully
584 (ACI)	Pulp and Paper	9	$C_8H_7O_3CL$ $C_5H_{11}SO_3Cl$	None	HRMS
7367 (B/N)	Amusements and Athletic Goods	10	$C_9H_{18}N_4O$	None	HRMS
13230 (ACI)	Mechanical Products	6	$C_9H_{19}Cl_2PO_2S$	None	HRMS, CI
19436 (ACI)	Plastics and Synthetics	9	$C_9H_{20}O_3$	None	HRMS
21258 (B/N)	Aluminum	5	$C_{16}H_{16}O_2$ $C_{15}H_{16}ON_2$ $C_{11}H_{16}O_4N_2$	None	HRMS
5855 (ACI)	Pesticides	5	$C_{10}H_{12}O_2$		HRMS, FT-IR
11338 (ACI)	Pesticides	29	$C_6H_4O_3F_2$	None	HRMS
11536 (B/N)	Inorganic Chemicals	5	None	None	
18959 (ACI)	Pulp and Paper	5	$C_8H_{16}O_4$	$CH_3(CH_2)_2CH(OH)-O-(CH_2)_3CO_2H$	HRMS, CI
20250 (BN)	Explosives	16	$C_{15}H_{24}O_4$	None	HRMS

REFERENCES

A. COMBINING GC/MS WITH GC/FTIR

CHARLES WILKINS, ET AL, UC RIVERSIDE, ANALYTICAL CHEMISTRY, 1981, 113; 1982, 2260; 1984, 1163.

RICHARD CRAWFORD, LAWRENCE LIVERMORE, ANALYTICAL CHEMISTRY, 1982, 817.

B. APPLICATIONS

DON GURKA AND DON BETOWSKI, EMSL-LV, ANALYTICAL CHEMISTRY, 1982, 1819.

KEN SHAFER, ET AL, BATTELLE COLUMBUS, ANALYTICAL CHEMISTRY, 1984, 237.

JOAN BURSEY, RTI, EPA-600/S4-84-072, 1984.

QUESTION AND ANSWER SESSION

MR. GORE: Bill Gore, American Cyanamid. There have been some recent reports of dramatically improving FTIR's sensitivity and resolution with cryogenic trapping techniques. Have you had a chance to do some experiments there and do you have any experience to draw on or comment?

MR. SHACKELFORD: No, I have no experience whatsoever with cryogenic trapping. I would say that the sensitivity that has been reported in the literature has been much better than what we've experienced, but it could be that in many of our cases we're talking about a small peak and a large background and that is perhaps interference rather than sensitivity problem.

MR. YOUNG: I'm Jim Young from the University of Arkansas. A few years ago we were working with complex samples, or samples of complex mixtures, and we found that the relative retention time of a compound would shift if you pass a sample through treatment so that you removed a lot of the junk. Did you find this to be a situation in your samples? I think you mentioned that you used relative retention time as a key

identifier, but it was a problem for us because it would shift significantly with treatment.

MR. SHACKELFORD: One of the things that we did do was to go through some cleanup stages to try to get a clean peak to go to the GC/FTIR and yes, after cleanup especially, when we're talking about same compound but a much different matrix injected, we did have significant changes in the relative retention times.

MR. YOUNG: Do you see that as a major problem in using this particular confirmation technique?

MR. SHACKELFORD: In the way we use it, no, because our samples in our data base management system would be kept separate from that of any later run acquired under different conditions.

DR. LESAGE: Suzanne Lesage, Environment Canada. You're talking about your data base management system. Is there any way of sharing with the world this kind of information? I think everybody doing industrial work has the same problem of the unknown spectrum and maybe the MBS library should contain unknowns rather than knowns.

MR. SHACKELFORD: Our system is built on a commercially available data base management system called Inform. That's not the most up-to-date data base management system. One of our problems is that so much of our data is packed column. Fortunately, there seems to be a consensus now on fused silica capillaries and perhaps in building a new data base, that would be more appropriate. But I certainly do think it would be worthwhile to perhaps discuss that, but I don't know that you would ever be able to get some kind of uniform system that everyone would feel like was appropriate for their own laboratory.

DR. ERICKSON: Mitch Erickson, Midwest Research. A kind of follow-up to the first question. Walt, as you know from this study, the throughput on GCIR tends to be maybe half to less, maybe a quarter, of the throughput you see on GCMS just because the data systems aren't nearly as facile as what we're experiencing on GCMS. With the interface on the cryogenic trapping, my understanding from their salespeople is that the throughput goes way down. The second problem with trying it out is that the interface alone is $115,000, so you have to want to invest in it.

MR. SHACKELFORD: If Bill had not moved us back into R&D, we'd probably have that kind of money, but...

DR. ERICKSON: I can write you a proposal.

MR. TELLIARD: Thank you, gentlemen. It's time for our school break. There's cookies and milk in the hallway. We're running late, as usual, so to get to the HMS Sinkfast, would you please get your cookie and so forth and get back in here in the allotted time? Thank you.

(WHEREUPON, a break was taken.)

MR. TELLIARD: Our third speaker, due to the departure of the HMS Sinkfast, will open tomorrow morning's session and in so doing, we'd like to start 15 minutes early which is a quarter to nine for those of you who can tell time.

One quick announcement. The HMS Sinkfast will be located at Waterside, which is down near Phillips, and be ready to leave at about 5 o'clock, plus or minus a couple of minutes. We will then break here. It's definitely a dress-down affair. The issuance of life rafts and so forth will then take place and you can then get on the boat. The best way to get to the boat would be to go downstairs either through the lobby bar or around behind it and out by the swimming pool and then just walk down the side. Captain John will be there. He'll be the derelict standing almost in front of the boat.

Now, to get our afternoon session going, I'd like to have Samuel To open with his presentation.

SAMUEL TO

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER ENFORCEMENT AND PERMITS

PROGRESS REPORT ON DMR QA STUDIES: QUALITY ASSURANCE PROGRAM FOR NPDES SELF-MONITORING

(Revised presentation submitted.)

Progress Report on DMR QA Studies
A Quality Assurance Program for NPDES Self-Monitoring Data

Samuel To

Municipal and industrial wastewater treatment facilities are regulated under the NPDES as mandated by the Clean Water Act. Each direct discharger is usually regulated by a unique NPDES permit, which specifies certain limits on pollutants in the discharge. The permittees are required to routinely sample and analyze their discharges and report the data in Discharge Monitoring Reports (DMR). The validity of the NPDES Program hinges on the quality of these DMR's.

Description of the Program

Through EMSL-Cincinnati, the Office of Water Enforcement and Permits has been conducting a Quality Assurance (QA) program to assure the quality of NPDES self-monitoring data. The program is designed to evaluate the major NPDES permittee laboratories' ability to analyze and report accurate NPDES self-monitoring data.

In 1979, DMR QA pilot studies were conducted in two States. Responses were good. As a result, a national study was initiated to include all 7500 major permittees. Since 1980, four national studies have been completed.

Major permittees under NPDES are sent performance evaluation samples containing constituents normally found in industrial and municipal wastewaters. The samples are then anlayzed using the methods employed for reporting NPDES self-monitoring data. Responding permittees subsequently receive an evaluation of their data, and are given guidance for checking error sources, advice for taking voluntary remedial action, and requests to communicate corrective actions in writing to the State for EPA Regional QA Coordinators.

Evaluation of Data Quality

This program has provided valuable data in assessing the quality of DMR's. As illustrated in Figures 1 and 2, improvements in the DMR QA data had been significant.

Since permittees in each study were largely the same, we can conclude that the quality of NPDES self-monitoring data has improved. Data analyses at the permittee level also made possible the identification of about 3000 permittees that have participated in all four studies. This group showed slightly higher success rates than the general population. (Figures 3 and 4)

Identification of the Standard Industrial Classification (SIC) code made possible the tracking of improvements by individual industries. Tables 1 and 2 summarizes the performance of major industries (i.e., with over 10 permittees in Study 4).

Other Uses of the Data

Besides measuring the quality of DMR data, this program also encourage of proper OA procedures. When permittees receive evaluation reports with unacceptable data, they are asked to check for sources for errors. An examination of follow-up information indicated that a substantial portions of errors were due to data management problems, such as transcription, calculation, erroneous units, or misplaced decimals. In Study 2, almost half of the errors were of this type. (Figure 5)

Understanding the source of errors is the first step to improvement. Data management errors are relatively easy to correct compared to analytical problems. It is, however, a very important, yet often neglected, part of a QA program. Such errors can be minimized by instituting proper data handling procedures. A comparison of sources of errors between (Figure 5) studies confirms this, as there was a much smaller percentage of data management errors in Study 3.

The DMR OA data were also useful in planning inspections and directing other follow-up. Since there are a large number of permittees, this program enables EPA and States to concentrate corrective actions on permittees with more needs. This results in increase efficiency of NPDES compliance monitoring.

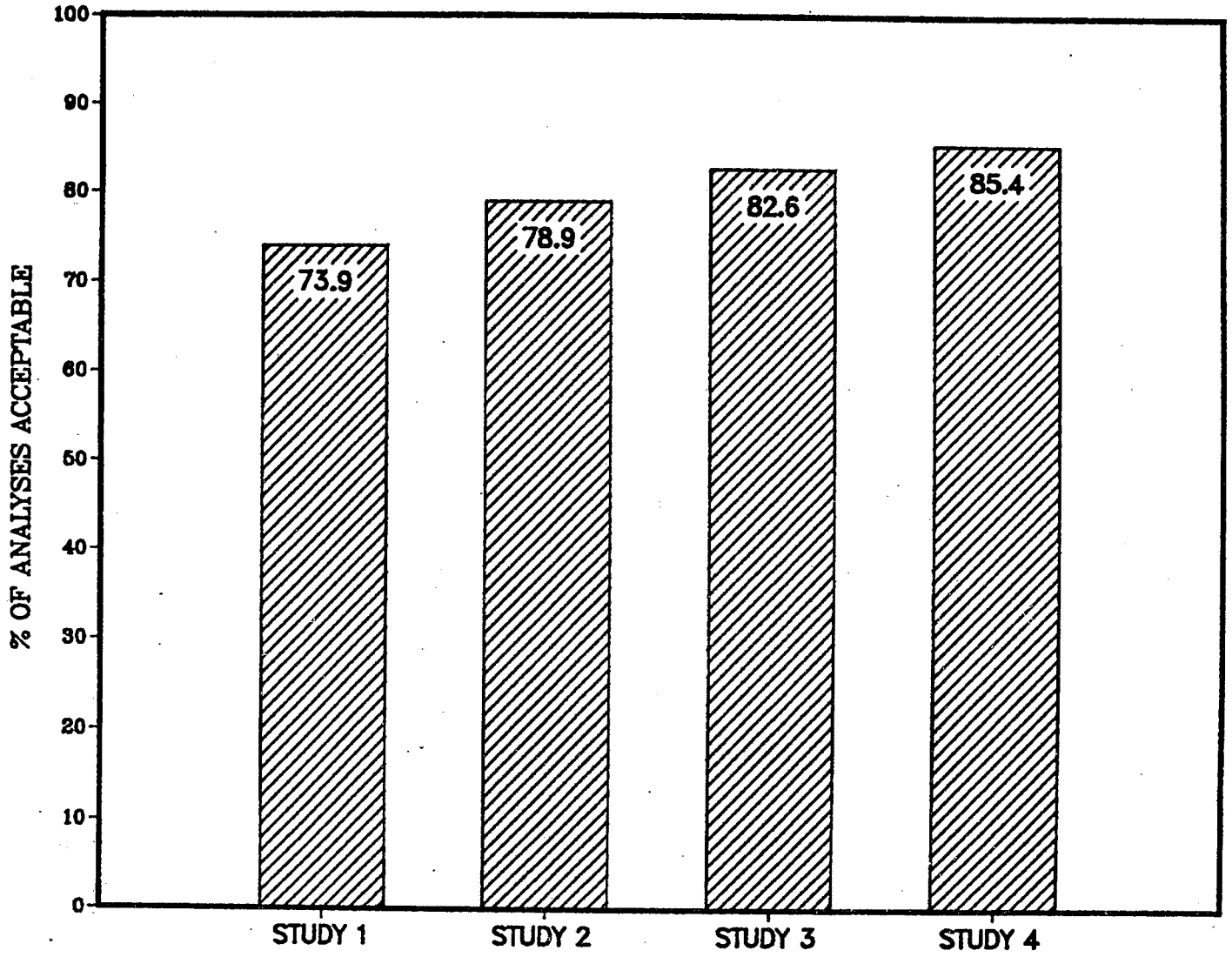


FIGURE 1 --PERCENT OF DMR QA ANALYSES ACCEPTABLE

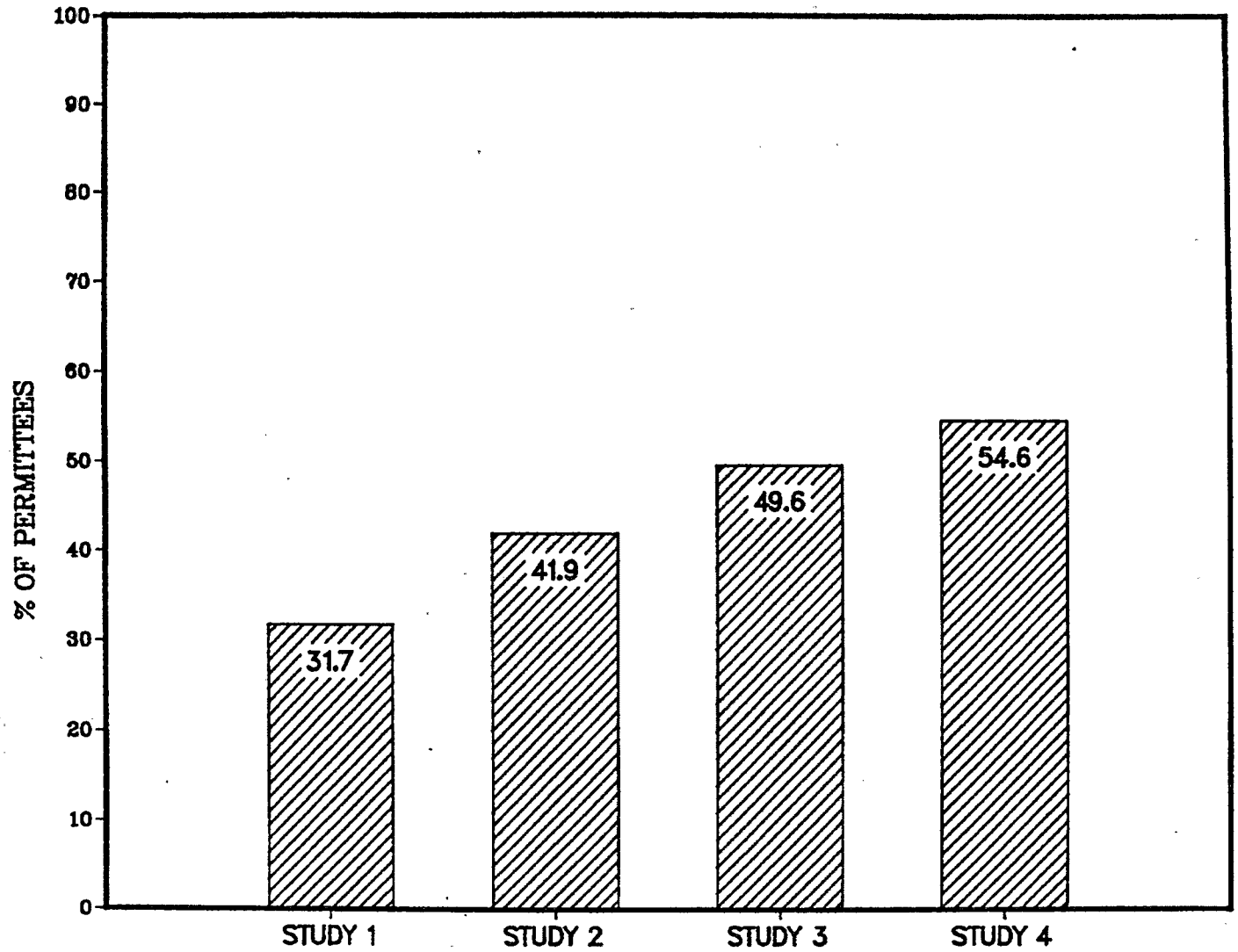


FIGURE 2 --PERCENT OF PERMITTEES WITH ALL DATA ACCEPTABLE

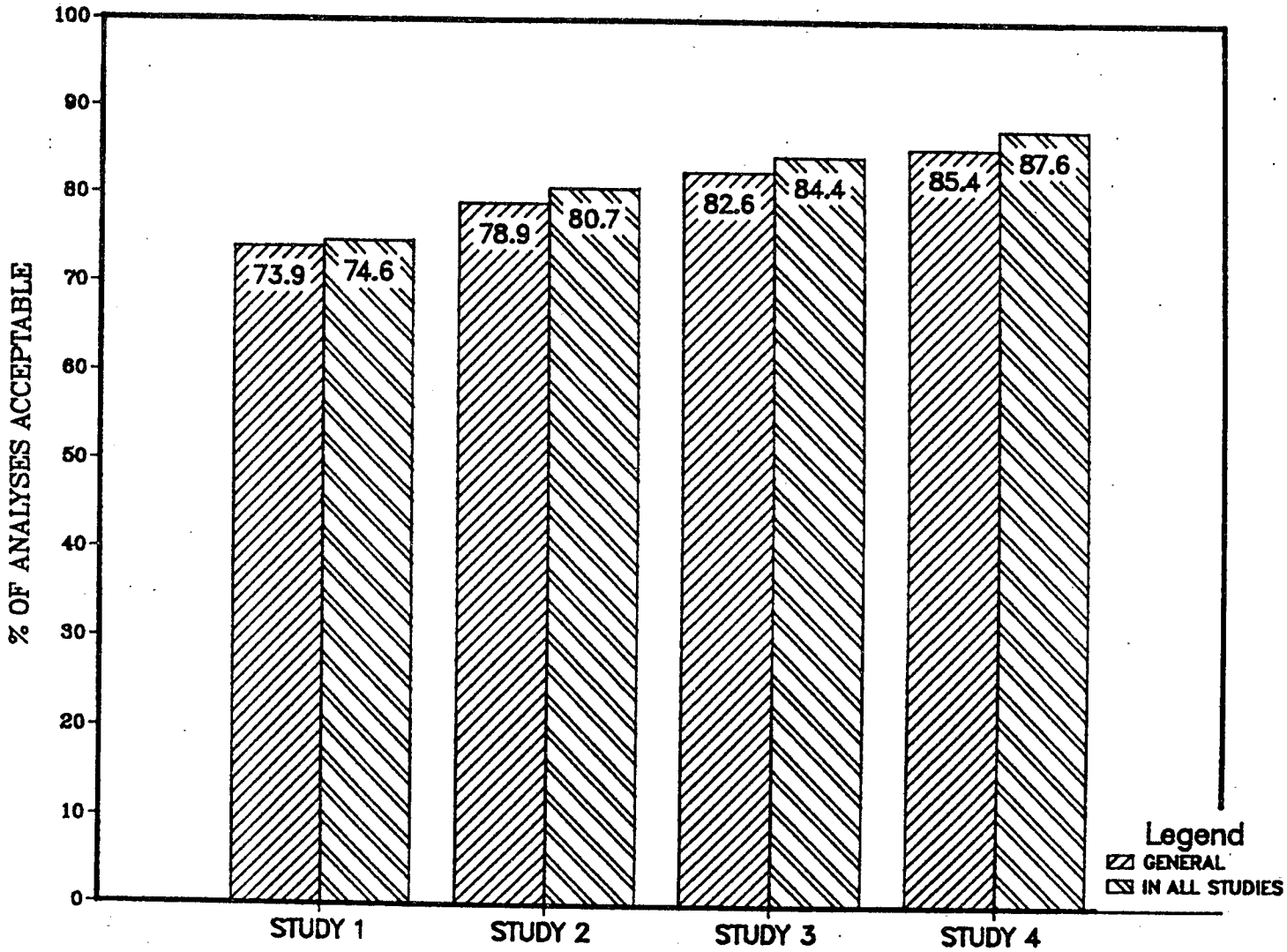


FIGURE 3--PERCENT OF DMR QA ANALYSES ACCEPTABLE

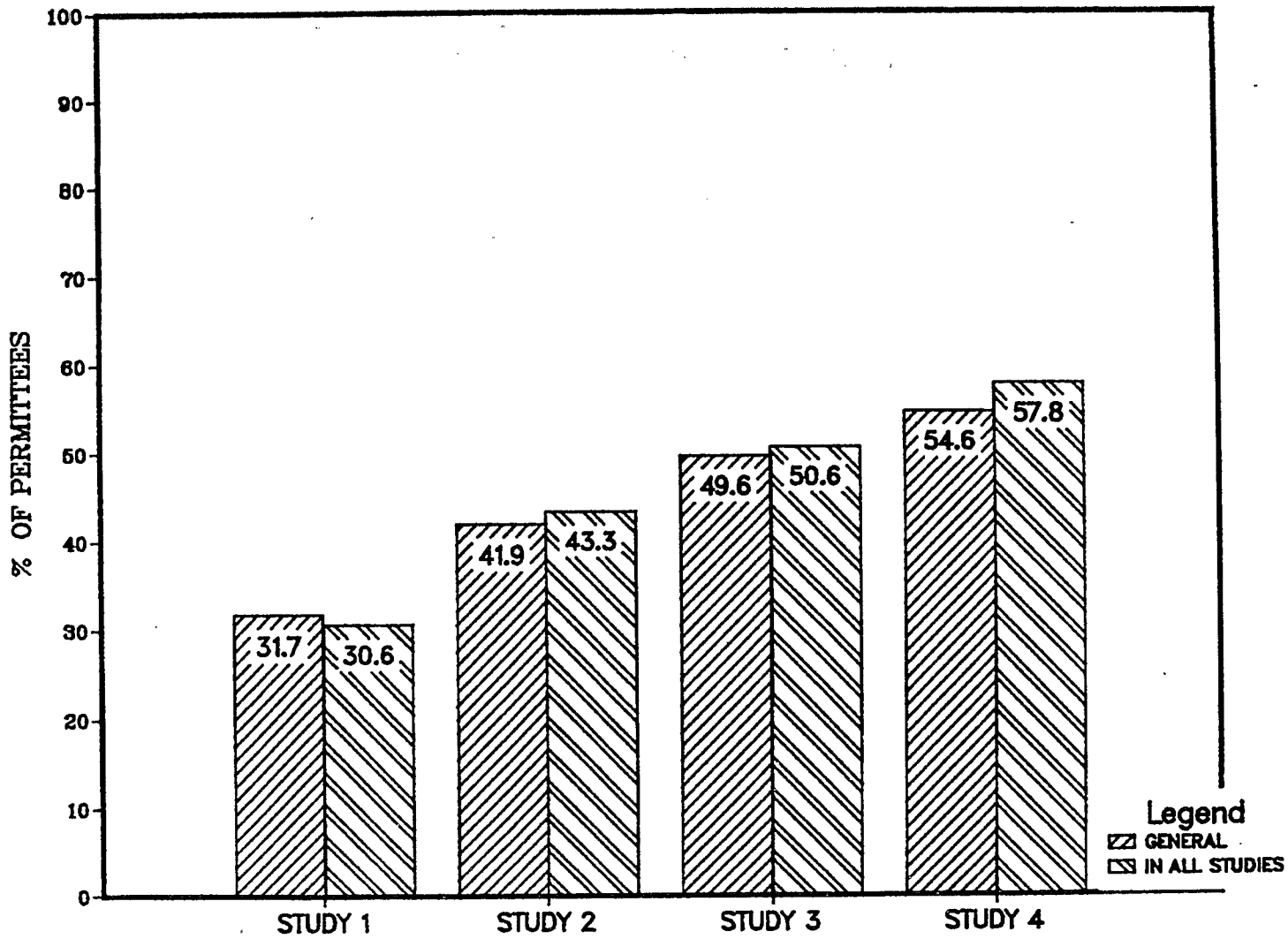


FIGURE 4--PERCENT OF PERMITTEES WITH ALL DATA ACCEPTABLE

Table 1 PERFORMANCE BY MAJOR INDUSTRY
PERCENT OF ANALYSES ACCEPTABLE

SIC CODE	SIC DESCRIPTION	STUDY 1 NUMBER ANALYSES	STUDY 1 % ANALYSES ACCEPTABLE	STUDY 2 NUMBER ANALYSES	STUDY 2 % ANALYSES ACCEPTABLE	STUDY 3 NUMBER ANALYSES	STUDY 3 % ANALYSES ACCEPTABLE	STUDY 4 NUMBER ANALYSES	STUDY 4 % ANALYSES ACCEPTABLE
.	NOT IDENTIFIED	695	72.66	165	82.43	167	71.8629	1266	75.36
1031	LEAD & ZINC ORES	107	78.50	167	90.41	122	88.5205	134	94.78
1094	URANIUM-RADIUM-VANADIUM ORES	150	58.00	306	66.66	125	82.4024	201	91.07
1211	BITUMINOUS COAL & LIGNITE	306	64.05	400	83.00	401	81.7925	433	87.30
1311	CRUDE PETROLEUM & NATURAL GAS	52	82.71	23	86.96	38	81.5816	53	79.23
1475	PHOSPHATE ROCK	126	88.89	46	82.61	54	94.4426	60	91.67
2011	MEAT PACKING PLANTS	195	74.35	65	79.99	75	82.6667	61	91.80
2016	POULTRY DRESSING PLANTS	151	76.82	72	81.94	78	79.4821	106	71.70
2063	BEET SUGAR	49	75.51	48	89.58	56	96.4304	48	89.59
2211	COTTON FABRIC MILLS	149	67.12	163	78.54	97	75.2608	107	83.17
2231	WOOL FABRIC MILLS	150	66.66	130	83.09	79	83.5481	87	89.64
2261	FINISHERS OF COTTON FABRICS	102	71.57	85	78.82	59	77.9695	69	85.50
2262	FINISHERS OF MAN-MADE FIBER	42	73.81	49	71.43	71	88.7324	63	90.48
2611	PULP MILLS	258	77.13	241	87.55	220	84.9995	235	91.07
2621	PAPER MILLS	575	76.17	429	82.99	434	87.3283	457	89.50
2631	PAPER BOARD MILLS	290	81.04	176	81.83	181	86.1945	192	87.51
2812	ALKALIES & CHLORINE	176	81.26	138	79.71	124	87.0992	151	91.39
2816	INORGANIC PIGMENT	98	63.27	122	77.87	100	90.9910	90	91.12
2819	INDUSTRIAL INORGANIC CHEMICAL	668	72.16	546	81.13	538	83.0849	621	85.18
2821	PLASTICS MATERIALS, RESINS	518	75.48	518	78.57	544	87.3079	573	85.34
2822	SYNTHETIC RUBBER	84	71.42	90	85.55	86	84.8767	90	87.78
2824	SYNTHETIC ORGANIC FIBERS	107	73.83	85	80.00	88	88.6330	92	88.04
2833	MEDICINAL CHEMICALS	108	71.29	91	79.12	138	82.6043	147	90.47
2834	PHARMEUTICAL PREPARATIONS	59	81.35	150	70.66	155	85.1535	192	85.41
2865	CYCLIC CRUDES & INTERMEDIATES	127	72.44	169	76.92	159	77.9836	154	83.76
2869	INDUSTRIAL ORGANIC CHEMICALS	889	71.88	820	79.39	897	82.3852	866	87.41
2873	NITROGENOUS FERTILIZERS	226	70.80	174	74.14	156	87.1808	158	88.61
2874	PHOSPHATIC FERTILIZERS	74	72.98	52	73.08	53	81.1264	67	92.54
2879	PESTICIDES & AGRI. CHEMICAL	61	60.66	77	81.82	68	91.1794	72	90.28
2899	PETROLEUM & COAL PRODUCTS	63	80.96	46	78.26	37	83.7784	60	88.33
2911	PETROLEUM REFINING	995	70.45	1053	73.88	1087	82.4154	1114	85.45
3312	BLAST FURNACES & STEEL MILLS	806	78.79	495	84.24	537	86.9918	618	88.35
3334	PRIMARY ALUMINUM	69	79.70	93	87.10	88	92.0489	94	89.36
3339	PRIMARY NON-FERROUS METALS	86	81.40	52	80.76	65	90.7631	69	94.20
3341	SECONDARY NON-FERROUS METALS	60	66.68	92	86.95	117	78.6239	147	92.52
3357	DRAWING NON-FERROUS WIRE	16	81.26	23	82.61	47	80.8447	45	75.55
3471	ELECTROPLATING	655	70.23	471	70.92	645	79.2225	642	87.54
3621	MOTORS & GENERATORS	16	93.75	18	83.33	44	90.9045	57	91.23
3679	ELECTRICAL COMPONENTS	48	89.58	115	72.16	154	82.4610	150	80.67
3714	MOTOR VEHICLE PARTS	68	80.88	79	82.29	104	85.5731	138	81.16
4911	ELECTRIC SERVICES	3245	78.58	3248	81.22	2882	85.8395	3076	89.72
4941	WATER SUPPLY	97	82.47	60	76.65	71	87.3183	70	88.58
4952	SEWAGE SYSTEMS	16571	73.49	13527	78.08	13752	81.4088	15837	84.22
4953	REFUSE SYSTEMS	138	74.65	143	75.53	147	87.7599	143	90.91
9711	NATIONAL SECURITY	314	71.35	214	75.24	124	88.7048	172	88.96

Table 2 PERFORMANCE BY MAJOR INDUSTRY
PERCENT OF PERMITTEES WITH ALL DATA ACCEPTABLE

SIC CODE	SIC DESCRIPTION	STUDY 1 NUMBER PERMITTEES	STUDY 1 % PERMITTEES 100%	STUDY 2 NUMBER PERMITTEES	STUDY 2 % PERMITTEES 100%	STUDY 3 NUMBER PERMITTEES	STUDY 3 % PERMITTEES 100%	STUDY 4 NUMBER PERMITTEES	STUDY 4 % PERMITTEES 100%
.	NOT IDENTIFIED	111	33.33	29	44.83	26	42.31	294	43.54
1031	LEAD & ZINC ORES	15	53.33	24	70.83	18	50.00	19	73.68
1094	URANIUM-RADIUM-VANADIUM ORES	20	25.00	28	17.86	28	50.00	21	57.14
1211	BITUMINOUS COAL & LIGNITE	58	12.07	92	57.61	88	53.41	90	60.00
1311	CRUDE PETROLEUM & NATURAL GAS	14	71.43	13	76.92	13	53.85	12	75.00
1475	PHOSPHATE ROCK	19	57.89	14	57.14	17	82.35	18	72.22
2011	MEAT PACKING PLANTS	28	14.29	12	33.33	14	42.86	12	58.33
2016	POULTRY DRESSING PLANTS	31	38.71	10	30.00	14	28.57	19	21.05
2063	BEET SUGAR	13	15.38	13	69.23	15	86.67	13	69.23
2211	COTTON FABRIC MILLS	25	20.00	25	36.00	23	34.78	23	52.17
2231	WOOL FABRIC MILLS	18	16.67	12	41.67	13	15.38	14	42.86
2261	FINISHERS OF COTTON FABRICS	15	20.00	17	35.29	12	50.00	12	58.33
2262	FINISHERS OF MAN-MADE FIBER	8	25.00	11	36.36	12	66.67	11	72.73
2611	PULP MILLS	47	42.55	52	61.54	51	60.78	56	75.00
2621	PAPER MILLS	118	39.83	101	53.47	105	63.81	113	68.14
2631	PAPER BOARD MILLS	68	44.12	50	46.00	52	63.46	55	69.09
2812	ALKALIES & CHLORINE	24	16.67	27	44.44	23	56.52	31	64.52
2816	INORGANIC PIGMENT	15	0.00	18	27.78	16	43.75	18	55.56
2819	INDUSTRIAL INORGANIC CHEMICAL	111	34.23	92	45.65	99	42.42	112	50.00
2821	PLASTICS MATERIALS, RESINS	80	35.00	98	36.73	109	62.39	114	56.14
2822	SYNTHETIC RUBBER	13	15.38	16	56.25	17	47.06	18	72.22
2824	SYNTHETIC ORGANIC FIBERS	18	33.33	16	31.25	17	58.82	19	57.89
2833	MEDICINAL CHEMICALS	16	18.75	15	46.67	19	47.37	20	75.00
2834	PHARMEUTICAL PREPARATIONS	13	38.46	28	42.86	33	48.48	33	54.55
2865	CYCLIC CRUDES & INTERMEDIATES	17	17.65	31	41.94	30	60.00	27	48.15
2869	INDUSTRIAL ORGANIC CHEMICALS	122	24.59	141	38.30	157	47.77	150	54.67
2873	NITROGENOUS FERTILIZERS	33	15.15	25	8.00	24	50.00	24	41.67
2874	PHOSPHATIC FERTILIZERS	15	13.33	12	25.00	12	50.00	15	66.67
2879	PESTICIDES & AGRI. CHEMICAL	11	18.18	14	42.86	13	61.54	14	71.43
2899	PETROLEUM & COAL PRODUCTS	11	36.36	8	37.50	6	50.00	11	63.64
2911	PETROLEUM REFINING	126	11.90	146	19.86	152	36.18	154	40.91
3312	BLAST FURNACES & STEEL MILLS	99	35.35	80	47.50	86	59.30	104	56.73
3334	PRIMARY ALUMINUM	17	47.06	25	56.00	24	70.83	26	69.23
3339	PRIMARY NON-FERROUS METALS	10	30.00	8	12.50	11	63.64	11	63.64
3341	SECONDARY NON-FERROUS METALS	8	37.50	14	50.00	21	28.57	26	65.38
3357	DRAWING NON-FERROUS WIRE	3	33.33	7	71.43	13	46.15	12	58.33
3471	ELECTROPLATING	78	19.23	54	22.22	80	30.00	85	44.71
3621	MOTORS & GENERATORS	2	50.00	6	50.00	15	73.33	13	61.54
3679	ELECTRICAL COMPONENTS	9	55.56	16	25.00	24	50.00	21	52.38
3714	MOTOR VEHICLE PARTS	10	40.00	11	45.45	19	52.63	25	48.00
4911	ELECTRIC SERVICES	413	31.96	472	41.10	435	52.41	490	56.12
4941	WATER SUPPLY	21	57.14	11	45.45	12	58.33	11	54.55
4952	SEWAGE SYSTEMS	2767	32.27	2697	42.20	2842	49.30	3185	54.57
4953	REFUSE SYSTEMS	12	8.33	12	33.33	12	16.67	13	38.46
9711	NATIONAL SECURITY	57	36.84	35	40.00	22	68.18	31	58.06

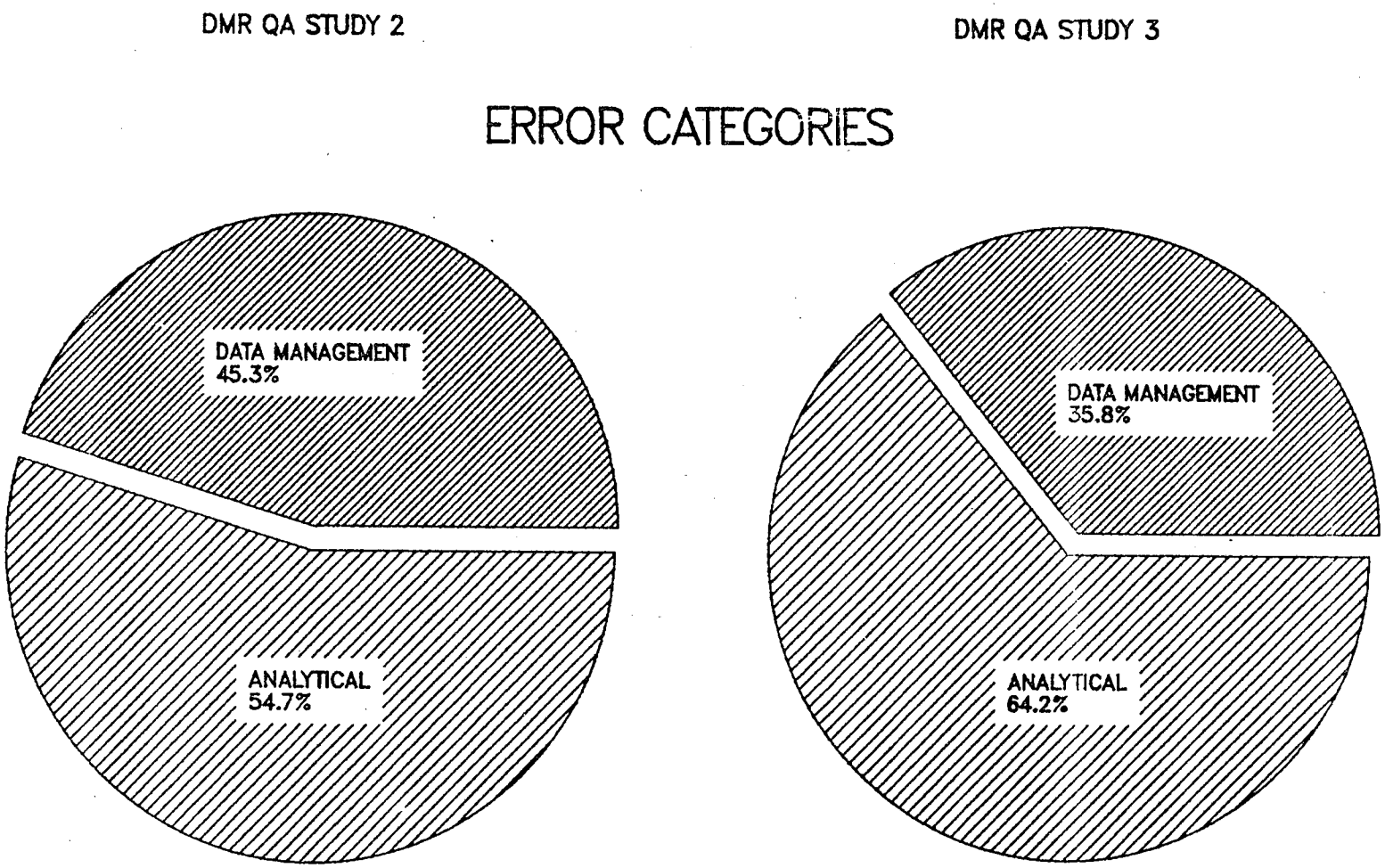


FIGURE 5 -- COMPARISON OF SOURCES OF ERRORS

PAUL BRITTON

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY

PROGRESS REPORT ON DMR QA STUDIES: QUALITY ASSURANCE PROGRAM FOR NPDES SELF-MONITORING DATA

MR. BRITTON: During my time here today, I want to cover two major topics. First, some special summary results that provide a better basis for direct comparison from one study to another; then some simulation results allowing comparison of several statistical estimation procedures when applied to data with known characteristics. Limits in DMR QA studies have been and will continue to be based upon a statistical estimation procedure equal or similar to one of these.

On the study summaries, Sam To gave the official study results and the percentage of acceptable data for each study. However, the basis for limits in each one of these studies differ. We wondered whether going to a standardized basis for limits and reevaluating the study results would lead to some difference in the percentage of acceptable data from study to study. Certainly it would lead

to percentages which could be more validly compared from one study to another.

For those who are not familiar with the basis for our limits, they are developed from two different kinds of information. First, regressions based on statistics from the six previous studies are developed directly from data generated by EPA and state laboratories in these studies on samples just like the ones in the DMR QA study. Statistical estimates are then developed directly from the study data; again, data produced by EPA and state laboratories analyzing the DMR QA samples.

By comparing limits developed directly from the study statistics against limits developed from regression-based statistical estimates, the limits to be used for each study are selected. In essence, the two limits are compared and if the limits from study statistics are completely enclosed within the regression-based limits, then the regression-based limits, the broader of the two, are used. If in any way, the regression-based limits fail to enclose the study statistics-based limits, then the study statistics-based limits are used directly, because there's no question about the relevancy of those limits to the study samples.

Obviously, from one DMR QA study to another, the six most recent studies would have changed and therefore, the regressions would have changed. In comparing and deciding on the broader of either the regression-based limits or the study statistics-based limits, there would also be a considerable change from one study to another in how the limits were developed. Consequently, we decided to go back and reevaluate all the study results using one set of fixed regressions to establish all appropriate limits for each of the studies. These regressions are based upon the six most recent studies which are related to the DMR QA samples.

Overhead 1 provides an example of one set of fixed regressions. It happens to be the set for aluminum, the first analyte, but gives you some idea of what the regressions are like. Each set includes relationships between the true concentration (T) of a sample and the mean (X) and standard deviation (S) that can be expected for analytical responses from multiple laboratories operating within control. The R2 values for these two fits, R2 being the percent of the total variability in the data that is explained by the regression, are also given. For the relationship

between X and T, R2 = 99.7 which is very high, showing that this relationship fits the data very well. Standard deviation being a little more imprecise in nature, only 89.5 percent of the total variability in the data were explained by the regression, but that is still a solid linear relationship and a reasonable basis for estimation.

The columns of residuals in Overhead 1 show how the estimates from the regressions relate to the observed statistics, sample by sample, again indicating that the standard deviation is more variable and that there are a few individual statistical estimates which vary somewhat from that line, but none which vary to any significant extent. This suggests good relationships and is characteristic of these regressions.

There are 26 analytes and two concentrations involved in each DMR QA study, so there were 26 sets of relationships like this which were applied to two different concentrations for each study. There was a total of four studies to go back and look at.

OVERHEAD 2

Using limits for each study that were strictly based upon the fixed regressions, we obtained the

results that are given in Overhead 2 for the DMR QA studies. The second column shows the time the study was actually run--basically an estimate of when the analysis was done. The next column shows the total number of reported values received in each study, which has been fairly consistent. There were more respondents in DMR QA Study 4 than in any of the other studies--probably the main reason why the total number of reported values was highest.

In the fourth column are the percentages of acceptable data using the special fixed regression limits. Surprisingly, they are not that much different from the official percent of acceptable data results, but the potential is certainly there for a difference. This apparently shows that the regression relationships have been fairly consistent for several years; not changing as much from one year to another as they did in the early years. The next column shows the change in percentages using the first study as a base, and that in DMR QA studies, the percentage of acceptable data has increased by 10.4 percent between the first and fourth studies.

The last column attempts to show the percentage of improvement in performance since the first study,

showing the DMR QA participants have reduced the amount of unacceptable data they reported by about 40 percent over these four studies. The EPA and state laboratory data from analyses of the same samples was similarly analyzed to give some idea of relative performance by these groups. For EPA laboratories, the percentage of data acceptable was 94 percent in the first study related to the DMR QA Study 1, and 96 percent for Study 4; improvement not unlike the 40 percent seen for the DMR QA studies. For state laboratories, the percentage starts out at 86.2 and goes to 91.4; a similar 37.7 percent improvement.

OVERHEAD 3

Overhead 3 is a graphic representation of the performance over the three and a half year period by those three laboratory groups. Again, it's fairly obvious that all show improvement with time, which is pretty much what was expected, and that they are all improving at about the same relative rate, which was somewhat surprising.

The second topic I want to discuss has to do with some simulations done on a series of different statistical estimation procedures, any of which could potentially have been used as the basis for

the background statistics on data from EPA and state laboratories, that become the basis for all limits.

OVERHEAD 4

Overhead 4 attempts to briefly depict the major difference between the four techniques. If the X-axis is the order of the ranked data, then the first procedure, the traditional calculation without any outlier removal, would allow every observation to have an equal weight of one in the statistical calculation. Where you have traditional estimation but do some outlier testing before you do the calculation itself, some of the observations, the extreme observations on either end, would have a weight of zero in the calculation. All other observations would have a weight of one.

If you use a robust estimation procedure--a procedure that does not use all of the observations in a data set in order to develop estimates of the mean and standard deviation of that set--the calculations are different, compensating for the fact that not all of the data are used.

The robust estimation procedure we are using involves outlier testing first, then a robust calculation on the middle 70 percent of the

retained data. In other words, any observations beyond the 15th and 85th percentiles of the retained data have weight zero, while data within this interval have full weight. The influence of observations transcending either percentile is prorated on the portion falling within the interval.

The fourth procedure is called the biweight estimator. It involves development of a weighting factor for every observation such that more extreme observations have less weight and, therefore, less influence on the biweight estimates.

We are currently investigating the effect of these estimation techniques on data from studies like ours and will probably continue to look for and investigate alternative ways in the future. This investigation is still in progress, so final conclusions are not available on whether we will change from the 70 percent robust procedure currently in use, however, some early results are presented in the following overheads.

OVERHEAD 5

The first set of simulation results in Overhead 5 involves a thousand samples taken from a normal population with mean 100, standard deviation 10, and 20 observations in each sample. The mean

and standard deviation of the underlying population were estimated from each of the thousand samples using the four estimation techniques.

The two columns under "EST. OF MEAN" show the traditional arithmetic mean and estimate of variability (the standard deviation) to give a general basis for comparison of the 1000 estimates of the population mean obtained using each of the four different estimation procedures. In general, the only difference among means produced by the four procedures involved their variability; as expected, the traditional estimates had slightly less variability. Estimates from the traditional procedure should always have the least variability when the population is normal. For practical purposes, you get the same estimate by all these procedures.

The last two columns characterize estimates of the standard deviation developed using the four procedures. As before, traditional has less variability, but there is really little practical difference, except for an apparent 5 percent high bias in the biweight estimates. Origins of the bias in biweight estimates of the standard deviation are unclear, but if used in its current form when the underlying population is a normal, it would slightly

overestimate the standard deviation and therefore lead to slightly broader limits.

Overhead 5 also contains results from 1,000 samples of size 50. As you would expect with a larger sample size, the variability of estimates is reduced for all procedures. The only real difference seems to be the bias on the estimate of the standard deviation from the biweight procedure; as before, it's about five percent high.

OVERHEAD 6

Okay, that shows what happens with normal distributions. What happens when you do not have a perfect underlying normal population? Certainly, we do not always see data from a perfect normal distribution during our studies. Overhead 6 shows results for simulations involving 1,000 samples again, but the data is a mixture of two underlying populations. For the first set of results, each sample of 50 contained 40 observations from a normal population with mean 100 and standard deviation 10. The other 10 observations in each sample came from another normal, with mean 130 and standard deviation 10. So, 20 percent of each sample reflected another distribution with a 30 percent higher mean.

We are really interested in estimating the characteristics of the 80 percent of each sample which represents the good data, and we want to look past the bad data which is represented by the data with a 30 percent bias. Therefore we would prefer our estimation procedure to give mean estimates of 100 and standard deviation estimates of 10, which reflect the capability represented in the good data here.

The mean estimate from traditional calculation techniques is 106.8 and is somewhat better with the outlier testing. Mean estimates from the two robust procedures are slightly better yet, although the difference is not dramatic. The estimates of the standard deviation are all similar; instead of being 10, they are all over 15. As a result, where we should have estimates of the mean of 100 and estimates of the standard deviation of 10, we've got estimates of the mean that run 105 or better, and estimates of the standard deviation that exceed 15. Obviously, evaluation limits developed from these estimates will be much broader than they should be to represent the bulk of the data.

The second set of results in Overhead 6 show

the effect of slightly increasing the bias of the 20 percent bad data in our simulation. The estimate of the mean from the traditional calculation has gone up to 112, whereas the biweight has already recognized all the bad data and has dropped the mean estimate down to 100.7. The other two procedures have both partially recognized the bad data, but haven't successfully ignored all of it. The standard deviation for the traditional calculation is over 26, for the next two is over 16, and for the biweight is down to 13. They should all be 10.

In the last set of results in Overhead 6, the bias of the bad data has been increased to 90 percent and the estimate of the mean from the traditional calculation has increased to 118. The other procedures have recognized virtually all 20 percent of the bad data and are estimating 100. Obviously, there are some residual effects on the mean estimates of the standard deviation, but by far the worst is the 38 produced by the traditional calculation as a mean estimate of the standard deviation.

A tremendous mixture of data comes in during these studies. As could be expected when anywhere from 15 to 25 percent of the data are considered

not acceptable, errors involving factors of 10, 100 or 1,000 are not unusual. Perhaps five percent of the data will reflect a decimal placement error of this kind. Factors of five, two and somewhat lower biases are also quite common. Although 20 percent of a particular kind of biased data is not a frequent occurrence in our studies, these simulations certainly demonstrate the effect of such data when you are really interested in the characteristics of the 80 percent that represent good performance.

OVERHEAD 7

We have seen what happens when the bias changes, but what happens when the standard deviation changes? In Overhead 7, 80 percent of each sample comes from a normal distribution with mean 100 and standard deviation 10, while for the first set of results, the remainder comes from a normal with mean 100, but a standard deviation of 30. The mean estimates, of course, are not influenced one way or the other, but this shows how the standard deviation estimates are affected. The estimate of the standard deviation from the traditional calculation is up to 16.2. For the others it is better, but still varies between 11.5 and

12.8.

Of course, you can have an alternative distribution which represents a very, very high quality group of performers. Under such circumstances, we were concerned that statistical estimates might be dramatically affected, causing evaluation limits to be much too narrow. Therefore, we looked at the effect when 20 percent of the data represented a normal distribution with a much smaller standard deviation of 3.3. The mean of the standard deviation estimates from all procedures are between 8.5 and 9.1, which is reasonably close to 10, the standard deviation of the general population representing average performance.

We plan to make comparisons of these procedures using real data. The disadvantage of using real study data, of course, is that its true nature is unknown. Its advantage, however, is in showing how much results from the estimation procedures would differ in actual use. We also plan to look at simulations using other mixtures of data, perhaps some more complex, but the ultimate is going to be how it performs on the real study data.

I'm not at all unhappy with results for the 70 percent robust procedure, the one we currently

use to produce the statistical estimates used as a basis for acceptance limits in DMR QA studies. If we do decide to change our statistical estimation procedure, it seems unlikely there would be a noticeable effect on limits in DMR QA studies.

That's basically the end of my presentation. I guess it's time for questions for Sam or I.

REGRESSION EQUATIONS FOR ALUMINUM

$\bar{X} = .962\ T + 43.1$ $\qquad$ $S = .0527\ T + 20.4$

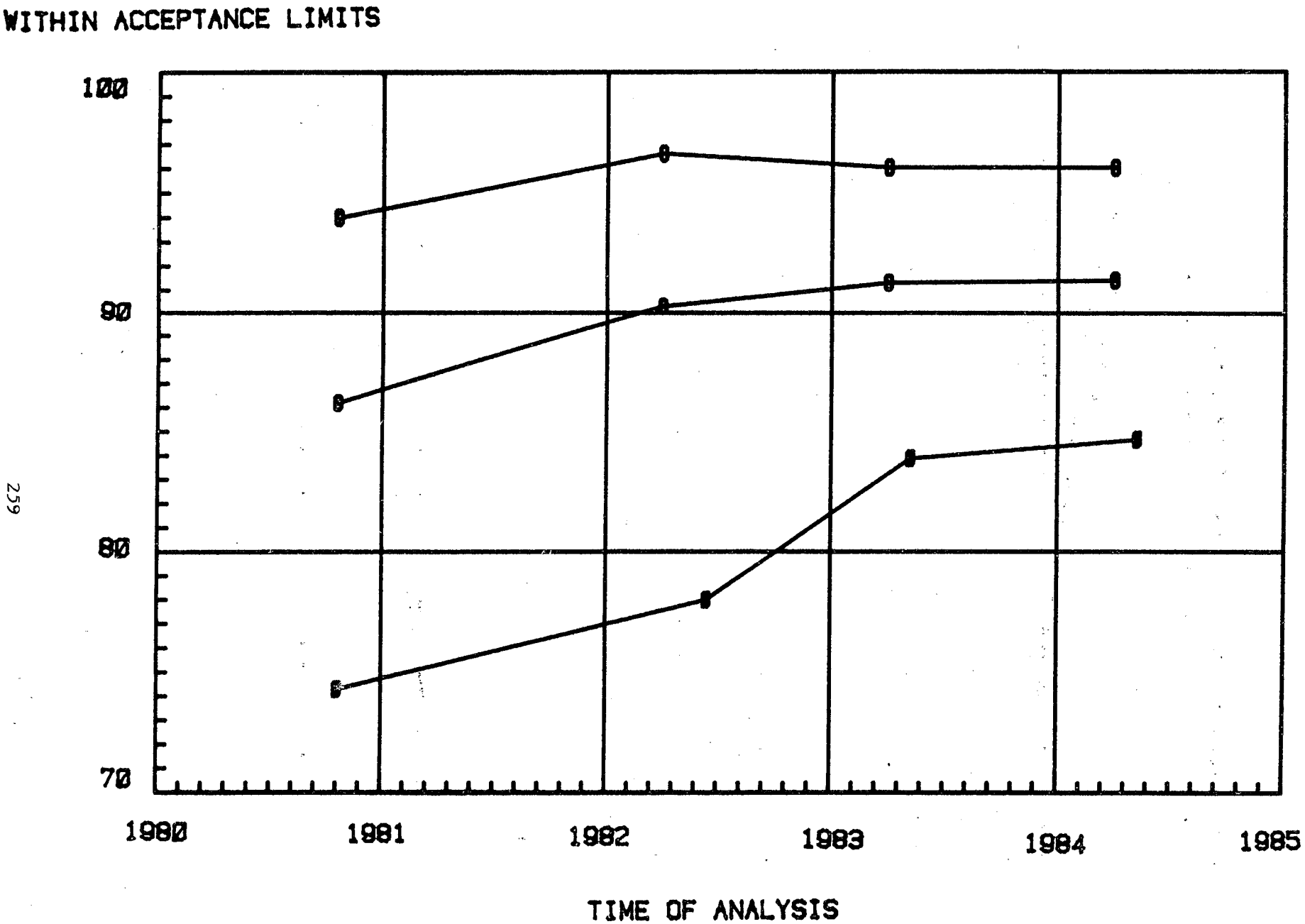
$R^2(\bar{X}) = .997$ $\qquad$ $R^2(S) = .895$

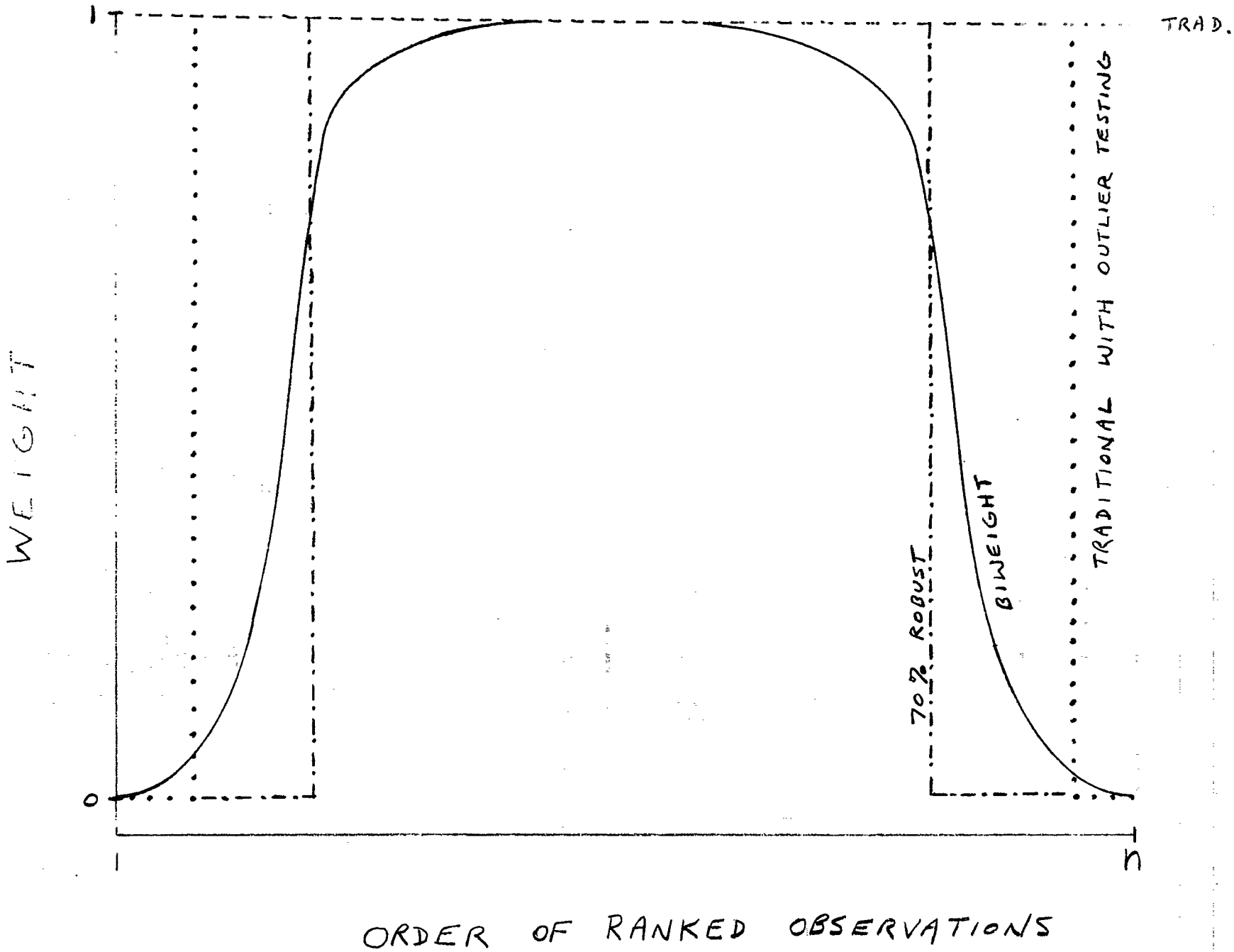
TRUE CONC. (T)	NUMBER OF OBS. REPORTED	MEAN RECOVERY ($\bar{X}$)	RESIDUAL FOR EST. OF $\bar{X}$	STD. DEV. (S)	RESIDUAL FOR EST. OF S
1860	46	1830	- 3.6	128.3	9.90
1310	46	1293	-11.2	95.5	6.07
1260	45	1186	-69.7	105.9	19.07
968	37	1003	27.9	82.7	11.26
930	43	925.1	-13.0	46.7	-22.69
662	42	697.3	17.1	57.0	1.68
607	43	614.7	-12.6	43.5	- 8.93
503	46	487.3	-39.9	51.0	4.08
340	35	397.5	27.2	50.3	11.94
98.1	38	134.3	- 3.2	22.9	- 2.75
94.6	35	145.4	11.3	21.4	- 4.02
43.0	32	82.6	- 1.8	23.9	1.17

PERFORMANCE IN DMR-QA AND RELATED STUDIES

STUDY	TIME	TOTAL NO. OF RESPONSES	% ACCEPT.	CHANGE SINCE FIRST STUDY	% IMPROVEMENT SINCE FIRST STUDY
DMR-1	10-11/80	35,056	74.3	---	---
DMR-2	5-6/82	33,208	78.0	3.7	14.4
DMR-3	4-5/83	33,730	83.9	9.6	37.4
DMR-4	4-5/84	39,324	84.7	10.4	40.5
FOR EPA LABS:					
WP006	10/80	515	94.0	---	---
WP008	3/82	590	96.6	2.6	43.3
WP010	3/83	582	96.0	2.0	33.3
WP012	3/84	595	96.0	2.0	33.3
FOR STATE LABS:					
WP006	10/80	3,312	86.2	---	---
WP008	3/82	2,952	90.3	4.1	29.7
WP010	3/83	2,960	91.3	5.1	37.0
WP012	3/84	2,872	91.4	5.2	37.7

PERFORMANCE IN DMR AND RELATED STUDIES




TRAD.
TRADITIONAL WITH OUTLIER TESTING
70% ROBUST
BIWEIGHT
WEIGHT
1
0
1
n
ORDER OF RANKED OBSERVATIONS

SIMULATIONS - EACH INVOLVING 1000 SAMPLES

POP.	N	EST. PROCED.	EST. OF MEAN MEAN	EST. OF MEAN VAR.	EST. OF STD. DEV. MEAN	EST. OF STD. DEV. VAR.
N(100, 10)	20	TRAD.	100.1	2.21	10.0	1.86
		W/O. T.	100.1	2.20	10.0	1.73
		70% R.	100.1	2.27	10.0	2.08
		BIWEIGHT	100.1	2.30	10.5	1.95
N(100, 10)	50	TRAD.	100.0	1.46	10.1	1.03
		W/O. T.	100.0	1.48	9.9	1.09
		70% R.	99.9	1.53	10.0	1.37
		BIWEIGHT	100.0	1.52	10.5	1.18

SIMULATIONS - EACH INVOLVING 1000 SAMPLES

POP.	N	EST. PROCED.	EST. OF MEAN MEAN	EST. OF MEAN VAR.	EST. OF STD. DEV. MEAN	EST. OF STD. DEV. VAR.
N(100, 10)	40	TRAD.	106.0	1.40	15.9	1.30
N(130, 10)	10	W/O. T.	105.7	1.54	15.3	1.53
		70% R.	105.1	1.65	15.8	2.27
		BIWEIGHT	104.4	1.71	15.9	1.65
N(100, 10)	40	TRAD.	112.0	1.44	26.5	1.38
N(160, 10)	10	W/O. T.	104.7	4.64	16.8	6.67
		70% R.	103.5	4.27	16.3	8.43
		BIWEIGHT	100.7	2.25	12.9	3.37
N(100, 10)	40	TRAD.	118.0	1.40	38.1	1.38
N(190, 10)	10	W/O. T.	100.1	1.80	10.0	2.02
		70% R.	100.1	1.76	10.1	2.24
		BIWEIGHT	100.0	1.59	11.5	1.45

SIMULATIONS - EACH INVOLVING 1000 SAMPLES

POP.	N	EST. PROCED.	EST. OF MEAN MEAN	EST. OF MEAN VAR.	EST. OF STD. DEV. MEAN	EST. OF STD. DEV. VAR.
N(100, 10)	40	TRAD.	100.1	2.30	16.2	2.98
N(100, 30)	10	W/O. T.	100.0	1.95	12.2	1.01
		70% R.	100.0	1.83	11.5	1.70
		BIWEIGHT	100.0	1.79	12.0	1.72
N(100, 10)	40	TRAD.	100.0	1.30	9.1	0.98
N(100, 3.3)	10	W/O. T.	100.0	1.34	8.7	1.11
		70% R.	100.0	1.30	8.5	1.39
		BIWEIGHT	100.0	1.30	9.0	1.16

QUESTION AND ANSWER SESSION

MR. RICE: Jim Rice.

Paul, all of those examples that you put up there could just as well have represented two different analytical methods, which is the case, for example, for where there are alternative methods given say for chromium or copper or whatever, one of them flame and one of them furnace. For instance, these two procedures or methods have considerably different precision statements when operating on the same metal sample. I mean, you could construct them so that everything you said there would work for that. Why not recognize that? Have you attempted to look and separate out and examine each method by the appropriate statistics on the data base separated into its components as you can identify them.

MR. BRITTON: Yes. You know that we did collect the information on the method that was used when these studies were run, and you also know that we do generate statistics by method. There would certainly be a policy issue involved in setting limits method-by-method, but I really think that's the only alternative, assuming

that indeed there is a significant mixture of methods and significantly different statistical characteristics for the various methods. I think that in general, we're not seeing that great a mixture of methods, and in general, we're not seeing tremendously different statistical results either.

I think one major exception is the group of nutrients where in the DMR QA studies, we observed that the percentage of unacceptable data in the nutrient group was characteristically about twice the average unacceptable data for the other groups. We started considering that issue and decided that it was probably caused by the fact that two-thirds of the EPA and state laboratories use automated methods for nutrients. We looked at the DMR QA group, and they use manual methods about 2:1. So we decided we had to make an adjustment, and from now on, in fact I think with Study 4, the limits for nutrients are to be based on statistics from all labs rather than the EPA and state laboratory group. This change has brought the percentage of unacceptable data for nutrients in DMR QA studies right down in line with all the other analyte groups, so things are balanced better now than they were.

In general, I don't anticipate looking at methodology and somehow compensating for statistical differences from one method to another. We really haven't had a chance to study the difference between methods in a rigorous sense, and I'm not real sure what I would do with the information. EPA policy, as I understand it, is that approved methods are all equivalent methods and therefore should be judged against the same general criteria.

MR. YOUNG: Jim Young from the University of Arkansas. I'm a little concerned that this approach might tend to cause people to think that this is the test precision that you are measuring, when in fact the precision within a particular laboratory should be much better than from the data you have collected and are analyzing. Is there any plan to look at intra-laboratory precision versus interlaboratory precision in this program?

MR. BRITTON: No, there isn't any plan to look at within-lab standard deviation in this program. That would require participants to do replicate analyses on the same sample, or analyze two samples that were identical or nearly so. But to do that, we would probably have to give

up having samples at two concentrations, in order to keep the analytical effort from increasing. I believe having information at two concentrations is more helpful for identifying where potential problems may be. If there appears to be similar bias at both concentration levels, follow-up personnel can look for a systematic error. If there are dramatically different errors at the two concentrations, then some kind of a precision problem is more likely. In general, we have not tried to get into within-laboratory standard deviation estimates in these studies. There are other sources for those estimates.

MR. STANKO: It would be difficult for me to look at the DMR QA program and criticize it on the fact that it has improved the quality of the data. It certainly has improved the quality of the data for the NPDES permit laboratories that are generating these data.

Last year at this conference, I gave a paper that was titled, Industry's Experience with the DMR QA program. The very specific point that I made last year was that the criteria acceptance limits identifies too many industrial and contract laboratories as bad performance. I would like to

show one slide for DMR QA Study 4, if I might.

SLIDE 1

This slide takes the EPA laboratory data for DMR QA Study 4, for the 52 parameters. The second column is the true value, the third column is the acceptance criteria limit that was used on industry for Study 4. The next column in identifies the number of federal and state laboratories that are using the program. The column after that is the number of outliers that were identified by the EPA statistical procedures. The next column identifies the additional number of EPA laboratories that fell outside of the 99 percent criteria. The last column identifies the percent of EPA laboratories that fall out of the 99 percent confidence interval. Statistics dictate that only approximately one percent of the laboratories should fall out of the 99 percent confidence limits.

If you will look at some of the bottom values, for total kjeldahlnitrogen, there was one identified outlier out of 53 observations, there were seven additional EPA laboratories that fell out of EPA criteria, for a total of 13.2 percent of EPA's own laboratories falling out of the 99 percent confidence interval. If I work my way up that column,

and what this column should be is 1 percent, we get 6.8, 9.1, 13.2, 9.4, 10.6, 6.1, 8.1, 8.1 and so on.

There is something wrong with the statistical procedures. I really don't care if you Winsorize, mesmerize, or hypnotize, but when you apply your criteria limit to your data set, only one percent of that data should be excluded. Thank you.

MR. BRITTON: You're right, George, something is wrong. The problem is your assumption that only one percent of the EPA and state data should fail our acceptance limits. We know that some of these data are not good and our limits are supposed to pass 99 percent of all good data. We would like all the bad data to fail, and to make this more likely, we are willing to pay by accepting rejection of one percent of the good data as well. Clearly, EPA and state laboratories are not perfect yet. They did generate some bad data in these studies. If they hadn't, then the failure rates you calculated would indeed have been one percent.

Revised Presentation

STATISTICAL BASIS FOR LABORATORY PERFORMANCE EVALUATION LIMITS

Paul W. Britton * and Daniel F. Lewis **

1. INTRODUCTION

The effectiveness of the water regulations enforced by the U.S. Environmental Protection Agency (USEPA) depends upon the quality of data generated by USEPA, state, local government, industrial, commercial and other non-USEPA laboratories. In an effort to improve the quality of data, USEPA conducts collaborative studies evaluating the ability of laboratories to analyze water samples and to produce data within specific evaluation limits. At present, five formal studies are conducted each year for USEPA by the Environmental Monitoring and Support Laboratory at Cincinnati, two for the drinking water laboratory certification program, two for point and non-point source discharge monitoring, and one for major NPDES permit dischargers. In addition, special performance evaluation studies are conducted for the Superfund and Solid Waste activities, and other USEPA programs involving contract laboratories.

Acceptable performance during these studies demonstrates a well-managed laboratory operating competently, while unacceptable performance indicates a laboratory which is likely to be having problems generating quality data, either for a particular analyte or in general. Unacceptable performance results in an investigation of the circumstances, and if appropriate, remedial action by USEPA. It should be mentioned, however, that even the best of laboratories will periodically develop analytical problems. It is perfectly reasonable for a quality laboratory to occasionally produce analytical data that are outside of evaluation limits, due either to an unfortunate result of random chance or to an actual analytical error.

During these performance evaluation (PE) studies, the Quality Assurance Branch of the Environmental Monitoring and Support Laboratory - Cincinnati sends participating laboratories a set of stable sample concentrates in sealed glass ampules, a data reporting form and appropriate instructions. Each laboratory produces the study samples by diluting a measured quantity of specific concentrates to volume with reagent water, then analyzes them using its routine procedures. The completed form is sent to USEPA for evaluation and a fully-detailed report is returned to each laboratory. The responsible state or USEPA office follows up with laboratories that demonstrate potential problems.

The effectiveness of these studies depends upon two things: first, whether the evaluation limits properly indicate data resulting from substandard analytical work; and second, the commitment of all participants to ensure that laboratories performing poorly receive appropriate follow-up work so that substandard laboratories either improve their performance or are excluded from producing further data. It should be noted that these studies serve only as an indicator of potential substandard ability and should not be used as the sole basis for a final decision as to the quality of a laboratory.

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2. DEVELOPMENT OF EVALUATION LIMITS

EPA's objective in developing evaluation limits is to be able to distinguish between data from analytical systems operating within state-of-the-art capabilities and data representing substandard performance. A statistical prediction interval at an appropriate significance level is clearly suitable if acceptable data have a normal distribution and if proper estimates of the arithmetic mean and standard deviation are available. (Note that a prediction interval is similar to a confidence interval except that where a confidence interval uses an assumed mean, a prediction interval uses an estimated mean. A prediction interval, therefore, is calculated the same as a confidence interval except that it requires an estimate of the standard deviation that has been adjusted to account for the variability of this estimated mean.) Since there are no independent sources of such information, it is necessary to investigate the data from the past or present studies themselves to establish a basis for the desired limits. Since even the best of data sets will contain an unpredictable and often substantial portion resulting from substandard analyses, it is necessary to use outlier tests to exclude extreme data points from the statistical process before producing estimates of the mean and standard deviation that will be robust to the effects of any outliers that may remain.

2.1 Estimating the Study Statistics Using Data From the Current Study

For most analytes in a sample, basic descriptive statistics are estimated from the data submitted by the participating USEPA and state laboratories, which usually number over 100. The statistics are estimated from these data because USEPA is more familiar with these laboratories and is confident that they will produce results generally representative of the current analytical procedures when properly applied. The statistical report for each analyte includes the following:

1. Identification of obvious outliers in the data set;

2. Traditional and robust estimates of the mean and standard deviation from the retained data;

3. Tests of the normality for the retained data;

4. A histogram of the retained data; and

5. A complete listing of the USEPA and state data with the obvious outliers noted.

Outliers are identified by a two-stage procedure. The first stage involves the rejection of values larger than 500 percent or less than 20 percent of the true value and is intended to remove all data resulting from a decimal placement error. Data retained through this first stage are used to calculate traditional estimates of the mean and standard deviation for the second stage tests. If there are 25 or fewer values left, the second stage involves successive Grubbs' tests at the two-tailed 1 percent significance level until a suspected value is retained. As described in Grubbs (1950 and 1969) and Standard Practice E-178 of the American Society for Testing and Materials, this test involves ordering the data, identifying the largest or smallest value as the suspected X -- the value farthest from the mean of all the data -- and calculating a statistic $T = (\bar{X} - X)/S$, where X is the traditional estimate of the mean and S is the traditional estimate of the standard deviation. The suspected value is discarded as an outlier if the absolute value of T exceeds the appropriate critical value, then $\bar{X}$ and S are recalculated from the remaining data prior to testing the next most extreme value. For sets of more than 25 retained values, any value beyond a 99 percent confidence interval, generated using the Student's t, is considered an outlier.

Final estimates of the mean and standard deviation are calculated from the n values retained through both stages of outlier testing. The traditional estimates are:

$$(1) \quad \bar{X} = \left(\sum_{i=1}^{n} X_i \right)/n$$

$$(2) \quad S = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{(n-1)}}$$

where X_i is the ith retained value.

Robust estimates are calculated from an ordered set of the retained data, using a 15 percent Winsorizing procedure to estimate the mean as described by Dixon and Massey (1969), and standard normal deviates to estimate the standard deviation.

After the retained data have been arranged in ascending order, the 15th and 85th percentile are calculated as:

(3) $P_{15} = X_j - (j - .15n - .5)(X_j - X_{j-1})$

where j = 1 + (the greatest integer portion of (.15n + .5)), and

(4) $P_{85} = X_k - (k - .85n - .5)(X_k - X_{k-1})$

where k = 1 + (the greatest integer portion of (.85n + .5)).

After the percentiles have been calculated, the Winsorized estimate of the mean is calculated as:

(5) $$\bar{X} = \frac{h(X_h) + (n+2-k)(X_{k-1}) + \sum_{i=h+1}^{k-2} X_i}{n}$$

where h = j - (the greatest integer portion of (j - .15n - .5)). Note that X_h is the smallest observation that is greater than or equal to P_{15}, and X_{k-1} is the largest observation that is less than or equal to P_{85}.

The robust estimate of the standard deviation is calculated from the distance across the middle 70 percent of the ordered retained data. Assuming an underlying normal distribution, this calculation is:

(6) $$S = \frac{P_{85} - P_{15}}{2.0729}$$

where 2.0729 is the number of standard deviations between the 15th and 85th percentiles of a normally distributed population. This value is obtained using an inverse normal function.

In order to produce prediction intervals, this estimate for standard deviation must be adjusted for the variability in the estimate of the mean, as follows:

$$(7) \quad S_{adj} = \sqrt{S^2 + S_{\bar{X}}^2} = \sqrt{S^2 + S^2/n}$$

$$= S \sqrt{1 + 1/n} \quad ,$$

where S_{adj} is the adjusted estimate of standard deviation to be used to construct a prediction interval, S is estimated using equation 6, and $S_{\bar{X}}$ is an estimate of the standard deviation of the mean and is equal to the square root of S^2/n for means calculated from n observations.

To provide information regarding normality, Kolmogorov-Smirnov (K-S) and Anderson-Darling (A-D) statistics are calculated for the full set of retained data and a K-S statistic is calculated for the middle 70 percent of the retained data. The K-S statistic is used because it is a highly regarded distance statistic that can be used to test normality of the middle 70 percent of the retained data as well as the full set. The A-D statistic is calculated for the full set of retained data because it is more sensitive to deviations from normality in the distribution tails, and was found to be one of the most powerful and easily calculated distribution tests studied by Stevens (1974) and Green and Hegazy (1976). Suitable critical values are provided at 1 and 5 percent significance levels using the approximations provided by Stevens (1974).

For analytes with rugged analytical procedures or with which the laboratories have considerable experience, there is no practical difference between the traditional and robust estimates of the mean and standard deviation. However, there are analytes for which a number of the USEPA and state laboratories still do not have their system fully under control, and so are generating data that do not represent competent performance. Under such conditions, the robust estimates are clearly superior to the traditional estimates since the robust estimates are not as likely to be influenced by persistent outliers and the outlier scheme will fail to identify all the outliers whenever analytical problems become too common. Because they are universally appropriate, the robust estimates of the mean and standard deviation are used to characterize acceptable performance.

The remainder of the statistical report is a histogram and an ordered listing of all the data from USEPA and state laboratories with outliers followed by an asterisk. The histogram provides a convenient visual impression of how the retained data are distributed and the ordered listing is necessary to document the actual data that the statistics were developed from. Figure 1 is an example of the report.

2.2 Estimating Study Statistics Using Regressions on Previous Study Statistics

When appropriate background statistics are available, estimates of the mean and standard deviation for the current study can be made from linear regressions generated from historical data. If these historically based estimates are universally beneficial to the participants, and they appear to be reasonable, they will be used as a basis for evaluation limits instead of the estimates generated from the current study.

These linear regressions, for $\overline{X}$ versus true value (TV) and S versus TV, are achieved by using the customary least-squares algorithm to fit:

(8) $$\frac{\overline{X}}{TV} = a + b\left(\frac{1}{TV}\right) \quad \text{and}$$

(9) $$\frac{S}{TV} = c + d\left(\frac{1}{TV}\right)$$

After the coefficients have been estimated, these equations may be simplified by multiplying through by TV, giving the desired forms:

(10) $$\overline{X} = a(TV) + b \quad \text{and}$$

(11) $$S = c(TV) + d$$

(Note that S is an estimate of standard deviation before it has been adjusted for the variability in the estimate of the mean. Which means, of course, that the historical data used to generate the regression equation for S will be unadjusted standard deviations from past studies.)

Using this regression technique leads to a minimization of the sum of the squares of the residuals as a percentage of the true value associated with each residual.

The chosen regression procedure was selected after a comparison of results with traditional linear, quadratic, cubic and power curve fits to the historical statistics. The nonlinear alternatives were rejected because they were more complex and did not generally produce regressions that fit the data better. The traditional linear least-squares alternative, i.e., directly fitting a linear relationship between $\overline{X}$ or S and TV, was rejected because the statistics for samples with high true values would tend to dominate the regressions, causing regressions which might provide misleading estimates of the mean and standard deviation for samples with low true values. The propriety of the chosen regression procedure is also consistent with theoretical expectations since variability in results tends to increase for most analytes in direct proportion to increasing concentration,and this regression model was designed to deal with that problem. Currently, residual randomness tests are performed and coefficients of determination are calculated for every regression equation as it is produced. In this way the regressions are continually monitored to assure they are appropriate and effective.

To produce the prediction intervals defined in the introduction to Section 2, the estimates of the standard deviation must be adjusted to account for the variability of the estimate of the mean. This is done as follows:

$$(12) \quad S_{adj} = \sqrt{S^2 + S_{\bar{X}}^2}$$

where S_{adj} is the adjusted estimate of standard deviation to be used to construct a prediction interval, S is the regression estimate of standard deviation from equation 9, and $S_{\bar{X}}$ is the standard deviation of the estimate of $\bar{X}$ obtained from equation 8.

2.3 Determining the Evaluation Limits

The first step in determining evaluation limits for an analyte is to calculate trial 95 percent confidence limits using the estimates from the current study as well as the historical estimates, both after the stated adjustments. Wherever the limits from the historical estimates do not completely encompass those from the current study estimates, the current estimates are used as a basis for calculating the final evaluation limits. Otherwise, the historical estimates are used as a basis for final limits. In recent studies, limits from current and historical statistics are generally quite similar, however limits based upon the historical statistics usually predominate.

There are several reasons why the estimates produced from regressions on historical statistics might be inappropriate. The true value for an analyte may be outside of the concentration range from which the regression was developed, the true value for the analyte may be in error, there may have been a significant change in the way the current sample was made, or one of the regressions may not represent the true relationship between the true value and the estimates of a statistic generated from past studies. Where there are no historically based estimates available for use in setting limits, either because the samples for previous studies were made differently or did not include particular analytes, limits can only be calculated using estimates made from the data of the current study.

To insure that these or other problems do not find their way into actual laboratory evaluations, the final limits as well as the estimates and procedures that lead up to them are inspected manually before any laboratories are evaluated. Suspected problems are thoroughly investigated.

3. PERFORMANCE EVALUATION REPORTS

Once appropriate standard deviations and mean recoveries are available, it is a simple matter to develop the 95 and 99 percent prediction limits (explained in the introduction to Section 2) to be used as evaluation limits, and proceed with evaluation of the study data. An individual report is generated for each participating laboratory showing the data reported, the related true values and limits, and an evaluation judgment for each reported value based upon its relationship to the appropriate limits. Each laboratory receives a copy of its report and a copy is sent to the responsible USEPA or state office for follow-up contact, as necessary, to resolve unacceptable performance.

4. DISCUSSION AND CONCLUSIONS

To date, USEPA has successfully completed 30 studies involving a total of 108 water analytes and thousands of participating laboratories. The results of these studies have been well received by the participating laboratories, the USEPA regional and participating program offices, and the states which depend on these reports to highlight laboratories requiring priority attention. When states have a parallel interest in an environmental regulation, they often request that their laboratories be included in the USEPA studies rather than developing a system of their own.

WS014 21: TOTAL TRIHALOMETHANE SAMPLE: 1 TRUE VALUE = 58.3

N	SUM	SUM OF SQUARES	MEAN	STD. DEV.	LOWER LIMIT	UPPER LIMIT	T
57	3276.0360	193449.1425	57.4743	9.6002	11.6600	291.5000	
56	3275.9800	193449.1394	58.4996	5.7295	43.2122	73.7871	2.6682
55	3201.9100	187962.7745	58.2165	5.3725	43.8721	72.5610	2.6700

STATISTICS BASED ON DATA RETAINED AFTER OUTLIER TESTS

15% WINSORIZED MEAN = 58.3496
STD. DEV. BASED ON MID 70% OF DATA = 5.5660 (CALCULATED USING DIF. BETWEEN 15TH AND 85TH PERCENTILE)

REGARDING NORMALITY OF THE MIDDLE 70% OF THE DATA:

THE KOLMOGOROV - SMIRNOV STATISTIC D = .0993
D > .1405 INDICATES NON-NORMALITY AT THE 5% SIG. LEVEL
D > .1625 INDICATES NON-NORMALITY AT THE 1% SIG. LEVEL

REGARDING NORMALITY OF THE DATA AFTER THE TRADITIONAL REMOVAL OF OUTLIERS.

THE KOLMOGOROV - SMIRNOV STATISTIC D = .0993
D > .1190 INDICATES NON-NORMALITY AT THE 5% SIG. LEVEL
D > .1376 INDICATES NON-NORMALITY AT THE 1% SIG. LEVEL

THE ANDERSON - DARLING STATISTIC A = .3759
A > .7870 INDICATES NON-NORMALITY AT THE 5% SIG. LEVEL
A > 1.0920 INDICATES NON-NORMALITY AT THE 1% SIG. LEVEL

MINIMUM VALUE	FREQ.	
45.1200	2	00
47.9574	2	00
50.7948	5	X0000
53.6322	10	XXXXXXXXXX
56.4697	16	XXXXXXXXXXXXXXXX
59.3071	6	XXXXXX
62.1445	8	XXXXXXOO
64.9819	3	000
67.8193	3	000

NON-BLANK LAB DATA VALUES:

SEQ	VALUE	METHOD CODE	SEQ	VALUE	METHOD CODE	SEQ	VALUE	METHOD CODE	SEQ	VALUE	METHOD CODE
79	.0560*	12	9	54.5000	19	44	58.3000	12	54	64.1600	12
50	45.1200	12	57	54.9800	19	8	58.4700	18	89	64.4000	12
85	47.3400	12	83	55.2000	12	76	58.6200	12	28	64.5000	18
94	49.4000	11	82	56.0000	11	81	58.8000	12	78	64.6500	19
26	49.7000	11	10	56.2800	13	107	58.9400	48	12	64.9700	11
96	50.9000	19	42	56.6000	11	75	59.0000	11	108	65.0000	12
110	51.9000	11	106	56.9000	12	56	59.3200	11	21	65.4000	11
84	52.5000	19	32	57.1200	19	69	59.3800	11	41	66.9000	12
114	53.0000	11	99	57.3800	11	16	59.9000	11	5	68.2000	18
53	53.0000	11	66	57.5300	11	24	59.9900	19	30	68.7000	12
113	53.6400	19	74	57.6300	12	4	60.7000	19	71	69.0000	99
65	53.7000	11	73	57.7700	12	61	60.7800	11	25	74.0700*	11
2	53.7000	99	52	57.9400	12	51	63.0000	12			
13	54.1000	99	38	58.0000	12	31	63.0000	11			
70	54.3000	11	95	58.3000	19	77	63.4000	11			

Figure 1. Example of the Statistical Report for Each Constituent in a Sample

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MR. TELLIARD: Thank you, Paul. Our next speaker is George Stanko. George is going to talk about somebody else's methods for a change.

GEORGE H. STANKO

SHELL DEVELOPMENT COMPANY

INTER- AND INTRALABORATORY ASSESSMENT OF SELECTED SW-846 METHODS FOR ANALYSIS OF APPENDIX VIII COMPOUNDS IN GROUNDWATER

MR. STANKO: I'd like to share with you the results of a Chemical Manufacturers Association study that evaluted some selected SW-846 methods for the analysis of Appendix VIII compounds in groundwater. I'd also like to point out that this is really a Chemical Manufacturers Association paper or project, and that I am co-author with Peter Fortini of the American Cyanamid Company.

On October 1st, 1984, the EPA proposed in the Federal Register to make the use of SW-846 methods mandatory under Subtitle C, 40 C.F.R., Parts 260 through 271. In 1983, they had published the second edition of SW-846. In May of 1983, the Chemical Manufacturers Association hired a contract laboratory, Environmental Testing and Certification Corporation, to do an evaluation of the effectiveness of SW-846 as a methods manual. The American Petroleum Institute also hired the Radian

Corporation to conduct a similar study on the same document. Both of these reports revealed that SW-846 was not adequate to guide an analytical lab due to the lack of sufficient information and details, technical inaccuracies and inconsistencies, and also, pointed to numerous problems with the compounds on the Appendix VIII list.

To verify the findings of the CMA study on the second edition, a project was developed in which three prominent laboratories participated. The prime objective of the project was an assessment of the inter- and intralaboratory precision and bias of some selected methods out of SW-846. A second objective was to determine how well the resulting data from this study would in fact define the contamination problem.

The study was designed to look at a simulated situation, a real environmental situation. We have an abandoned waste site. Up gradient from this waste site is a service station that's been closed. Several years earlier there was a known leak of product, gasoline, and it would be reasonable to expect that it made its way into the groundwater and compounds such as benzene, isobutyl alcohol, toluene, might be expected to be found in the

groundwater, up gradient of the dump site.

Also up gradient from this dump site is a subdivision that uses septic tanks. They have been using liquid drain cleaners, 1,1,1-trichloroethane primarily, and one characteristic of this simulated environmental situation is that all the organics that went into this dump site are absolutely known. It is reasonable to expect after 25 years that all of the drums would have been leaking and the compounds we know we put there probably showed up in the water table. For a person who would have to assess this environmental situation, it would be reasonable to put one up gradient well and three down gradient wells, collect water samples and analyze these water samples.

CMA environmental monitoring task group prepared a list of some 34 compounds from the Appendix VIII list, and also prepared a list of distribution across these wells in this environmental situation I just described. CMA sent requests for proposals to four prominent laboratories and each of the laboratories were asked to maintain the integrity of the list and the samples. CMA task group suggested some nine methods that were listed in SW-846 as possible candidates to define this environmental

situation. Three bids were received from the four laboratories and all three of those laboratories concurred that method 8240, 8270, which are GC/MS methods, and 8330, which is an HPLC method, would be suitable to identify all the compounds on the list. This was not surprising since the list was prepared, more or less, with that in mind. Many of us wanted to test the best procedures we thought were in SW-846 and we arbitrarily picked one HPLC procedure that no one had any experience with HPLC for the Appendix VIII list type compounds.

CMA accepted proposals from all three of these laboratories and they selected one of these laboratories to prepare the simulated environmental situation for analysis by all laboratories. The list that we originally sent out of the RFP was altered--some of it. Also, the concentrations that were initially listed had been changed a little bit. Each lab was advised to use the three methods that they themselves indicated would be suitable, and each laboratory was also advised that there were some Appendix VIII compounds not listed in the two methods, but amenable to the procedures, according to the EPA. Three laboratories analyzed these samples independently and submitted the data

to CMA. One of the laboratories was selected to collect the data, compile it and do statistical analysis.

The actual water used for this sample was a groundwater sample collected in the coastal plain region of Texas. It was provided by one of the member companies of the CMA environmental monitoring task group.

SLIDE 1

This particular slide identifies the physical and chemical properties of that groundwater. We also analyzed a portion of this water to verify that none of the organics of interest for this study was indeed present in the sample. It turns out that the water in this well had no organics.

The study had four samples, and because we were interested in inter- and intralaboratory precision, bias, we elected to run these samples as blind triplicates. We used a random number generator, my wife. We assigned a number to each one of the four samples, and each of the three laboratories received 12 samples with 12 different numbers. The only identification on the sample was a number.

The samples that were prepared for this study followed procedures similar to those described in

Section 7, "Calibration" of Method 624. Sample 1 was a background and contained only four volatile organics; benzene, toluene, 1,1,1-trichloroethane--and the slide will tell us what the next one is.

SLIDE 2

Isobutyl alcohol. The three other samples were prepared to actually simulate what might be expected in the down gradient wells. The slide shows the compounds that were used for the volatile list. Sample 1 contained four volatiles in a concentration that varied from 25 parts per billion to 50 parts per billion. Sample 2 contained approximately half of the volatiles. Sample 3 contained the other half of the volatiles, and Sample 4 was the one that was loaded up and contained each one of the volatile organic compounds.

SLIDE 3

This is a list of the semi-volatile compounds or the compounds that were amenable to Method 8270. As you can see, Sample 1 was the background. It only contained volatile materials. Sample 2 contained the lowest concentration and about half of the semi-volatile compounds. Sample 3 contained about the other half, and and here again, Sample 4 contained all of the compounds. The concentrations

were highest in Sample 4.

SLIDE 4

This slide identifies the three compounds that were taken off of the 8330 list, more or less amenable to this procedure. Sample 1, again, did not contain any. Samples 2, 3 and 4 contained various concentrations ranging from 100 Mg/L to 500 Mg/L.

The data from the study was statistically analyzed to assess precision and bias. The assessment of bias procedure was taken from the quality assurance section of SW-846, Methods 8240, 8270. In this procedure, one calculates the percent recovery of spiked compounds; you calculate the standard deviation of the percent recovery, assuming a normal distribution; and you calculate the percent relative standard deviation. Mean percent recovery and percent relative standard deviation define bias and precision as stated by the EPA for a given compound.

SW-846 Method 8240 and 8270 also have the performance criteria to be met in Section 8.2.4. The mean recovery must be greater than 20 percent for all compounds to be measured, greater than 60 percent for all surrogate compounds, and the percent relative standard deviation must be less than 20

percent for all compounds to be measured, including the surrogates.

Section 8.1.1 of the quality assurance section states, before performing any analyses, the analyst must demonstrate ability to generate acceptable accuracy and precision with this method. Ability is established in Section 8.2 that I just described. Calculations from the study were made for each of the laboratories and each of the compounds.

SLIDE 5

This is a summary table that was prepared because you wouldn't be able to read all the small numbers. What this does is an assessment of how well these prominent laboratories met EPA criteria stated in Method 8240 and 8270. For Method 8240, there were 17 compounds. Laboratory A did not meet them for 29 percent, B for 29 percent. Laboratory C did not meet them for 94 percent of the compounds. For 8270--31, 69 and 81. For Method 8330, there were three compounds studied. The laboratories, all three, 100 percent, did not meet the criteria.

It's difficult to explain why such a high percentage of the data did not meet the EPA criteria since these were experienced labs, experienced operators and useed very good equipment. I'd also

like to point out that there is no guidance listed in either one of the analytical procedures to tell you what to do when you do not meet the criteria.

An assessment of precision that we selected to use was different from the EPA. We did not use percent relative standard deviation. We used lognormal distribution theory to assess the precision. As a measure of a precision, we selected to use variability factors and repeatability factors. These define 95 percent confidence intervals. This defines variability factor, Vu, and as an example, if you have a variability factor of 1.68 for an organic compound and the true or the mean concentration is known to be 100 parts per billion, the 95 percent confidence interval of all observations should fall between 60 parts per billion and 168 parts per billion, using these calculations.

When you don't know the true value or the mean value, and you have a single observation, and you're trying to define the distribution of a second observation, you use a repeatability factor. Here again, I use the same example. The range is expanded; 208 parts per billion, 48 parts per billion. That describes, more or less, how variability and repeatability factors are used to define precision

of the method.

In our statistical analysis of the data, we considered looking at the nondetected observation--we put the stuff in the samples and it should have had a value--and we elected not to include non-detects in our data. It would have made things look a lot worse if we had.

There were two unusual values for diphenylamine on different samples by one of the laboratories, and a single value for methyl ethyl ketone by a different laboratory. We excluded three outliers out of somewhere between 500 to 600 observations. I don't think we overdid it. The standard deviations from the triplicate analyses were pooled over the laboratories--samples, compounds and specific basis.

I have a number of copies available of this paper and there is a Table 9 in there. If you're interested in finding out for each one of the compounds, it's in there. I will only show a summary of the intra-laboratory data on this slide.

SLIDE 6

This is a summary based on the Method 8240, 8270, for the three particular laboratories. You can look at the Vu values or the Ru, whatever you care to, the number of degrees of freedom are

listed. You can see that for the different compounds, the laboratories did not perform equally as well. For 8240, Laboratory B had the smallest Vu value or variability factor, which means it has the best precision. For the 8270 methodology, Laboratory A had better precision. If you want to look at 8330, one laboratory didn't see anything, and I would really disregard all the results, the Vu and Ru; there's just not enough values there.

We also took the data and handled it on the inter-laboratory basis. In other words, we pooled all the data and these are the results. Here again, in a copy of the paper you can look at individual compounds on a laboratory basis. This is a summary of values in the paper itself. You can see for Method 8240, the variability factor is 2.31; for 8270, 2.40, exclusive of a couple of outliers--and there's not a whole lot of difference between the precision of these two methods on an average. I would like to direct your attention to the Ru range. For an Ru value of 3.27, the actual range of Ru values is 1.70 to 9.01. It is very sensitive to laboratories as well as compound specific.

There were other problems with the SN-846 methods. There were 11 compounds which one or

more laboratories failed to detect in any of the samples.

This brings up my favorite topic--false positive, false negative observations. These are two important properties to actually describe the qualitative aspects of an analytical method. The CMA study was designed to allow a very good assessment of false positive and false negative observations. We knew what compounds we had spiked into water. We looked at the data.

SLIDE 7

These are the false positive, false negative observations for Method 8240 volatile compounds. For Sample 1, we had seven false positive observations by two laboratories. It's interesting, if you look at the actual raw data, which is also included with the report, that the total concentrations of false positives exceeded what we put in the sample initially.

The false negative observations from the list of compounds that we used for the study, are listed on this slide. Three laboratories could not find isobutyl alcohol at a concentration range between 50 and 250 mg/L in all 12 of the samples. That's a false negative. Three laboratories also could not

detect the presence of 1,4-dioxane in the six samples that it had been spiked in. The concentration level here was 200 to 400 parts per billion. Here again, I'm not going to go through all these, but of the 17 compounds that were spiked into two or more samples, there were seven cases of false negatives.

SLIDE 8

This is the tally sheet of false positives and false negatives for Method 8270. There are eight false positives by one or more of the laboratories. There was an interesting situation where two of the laboratories had a false positive of N-nitrosodiphenylamine in six samples. I would hate to put somebody in jail on that kind of evidence and knowing that compound was not spiked in the sample and not present. Two laboratories confirmed it was.

One interesting thing was noted in the data. One laboratory did not experience any false negatives with Method 8270. The tally sheet shows what the other two laboratories did on a compound specific basis. Some had problems with methyl methacrylate, acetophenone. The one surprising thing, one of these is a priority pollutant, chlorobenzene. One laboratory could not find it in six samples at a

concentration between 100 and 200 Mg/L.

A second objective of the study was how well the resulting data defined the groundwater situation that I described earlier. The results from the study were reviewed from the perspective of those conducting an environmental assessment and not from an analytical point of view. Because the true concentration and identity was known, it was possible to evaluate the data on how well groundwater contamination at the hypothetical situation was really defined.

The analytical results were arbitrarily classified for the spiked components only and did not deal with false positives. You either failed to detect the compound, which is a false negative... If you detected a compound, we had two categories. You detected it and it was within a factor of two of what was actually spiked into the samples, or you detected it and there was a factor greater than two than the actual spiked amount.

SLIDE 9

Table 9 in the handout lists this information for all of the compounds. This is a summary table right here. What this tells you is that we do better for some compounds than we do for others.

This actually identifies agreement between the analysis run by two laboratories for Samples 2, 3 and 4 only. Percent of the compounds detected in the first laboratory will be found by the second laboratory.

For the 8240 compounds, the non-chlorinated, you can see that for 49 percent...the bulb went? I'll have to ad lib from here on out without slides. Essentially what this says, is that if a first laboratory found a compound, the chances of a second laboratory...you have one out of two chances that a second laboratory would indeed find it. I can't go through all of the data on that basis. It is included in the report.

I think I'll just drop down to the conclusions and the recommendations from CMA. The results from the CMA SW-846 Assessment Study revealed that even the best GC/MS methods contained in SW-846 are somewhat inadequate for the analysis of compounds reportedly amenable by Method 8240 and 8270 from the Appendix VIII list. Method 8330 was found to be completely inadequate for the three compounds included in the study. This observation was confirmed by all three laboratories. The statement that SW-846 contains analytical methods for all

375 compounds from the Appendix VIII list simply is not true.

Review of the results from the assessment of bias indicated that the three laboratories were not able to meet the EPA criteria to demonstrate one's ability to generate acceptable accuracy and precision for a large percentage of the compounds included in the study. This observation was unexpected since three prominent laboratories--and these were experienced laboratories--took part in the study, and the samples contained only pure compounds in a relatively simple groundwater matrix.

There were a large number of false positive and negative observations by all laboratories to varying degrees. This observation is of particular concern since GC/MS methodology was employed for rather simple samples in a rather simple groundwater matrix. With less specific detectors such as FID (GC) or UV (HPLC), the problem of false positive and negative observations would be expected to be even more severe. The resulting data for Method 8330 supports this conclusion.

The calculated Vu and Ru values, repeatability and variability factors, which express intra- and interlaboratory basis, revealed precision varies

from laboratory to laboratory for different methods and for specific compounds. These facts must be considered when analytical data are being interpreted to assess an environmental situation.

It is reasonable to conclude that the numerous problems observed for Method 8240, 8270 and 8330 for the samples included in the CMA SW-846 Assessment Study, may be expected for other methods in SW-846 not evaluated by SW-846. SW-846 certainly has not reached the level of development whereby its use should be mandated by law. Until all of the methods contained in SW-846 are adequately validated, SW-846 is nothing more than a collection of methods that may or may not work. It is only suitable as a reference document. Even as a reference document, SW-846 has limitations.

If one reviews the resulting data from the CMA study from a data user's perspective, one would conclude that the analytical community could indeed identify groundwater contamination, but not very well both qualitatively and quantitatively. The problem of false positive and false negative observations would be of major concern in determining intermediate corrective action, as well as the potential for future problems.

CMA recommends that the EPA should not promulgate the mandatory use of SW-846 for testing for Appendix VIII compounds under Subtitle C of RCRA. The methods have only been validated for a few of the Appendix VIII list and further validation is required before promulgation.

The EPA should concentrate its efforts initially to the validation and development of GC/MS Methods 8240 and 8270. The list of Appendix VIII compounds amenable to these procedures needs to be established, along with supporting precision and bias data.

For Appendix VIII compounds not amenable to 8240 and 8270, EPA needs to validate other methods in SW-846 to confirm they actually work for some matrix with a reasonable expectation that they may work in other matrices.

Multilaboratory studies of all methods similar to the CMA SW-846 Assessment Study would be advisable prior to including a method into a manual such as SW-846, and prior to considering promulgation of a method for mandatory compliance testing. Results from such studies should be subjected to the analytical peer review process and appropriate statistical evaluation, both within and outside the Agency.

I'd like to give an acknowledgement to the CMA

Environmental Monitoring Task Group who contributed greatly to this paper, and particularly to CMA Staff Excecutive, Sharon Kneiss, who happens to be in the audience, and to Becky Wilson, the CMA Administrative Assistant.

Thank you.

MR. TELLIARD: I'm glad you're behind us again, George. Any questions?

Inter– and Intralaboratory Assessment of Selected SW–846 Methods for Analysis of Appendix VIII Compounds in Groundwater

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08611-1

Physical and Chemical Properties of Groundwater
CMA Assessment Study

Property	*Results*
Appearance	Very Turbid, Sandy Solids
pH	6.9
Total Suspended Matter	1060 mg/L
Total Dissolved Solids*	580 mg/L
Chloride, Cl	70 mg/L
Hardness, as $CaCO_3$	318 mg/L

Filtered thru 0.45μ membrane.

08611-2

Sample Code Used for CMA Assessment Study

Samples	*Assigned Numbers*
Sample 1	10, 7, 6
Sample 2	8, 11, 2
Sample 3	5, 1, 9
Sample 4	3, 12, 4

08611-3

CMA SW–846 Assessment Study (8240)
Sample Identity and Concentration Information

	Samples (µg/L)			
Compounds	*1*	*2*	*3*	*4*
Acetone	–	200	–	400
Acetonitrile	–	200	–	400
Benzene	50	50	50	100
Carbon Tetrachloride	–	–	200	300
Chloroform	–	25	–	50
1,1–Dichloroethane	–	25	–	50
1,2–Dichloroethane	–	100	–	200
1,2–Dichloropropane	–	100	–	200
Ethylbenzene	–	–	200	200
Tetrachloroethane	–	–	200	300
1,1,1–Trichloroethane	25	25	25	325
Trichloroethene	–	–	200	300
Toluene	25	25	25	75
1,1,2,2–Tetrachloroethane	–	–	200	300
Methyl Ethyl Ketone	–	200	–	400
Isobutyl Alcohol	50	50	250	250
1,4–Dioxane	–	200	–	400

08611-4

CMA SW–846 Assessment Study (8270)
Sample Identity and Concentration Information

	Samples (µg/L)			
Compounds	*1*	*2*	*3*	*4*
Aniline	–	25	–	250
p–Chloro–m–Cresol	–	100	–	200
2–Chlorophenol	–	–	300	350
Di–n–Octyl Phthalate	–	–	300	500
Diphenylamine	–	100	–	250
Methylmethacrylate	–	–	300	500
Naphthalene	–	100	–	250
4–Nitrophenol	–	–	300	500
Phenacetin	–	–	300	500
2,4,6–Trichlorophenol	–	–	300	350
2,4,5–Trichlorophenol	–	–	300	350
4–Chlorophenol	–	–	300	350
Acetophenone	–	25	–	250
1,3–Dichlorobenzene	–	25	–	200
Chlorobenzene	–	100	–	200

08611-5

CMA SW–846 Assessment Study (8330)
Sample Identity and Concentration Information

	Samples (µg/L)			
Compounds	***1***	***2***	***3***	***4***
Thiourea	–	–	300	500
N–Phenylthiourea	–	100	300	500
1–Acetyl–2–Thiourea	–	100	300	500

08611-6

Percent of Recovery Observations *Not* Meeting EPA Criteria

	Laboratory		
SW–846 Method	*A*	*B*	*C*
8240 (17)*	29	29	94
8270 (16)*	31	69	81
8330 (3)*	100	100	100

**Total number of compounds included in calculations.*

08611-7

Variability Factor

Vu = Exp (2 S) for the Upper Limit

Vl = Exp (–2 S) for the Lower Limit

Example

Factor = 1.68
True or Mean = 100 ppb

Vu = 100 ppb • 1.68 = 168 ppb

Vl = 100 ppb/1.68 = 60 ppb

08611-8

Repeatability Factor

$Ru = Exp(2 \cdot \sqrt{2}S)$ for the Upper Limit

$Rl = Exp(-2 \cdot \sqrt{2}S)$ for the Lower Limit

Example

Factor = 2.08
First Analysis = 100 ppb

$Ru = 100 \text{ ppb} \cdot 2.08 = 208 \text{ ppb}$

$Rl = 100 \text{ ppb}/2.08 = 48 \text{ ppb}$

08611-9

CMA SW–846 Assessment Study

Intralaboratory Precision: Within Compound and Sample Pooled

Method	*Laboratory*	*Degrees of Freedom*	*Vu*	*Ru*
8240	A	70	1.29	1.44
	B	60	1.21	1.32
	C	53	2.14	2.94
8270	A	64	1.63	2.00
	B	43	3.34	5.50
	C	50	2.40	3.45
8330	A	3	1.63	2.00
	B	6	2.43	3.52
	C	0	–	–

08611-10

CMA SW–846 Assessment Study
Interlaboratory Precision, Combined*

Method	*Vu*	*Ru*	*Ru Range*
8240	2.31	3.27	1.70–9.01
8270	2.40**	3.46**	1.63–7.63
8330	–	–	–

* *Based on average variance.*
** *Excluding 4–nitrophenol, 2,3,5–trichlorophenol.*

08611-11

False Positive and Negative Observations

Method 8240 (Volatiles)

7 False Positives, Sample 1, 2 Labs

False Negatives

3 Labs, Isobutyl Alcohol	50–250	µg/L	(12)
3 Labs, 1,4–Dioxane	200–400	µg/L	(6)
2 Labs, Acetone	200–400	µg/L	(6)
2 Labs, Acetonitrile	200–400	µg/L	(6)
2 Labs, Methyl Ethyl Ketone	200–400	µg/L	(6)
1 Lab, 1,1,1–Trichloroethane	25	µg/L	(9)

Of 17 Compounds Spiked into 2 or More Samples, There Were 7 Cases of False Negatives

08611-12

False Positive and Negative Observations

Method 8270 (Semi–Volatiles)

8 False Positives, 1 or More Labs

2 Labs N–Nitrosodiphenylamine in Same 6 Samples

1 Lab, No False Negatives

2 Labs, Methylmethacrylate	300–500 µg/L	(6)
2 Labs, Acetophenone	25–250 µg/L	(5)
1 Lab, Diphenylamine	100–250 µg/L	(6)
1 Lab, Phenacetin	300–500 µg/L	(6)
1 Lab, Chlorobenzene	100–200 µg/L	(6)

08611-13

Classification of Detection
(Samples 2, 3, 4 Only)

Compound Class	% of Detections Which Are: False Positive	Present but not Within Factor of X2	Present Within X2	(# Detection)
8240 Nonchlorinated*	16	24	60	(55)
Chlorine Containing**	0	4	96	(134)
8270 Nonchlorinated	25	20	55	(138)
Chlorine Containing	4	26	70	(142)
8330 Sulfur Containing	76	24	None	(62)
Combined Performance	18	19	63	(531)

* *Benzene, toluene, isobutyl alcohol background compounds not included.*
** *1,1,1 trichloroethane not included. Methylene chloride false detections not included.*

08611-14

Agreement Between Analyses Run by Two Laboratories
(Samples 2, 3, 4; All Pairs with Same Sample, Different Laboratory)

Compound Class*	% of Compounds Detected by the First Laboratory Which Will Be:		
	Not Detected by Second Lab	Found but not in Quantitative Agreement	Found, in Amount Within a Factor of X2 of Original Determination
8240 Nonchlorinated	49	21	30
Chlorine Containing	8	16	76
8270 Nonchlorinated	42	27	31
Chlorine Containing	10	22	68
8330 Sulfur Containing	88	11*	1**
Combined Performance	31	20	49

* *Notes under Table 10 apply. Thus, background and methylene chloride data are excluded.*
** *Confirmed findings were, in fact, false positives.*

08611-15

Acknowledgements

CMA Environmental Monitoring Task Group

Sharon H. Kneiss, CMA Staff Executive

Becky Wilson, CMA Administrative Assistant

08611-16

QUESTION AND ANSWER SESSION

MR. BRITTON: What were the outlier criteria that you used in that study, George?

MR. STANKO: It failed all of them. Grubbs was the primary one. Those three values were so far out that they were out by a factor of 10 to 15 from the rest; very isolated cases. We didn't chase down what caused the outlier.

MR. BRITTON: So you basically ignored only those observations which were roughly a factor of 10 larger than they should have been?

MR. STANKO: We applied the Grubbs test, but that's how far out they were, to give you some idea of the magnitude of how far out they were.

MR. BRITTON: Of course it depends on the confidence level.

MR. STANKO: Right.

MR. BRITTON: That's one of the reasons why there'd be a great difference between results from your analysis and results from our analysis, even on the same data. We have a bit

of a difference in philosophy, I think, as to whether we are concerned with characterizing the data that would reflect in general use of the method, or whether we're interested in characterizing how the method data would look when performed properly. That philosophical difference is tremendous. It's one of the reasons why the log transformation...log transformation is appropriate if you're trying to encompass virtually all the data that would be generated by use of that method in practice but would basically accomodate all of the data that represents inadequate performance. But if you are interested in what the method would look like, what data from the method would look like when performed properly, then you have to try and look past that data. It's a very big trick cause none of us know exactly which numbers are bad and which numbers aren't. We want to look past those bad numbers.

MR. TELLIARD: John.

MR. McGUIRE: John McGuire, EPA. George, one question. You said...probably I wasn't paying attention. You said that you had filtered the samples. Was that before or after spiking.

MR. STANKO: No, the samples were not filtered.

MR. McGUIRE: I thought the groundwater was filtered.

MR. STANKO: No. That groundwater sample contained solids. But we looked at the data to see if the solids had any affect on the analysis of the compounds and in all honesty, I could not detect any irreversible absorption of the compounds we used in this study.

MR. McGUIRE: Okay. There was no attempt made to extract the solid itself, then?

MR. STANKO: The solids were extracted, yes.

MR. McGUIRE: They were?

MR. STANKO: With the water, in situ.

MR. McGUIRE: Okay, but I mean totally extracted?

MR. STANKO: Total extraction.

MR. McGUIRE: Thank you. I certainly hope I can get a copy of that.

MR. TELLIARD: In the back.

Any other questions? All right, folks, thank you very much.

Point of interest. Tomorrow at a quarter to nine here. The boat will be leaving about 5:30-ish. That gives you about 20 minutes to get your snuggies on and get out there. Waterside, down there by Phillips. Thank you.

(WHEREUPON, the 4-3-85 session was adjourned.)

Revised Presentation

INTER- AND INTRALABORATORY ASSESSMENT OF SELECTED SW-846 METHODS FOR ANALYSIS OF APPENDIX VIII COMPOUNDS IN GROUNDWATER

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Analysis of Pollutants in the Environment
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ABSTRACT

EPA Methods 8240, 8270 (GC/MS), and 8330 (HPLC) from SW-846 were evaluated at three prominent laboratories for a select list of 36 compounds that were spiked into a relatively simple groundwater matrix. Sample preparation was designed to simulate a groundwater contamination problem at an abandoned waste site and samples were prepared as blind triplicates to facilitate statistical analyses for precision and bias. Results from the assessment of bias (accuracy, recovery) indicated that the three laboratories that took part in the study were not able to meet EPA's quality assurance criteria for a large percentage of the 36 compounds included in the study. The assessment for precision revealed precision varies from laboratory to laboratory, for different methods, and for specific compounds. A large number of false positive and negative observations resulted at all laboratories to varying degrees. The list of Appendix VIII compounds reportedly amenable to Methods 8240 and 8270 was found to be somewhat less than reported by EPA. Method 8330 was found to be completely inadequate for the detection and quantification of the three compounds included in the study. The statement by EPA that SW-846 contains the analytical methods for all (375) Appendix VIII compounds (excluding exotics and water reactive compounds) simply is not true.

RLL8506605

INTER- AND INTRALABORATORY ASSESSMENT OF SELECTED SW-846 METHODS FOR ANALYSIS OF APPENDIX VIII COMPOUNDS IN GROUNDWATER

1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) published "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods" (SW-846) to serve as a methods manual for the sampling of groundwater or leachate and analyses of 375 parameters listed in Appendix VIII of Title 40, Part 261 of the code of Federal Regulations (40 C.F.R. 261). On October 1, 1984 (Fed. Reg. pp. 38786-38809), the EPA proposed to amend its hazardous waste regulations under Subtitle C of the Resource Conservation and Recovery Act (RCRA) to make SW-846 methods mandatory for all testing and monitoring activities required under Subtitle C, as specified in 40 C.F.R. Parts 260-271. The EPA identified the principal issue of the proposed rule as "how" testing under RCRA should be carried out, and the proposed rule specifically addresses SW-846 to be used to, "ensure accurate, consistent, and comparable testing results--year to year, facility to facility, and region to region".

In May 1983, Environmental Testing and Certification Corporation (ETC) prepared a report[1] for the Chemical Manufacturers Association (CMA) from evaluating the effectiveness of SW-846 (2nd Edition) in assuring that users would be guided to select consistent analytical and sampling procedures for each parameter. The American Petroleum Institute had a similar report[2] prepared by Radian Corporation. Both of these reports revealed that SW-846 was not adequate to properly guide an analytical laboratory due to lack of sufficient information and details, technical inaccuracies, and inconsistencies. The evaluations by ETC and Radian also pointed to numerous problems associated with compounds identified by the Appendix VIII list. To verify these findings, CMA conducted a limited study at three prominent laboratories involved in environmental analyses. The prime objective of the CMA study was an assessment of inter- and intralaboratory precision and bias (accuracy) of selected analytical methods from SW-846. A second objective was to determine how well the resulting data would define groundwater contamination where the identity and levels of all pollutants were known.

2. STUDY DESIGN

The CMA limited study was designed to simulate an environmental situation that could very well exist, and someone would have to install wells; collect and analyze groundwater samples; and finally assess the nature and magnitude of the problem. The environmental situation was as follows:

> There is an old waste disposal site where groundwater contamination is suspected. The direction of flow of the grourdwater is known and a decision was made to install four monitoring wells. One of these wells is located as a background well and three are down gradient from the disposal site. Up gradient from the background well is a gasoline service station that experienced a leaking storage tank in the past;

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however, groundwater contamination from the leak was never assessed. A small subdivision up gradient from the waste site uses septic tanks. Chlorinated drain cleaner (1,1,1-trichloroethane) has been used for years. All of the organic compounds that were disposed of at the site (unlined) are known. It is reasonable to assume that the drums have been leaking for sometime, and it would be reasonable to believe that all of the compounds have migrated into the groundwater.

Four samples were designed for the CMA study to simulate the environmental situation described. A list of 34 organics of interest from Appendix VIII was developed and the distribution and approximate concentrations for the four samples was prepared for a request for proposal (RFP) from four prominent contract laboratories. The laboratories were contacted about the CMA RFP and advised to maintain the integrity of the compound list as well as the sample list. As part of the RFP, nine SW-846 methods were suggested to be used to conduct the analyses. CMA received bids from three laboratories for the study. All three laboratories concurred and recommended that SW-846 methods 8240 (GC/MS for volatiles), 8270 (GC/MS for semi-volatiles), and 8330 (HPLC method, sulfur compounds) be used to analyze for the select list of compounds. This concurrence was not surprising since the compound list was originally designed to test the GC/MS methods which were considered the best and most reliable in SW-846. Method 8330 was randomly selected for evaluation of an HPLC procedure from SW-846 and compounds listed in the method were included in the compound list.

CMA accepted proposals from three laboratories to analyze the samples for the study and selected one laboratory to prepare the samples and to prepare a report which included data from all laboratories. To maintain the integrity of the study, the CMA list of compounds was slightly altered with respect to compounds listed as well as concentrations in various samples. In the case of the laboratory that prepared the samples, special precautions were taken to make sure the confidential information was not available to the analysts. Each of the laboratories was instructed by CMA to analyze each sample by SW-846 Methods 8240, 8270, and 8330. The laboratories were also instructed that there were other Appendix VIII compounds not listed in Methods 8240 and 8270, but were amenable to these methods according to EPA. Three laboratories performed the analyses independently and submitted the data to CMA. When all data were reported, CMA sent the data to the one laboratory selected to prepare a report. Statistical analysis of all the data was performed to determine inter- and intralaboratory precision and bias. These statistical results from the contract laboratory were included and were the basis for this report.

3. EXPERIMENTAL

3.1 Groundwater Matrix

Groundwater for the study was provided by a CMA member company. The water was collected from a monitoring well located in the coastal plain region of Texas. Some physical and chemical properties for the water are listed in Table 1.

Table 1
Physical and Chemical Properties of Groundwater - CMA Assessment Study

Property	Results
Appearance	Very turbid, sandy solids
pH	6.9
Total Suspended Matter	1,060 mg/L
Total Dissolved Solids[a)]	580 mg/L
Chloride, Cl	70 mg/L
Hardness, as $CaCO_3$	318 mg/L

a) Filtered thru 0.45μ membrane.

A portion of the groundwater was also analyzed prior to preparation of samples to establish the presence or absence of organics. The results demonstrated the well sample to be free of organics and specifically the list of organics of interest from the Appendix VIII list. Since the groundwater was free of organics, method bias (recovery, accuracy) could easily be assessed from the spiking levels (true value = spiking value).

3.2 Sample Identification

Four samples were used for the CMA study. To obtain the necessary data, these four samples were analyzed as blind triplicates. Sample numbers were randomly generated and assigned to the samples. Table 2 identifies the sample numbers employed.

Table 2
Sample Code Used for CMA Assessment Study

Samples	Assigned Numbers
Sample 1	10, 7, 6
Sample 2	8, 11, 2
Sample 3	5, 1, 9
Sample 4	3, 12, 4

Each laboratory received 12 samples identified only by a single number. For each sample number they received two one-liter containers and two VOA vials. The study was coordinated in a way that analysis of samples was initiated on the same day.

3.3 Sample Preparation

Table 3 reveals the identity and spiking levels of compounds in each of the four samples. Complete details for the sample preparation have not been included in this report. The groundwater matrix was spiked following procedures described in Section 7, "Calibration" of EPA Method 624. The samples were prepared to simulate what might be expected for the four hypothetical monitoring wells described in the enviornmental situation. Sample 1 (identified by numbers 6, 7, and 10) was prepared to represent the

TABLE 3

CMA SW-846 ASSESSMENT STUDY
SAMPLE IDENTITY AND CONCENTRATION INFORMATION

METHODS	COMPOUNDS	3	4	12	1	5	9	2	8	11	6	7	10
8240	Acetone	400	400	400	-	-	-	200	200	200	-	-	-
	Acetonitrile	400	400	400	-	-	-	200	200	200	-	-	-
	Benzene	100	100	100	50	50	50	50	50	50	50	50	50
	Carbon Tetra-chloride	300	300	300	200	200	200	-	-	-	-	-	-
	Chloroform	50	50	50	-	-	-	25	25	25	-	-	-
	1,1-Dichloro-ethane	50	50	50	-	-	-	25	25	25	-	-	-
	1,2-Dichloro-ethane	200	200	200	-	-	-	100	100	100	-	-	-
	1,2-Dichloro-propane	200	200	200	-	-	-	100	100	100	-	-	-
	Ethylbenzene	200	200	200	200	200	200	-	-	-	-	-	-
	Tetrachloro-ethane	300	300	300	200	200	200	-	-	-	-	-	-
	1,1,1-Tri-chloroethane	325	325	325	25	25	25	25	25	25	25	25	25
	Trichloro-ethene	300	300	300	200	200	200	-	-	-	-	-	-
	Toluene	75	75	75	25	25	25	25	25	25	25	25	25
	1,1,2,2-Tetra-chloroethane	300	300	300	200	200	200	-	-	-	-	-	-
	Methyl Ethyl Ketone	400	400	400	-	-	-	200	200	200	-	-	-
	Isobutyl Alco-hol	250	250	250	250	250	250	50	50	50	50	50	50
	1,4-Dioxane	400	400	400	-	-	-	200	200	200	-	-	-
8270	Aniline	250	250	250	-	-	-	25	25	25	-	-	-
	p-Chloro- m -Cresol	200	200	200	-	-	-	100	100	100	-	-	-
	2-Chlorophenol	350	350	350	300	300	300	-	-	-	-	-	-
	1,2-Dichloro-benzene	200	200	200	-	-	-	25	25	25	-	-	-
	Di-n-Octyl phthalate	500	500	500	300	300	300	-	-	-	-	-	-
	Diphenylamine	250	250	250	-	-	-	100	100	100	-	-	-
	Methylmeth-acrylate	500	500	500	300	300	300	-	-	-	-	-	-

SAMPLE IDENTITY AND CONCENTRATION INFORMATION

TABLE 3 cont'd.

METHODS	COMPOUNDS	3	4	12	1	5	9	2	8	11	6	7	10
8270 cont'd	Naphthalene	250	250	250	-	-	-	100	100	100	-	-	-
	4-Nitrophenol	500	500	500	300	300	300	-	-	-	-	-	-
	Phenacetin	500	500	500	300	300	300	-	-	-	-	-	-
	2,4,6-Tri-chlorophenol	350	350	350	300	300	300	-	-	-	-	-	-
	2,4,5-Tri-chlorophenol	350	350	350	300	300	300	-	-	-	-	-	-
	4-Chloro-phenol	350	350	350	300	300	300	-	-	-	-	-	-
	Acetophenone	250	250	250	-	-	-	25	25	25	-	-	-
	1,3-Dichloro-benzene	200	200	200	-	-	-	25	25	25	-	-	-
	Chlorobenzene	200	200	200	-	-	-	100	100	100	-	-	-
8330	Thiourea	500	500	500	300	300	300	-	-	-	-	-	-
	N-Phenyl-thiourea	500	500	500	300	300	300	100	100	100	-	-	-
	1-Acetyl-2-Thiourea	500	500	500	300	300	300	100	100	100	-	-	-

background well and was spiked with benzene, toluene, isobutyl alcohol (leaking tank), and 1,1,1-trichloroethane (septic tank). These same compounds were spiked in all samples. Samples 2, 3, and 4 were prepared to simulated the three down gradient wells at the hypothetical waste site. As noted in Table 3, samples 2 and 3 contained approximately half of the list of 36 compounds in each sample. Sample 4 contained all compounds at the highest concentration levels. The study was designed so each of the 36 selected compounds was present in a minimum of two samples.

3.4 Analytical Results

The analytical results from the three contract laboratories were summarized into the five tables included in Appendix A. The data were put in a format to facilitate statistical analysis. The tables identify laboratories by code (A, B, C), the coded samples, compounds spiked into samples, and the reported values for the study. Reviewers of Appendix A are cautioned that the purpose for the study was not to compare the performance of a given laboratory with respect to other laboratories, but to assess analytical methods contained in SW-846 for the analysis of Appendix VIII compounds in a real groundwater matrix. It should also be noted that the three laboratories are prominent laboratories that employ modern instrumentation, have years of experience with GC/MS methodology, and certainly have qualified and experienced analyst. There is every reason to believe that the analytical results obtained for the study represent the current state-of-the-art with respect to low level environmental analyses.

3.5 Statistical Analysis of Data

The analytical data were statistically analyzed to assess the two most important criteria of analytical methods, precision and bias (accuracy, recovery). There are numerous acceptable ways that this could be done. One of the procedures employed was taken from procedures described in the EPA methods as part of the quality assurance sections. Every effort was made not to distort the statistical results, but to be conservative and reasonable where arbitrary decisions or assignments were required.

3.5.1 Assessment of Bias (Recovery, Accuracy)

The statistical procedures used to assess bias were taken from the quality assurance section of SW-846 Methods 8240 and 8270. One is directed to calculate percent recovery of spiked compounds, then calculate standard deviation for the percent recoveries assuming a normal distribution for the recovery values. The resulting standard deviation is then used to calculate a percent relative standard deviation (%RSD). The mean percent recovery and %RSD of the percent recovery defines the bias for a given compound by a specific method. For the CMA study, these calculations were done for each laboratory and for all laboratories combined. It should be noted that the %RSD defines the dispersion of values of percent recovery with respect to the simple mean percent recovery. The %RSD also represents one measure for precision as well. While the %RSD values were calculated

TABLE 4

CMA SW-846 ASSESSMENT STUDY
STATISTICAL RESULTS FOR BIAS (RECOVERY, ACCURACY)

Compounds	Spike Range	LAB A			LAB B			LAB C			ALL LABORATORIES			
	ppb	No. Observations	Mean Recovery %	% RSD	No. Observations	Mean Recovery %	% RSD	No. Observations	Mean Recovery %	% RSD	No. Observations	% Recovery Range	Mean Recovery %	% RSD
Method 8240														
Acetone	200-400	6	100	91	6	0	-	6	31	68	18	0-13[illegible]	44	105
Acetonitrile	200-400	6	33	111	6	0	-	6	0	-	18	0- 70	-	-
Benzene	50-100	12	101	15	12	112	7.7	12	123	28	36	64-172	112	21
Carbon Tetrachloride	200-300	6	99	8.0	6	194	15	6	0	-	12	90-220	145	36
Chloroform	25- 50	6	94	14	6	108	6.8	6	73	14	18	66-120	92	19
1,1-Dichloroethane	25- 50	6	87	15	6	124	10	6	55	59	18	0-140	88	40
1,2-Dichloroethane	100-200	6	126	12	6	144	5.6	6	86	52	18	53-153	118	26
1,2-Dichloropropane	100-200	6	83	7.5	6	126	11	6	60	115	15	75-150	107	24
Ethylbenzene	200	6	75	18	6	108	5.5	6	40	67	18	0-115	74	44
Tetrachloroethane	200-300	6	78	6.9	6	110	10	6	95	34	18	60-150	94	25
1,1,1-Trichloroethane	25-325	12	75	17	12	112	9.5	12	26	78	36	0-129	71	59
Trichloroethene	200-300	6	92	2.4	6	133	8	6	89	26	18	70-146	105	24
Toluene	25- 75	12	82	14	12	98	7.6	12	89	28	36	56-152	90	19
1,1,2,2-Tetrachloroethane	200-300	6	61	5.2	6	163	14	6	70	42	18	31-197	98	53
Methyl Ethyl Ketone	200-400	6	71	23	6	2	-	6	49	58	12	23-105	60	41
Isobutyl Alcohol	50-250	6	0	-	6	0	-	6	0	-	18	-	0	-
1,4-Dioxane	200-400	6	0	-	6	0	-	6	0	-	18	-	0	-
Method 8270														
Aniline	25-250	6	64	23	3	91	47	3	60	7	12	28-140	63	45
p-Chloro-m-Cresol	100-200	6	72	44	6	53	6.4	6	56	27	18	35-120	60	35
2-Chlorophenol	300-350	6	76	11	6	79	7.0	6	66	19	18	43- 90	74	14
1,2-Dichlorobenzene	25-200	6	70	18	6	95	18	6	42	24	18	28-128	69	37
1,3-Dichlorobenzene	25-200	6	71	18	6	89	14	6	39	26	18	24-112	66	36
Di-n-Octyl Phthalate	300-500	6	75	16	6	50	64	6	110	50	18	0-187	78	55
Diphenylamine	100-250	6	97	11	6	110	77	6	0	-	12	17-210	104	56
Methylmethacrylate	300-500	6	68	11	6	0	-	6	0	-	18	0- 77	-	-
Naphthalene	100-250	6	64	12	6	61	24	6	48	22	18	33- 77	58	22
4-Nitrophenol	300-500	6	14	51	6	170	29	6	38	50	18	2-255	74	103
Phenacetin	300-500	6	83	13	6	0	-	6	40	27	12	24-100	61	40
2,4,6-Trichlorophenol	300-350	6	73	6.4	6	84	8.6	6	85	27	18	57-117	82	18
2,4,5-Trichlorophenol	300-350	6	105	9.0	6	24	51	6	82	23	18	4-117	70	53
4-Chlorophenol	300-350	6	46	36	6	35	80	6	72	18	18	0- 87	51	43
Acetophenone	25-250	6	67	15	6	60	156	6	0	-	12	0-184	-	-
Chlorobenzene	100-200	6	47	26	6	0	-	6	20	24	12	15- 60	34	49
Method 8330														
Thiourea	300-500	6	0	-	6	0	-	6	0	-	18	-	0	-
N-Phenylthiourea	100-500	9	0	-	9	5	44	9	0	-	27	0- 10	-	-
1-Acetyl-2-Thiourea	100-500	9	1	100	9	0	-	9	0	-	27	0- 3	-	-

and included in the report (Table 4), an alternate procedure was employed to assess precision.

Table 4 was prepared to summarize all the statistical information for bias (accuracy) following the procedures specified by EPA in Section 8.2.4 of SW-846 Methods 8240 and 8270. Table 4 shows the bias results for each compound included in the CMA study by laboratory, by method, and for all three laboratories combined. The number of observations included in each recovery calculation was also listed. Some discretion was used in preparation of Table 4. No mean recovery value for all laboratories was calculated when only one laboratory reported values for a compound. There were several instances where a laboratory reported values for one set of samples but did not find the compound in a second set. For these cases, the zero percent recovery observations were not included in calculations since they would have influenced bias in a way that did not appear justified nor useful.

The performance criteria set forth in Section 8.2.4 of the Quality Control section of SW-846 Methods 8240 and 8270 is identical. "The average percent recovery must be greater than 20 (%) for all compounds to be measured and greater than 60 (%) for all surrogate compounds. The percent relative standard deviation (%RSD) must be less than 20 (%) for all compounds to be measured and all surrogate compounds." Section 8.1.1 states, "Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method (8240, 8270). This ability is established as described in Section 8.2." Table 4 was reviewed to determined the "ability" of the three laboratories to "generate acceptable accuracy and precision" using the EPA criteria. The results are summarized in Table 5.

The data summarized in Table 5 indicate that these three laboratories did not meet the criteria for a large percentage of compounds to demonstrate their ability to generate acceptable data. Considering that the three laboratories are prominent laboratories with considerable GC/MS experience, it is doubtful that any laboratory can meet the criteria for all compounds amenable to the three SW-846 Methods. It should also be noted that the methods give no guidance or instructions on what is to be done when the criteria are not met.

TABLE 5

Percent of Recovery Observations NOT Meeting EPA Criteria

SW-846 Method	Laboratory		
	A	B	C
8240 (17)[a]	29	29	94
8270 (16)[a]	31	69	81
8330 (3)[a]	100	100	100

a) Total number of compounds included in calculations.

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3.5.2 Assessment of Precision

The resulting data from the CMA study were statistically analyzed using lognormal distribution theory to assess precision for each method (i.e., 8240, 8270, 8330). As measures of intralaboratory and interlaboratory precision, variability factors (V) and repeatability factors (R) were calculated.

Upper and lower variability factors are defined by equations (1) and (2) where S is a pooled standard of logarithms of amounts found by analysis.

(1) $Vu = \exp(2S)$ for the upper limit

(2) $Vl = \exp(-2S)$ for the lower limit

These variability factors define 95% confidence limits for the ratio of an observed to a known mean or true value. If the true or mean value is expressed as (X), then 95% confidence limits on an observed value are calculated using equations (3) and (4).

(3) $X \circ Vu$ = upper limit

(4) X/Vu = lower limit

For example, for a mean or true value of 100 ppb and a Vu value of 1.68, 95% of the results for a sample would fall in a range of 60 ppb (100/1.68) to 168 ppb (100 o 1.68).

Upper and lower repeatability factors are defined by equations (5) and (6). These define 95% limits for an observation when the true or mean value is not known but only estimated from a single previous observation (x).

(5) $Ru = \exp(2\sqrt{2}\,s)$ for the upper limit

(6) $Rl = \exp(-2\sqrt{2}\,s)$ for the lower limit

The 95% prediction limits for the second observation are given by equations (7) and (8).

(7) $X \circ Ru$ = upper limit

(8) X/Ru = lower limit

Thus, if the first analysis of a sample gives a value of 100 ppb and the Ru value is 2.08, then with 95% probability, the results for the second analysis will fall in the range of 48 ppb (100/2.08) to 208 ppb (100 o 2.08).

Variability and repeatability factors expressing intralaboratory precision for each compound in the CMA study are given in Table 6.

Reported results were transformed to the standard deviations of log values (amount found) were calculated for each laboratory, compound, and sample from results reported for the (up to) three triplicates. Non-detected observations were omitted from statistical analysis. Two unusual values for diphenylamine on different samples reported by laboratory B, and the single value for methyl ethyl ketone by laboratory B were also excluded as outliers. Standard deviations from triplicate analyses samples were pooled over laboratories and sampled on compound-specific basis and Vu and Ru calculated from equations (1) and (5) using the pooled standard deviations. Also included in Table 6 are the number of samples in which the compound was detected, divided by the number of samples in which it was present; the degrees of freedom in the estimate of (S) used to calculate Vu and Ru; the geometric mean level; and the percent recovery. The geometric mean levels were calculated using equation (9).

(9) Geometric $X = \exp(1/n \Sigma \log X)$

The percent recovery reported is this geometric mean divided by the geometric mean of actual levels in the samples where the compound was detected.

The Vu and Ru calculated in Table 6 represent somewhat of an average within laboratory variability for the three laboratories. These Vu and Ru values can be used to estimate the range of values one might expect (95% confidence interval) for any of the laboratories with respect to a true or mean value (Vu) or first observation (Ru). For example, the value of Ru listed in Table 6 for 1,2 dichloroethane is 2.02. If a first determination for that dichloroethane was 100 ppb, then the range of values for a second observation would be 50 ppb (100/2.02) to 202 ppb (100.2.02) when the same laboratory analyzed the sample a second time. Precision does vary from compound to compound and that this variability must be considered when interpreting the analytical data.

The Vu and Ru values shown in Table 7 quantify the variability each laboratory experienced for the group of compounds using a specific method. Standard deviations for triplicate samples were pooled over compounds detected by each method for each of the three laboratories and Vu and Ru values calculated for each method and laboratory using equations (1) and (5). Using Ru values it can be seen that Laboratory B had the best precision with Method 8240 and the poorest for Method 8270. Laboratory A had the best precision for Method 8270. The precision observed for Laboratory C was fairly consistent for both Methods 8240 and 8270. Although the values for Ru and Vu were calculated for Method 8330 and included in Table 7, they are of very limited value due to the low rate of detection for all laboratories.

Interlaboratory estimates of precision were also computed for the CMA Study and expresssed as Vu and Ru values in Table 8. This was done on a method and compound specific basis. Actual reported values were transformed to the logarithm scale. For each compound, the root mean square different (in this scale) between determinations of different laboratories on equivalent samples was calculated using equation (10).

TABLE 6

CMA SW-846 ASSESSMENT STUDY

Precision Of Methods 8240, 8270, 8330: Within Laboratory and Sample, Pooled

Method, Compound	(ppb) Spike Range	Fraction Detections	Geom $\bar{x}$ (% Rec.)	Degree of Freedom	Vu	Ru
8240						
Acetone	200-400	10/18	183 (60%)	6	1.90	2.48
Acetonitrile	200-400	3/18	129 (65%)	2	1.32	1.48
Benzene	50-100	36/36	65 (110%)	24	1.56	1.87
Carbon Tetrachloride	200-300	12/18	332 (136%)	8	1.28	1.42
Chloroform	25- 50	18/18	32.2(91%)	12	1.29	1.43
1,1-Dichloroethane	25- 50	17/18	32.3(89%)	11	1.47	1.72
1,2-Dichloroethane	100-200	18/18	160 (113%)	12	1.64	2.02
1,2-Dichloropropane	100-200	15/18	138 (105%)	10	1.34	1.51
Ethyl Benzene	200	17/18	145 (72%)	11	1.89	2.46
Tetra chloroethene	200-300	18/18	225 (92%)	12	1.53	1.82
1,1,1-Trichloroethane	25-325	29/36	47.0(85%)	18	1.36	1.55
Trichloroethene	200-300	18/18	249 (102%)	12	1.35	1.54
Toluene	25- 75	36/36	29.0(88%)	24	1.42	1.65
1,1,2,2-Tetrachloroethane	200-300	18/18	211 (86%)	12	1.85	2.39
Methyl Ethyl Ketone	200-400	13/18	135 (49%)	8	2.40	3.45
Isobutyl Alcohol	50-250	0/36	-	0	-	-
1,4-Dioxane	200-400	3/18	7.5 (3%)	1	2.06	2.78
8270						
Acetophenone	25-250	8/18	67.0(85%)	4	1.46	1.71
Aniline	25-250	13/18	70.6(57%)	8	1.80	2.30
p-Chloro-m-Cresol	100-200	18/18	81.0(57%)	12	1.51	1.78
Chlorobenzene	100-200	12/18	42.0(30%)	8	1.49	1.75
2-Chlorophenol	300-500	18/18	236 (73%)	12	1.32	1.49
4-Chlorophenol	300-350	16/18	178 (55%)	10	1.71	2.14
1,2-Dichlorobenzene	25-200	18/18	45.3(64%)	12	1.53	1.82
1,3-Dichlorobenzene	25-200	18/18	43.3(61%)	12	1.49	1.76
Di-n-Octylphthalate	300-500	17/18	292 (74%)	11	2.71	4.09
Diphenylamine	100-250	12/18	121 (77%)	8(6)	10.56(1.54)	28.05(1.84)
Methyl Methacrylate	300-500	6/18	260 (67%)	4	1.24	1.36
Naphthalene	100-250	18/18	88.9(56%)	12	1.59	1.93
4-Nitrophenol	300-500	18/18	146 (38%)	12	4.69	8.89
Phenacetin	300-500	12/18	218 (56%)	8	1.56	1.87
2,4,6-Trichlorophenol	300-350	18/18	261 (80%)	12	1.49	1.75
2,4,5-Trichlorophenol	300-350	18/18	178 (55%)	12	2.66	3.98
8330						
Thiourea	300-500	0/18	-	0	-	-
N-Phenylthiourea	100-500	9/27	12.3(5%)	6	2.43	3.52
1-Acetyl-2-Thiourea	100-500	6/27	8.8(3%)	3	1.63	2.00
8240						
Combined	25-400	281/378	86.7(87%)	183	1.57	1.90
8270						
Combined	25-500	240/288	119 (59%)	157	2.36	3.38
8330						
Combined	100-500	15/72	10.7(4%)	9	2.18	3.09

TABLE 7

CMA SW-846 ASSESSMENT STUDY

INTRALABORATORY

Precision of Methods 8240, 8270, 8330: Within Compound and Sample Pooled

Method, Laboratory		Fraction Detections	Geom $\bar{X}$ (% Rec.)	Degrees of Freedom	Vu	Ru
8240	Lab A	105/126	86.7 (84%)	70	1.29	1.44
	Lab B	91/126	105.4 (118%)	60	1.21	1.32
	Lab C	85/126	70.4 (66%)	53	2.14	2.94
8270						
	Lab A	96/96	117 (61%)	64	1.63	2.00
	Lab B	69/96	133 (69%)	43	3.34	5.50
	Lab C	75/96	109 (50%)	50	2.40	3.45
8330						
	Lab A	5/24	9.8 (24%)	3	1.63	2.00
	Lab B	10/24	11.2 (5.0%)	6	2.43	3.52
	Lab C	0/24		0	-	-

TABLE 8

CMA SW-846 ASSESSMENT STUDY

Interlaboratory Precision Of Methods 8240, 8270, 8330

Method, Compound	ppb Spike Range	Labs Reporting	Vu	Ru
8240				
Acetone	200-400	A,C	4.73	9.01
Acetonitrile	200-400	A	-	-
Benzene	50-100	A,B,C	1.51	1.79
Carbon Tetrachloride	200-300	A,B	2.63	3.92
Chloroform	25- 50	A,B,C	1.72	2.15
1,1-Dichloroethane	25- 50	A,B,C	2.12	2.89
1,2-Dichloroethane	100-200	A,B,C	2.06	2.77
1,2-Dichloropropane	100-200	A,B,C	1.73	2.17
Ethyl Benzene	200	A,B,C	2.80	4.30
Tetra chloroethene	200-300	A,B,C	1.68	2.09
1,1,1-Trichloroethane	25-325	A,B,C	2.05	2.75
Trichloroethene	200-300	A,B,C	1.70	2.11
Toluene	25- 75	A,B,C	1.45	1.70
1,1,2,2-Tetrachloroethane	200-300	A,B,C	3.38	5.61
Methyl Ethyl Ketone	200-400	A,C	2.59	3.84
Isobutyl Alcohol	50-250	-	-	-
1,4-Dioxane	200-400	C	-	-
8270				
Acetophenone	25-250	A,B	4.21	7.63
Aniline	25-250	A,B,C	1.81	2.31
p-Chloro-m-Cresol	100-200	A,B,C	1.75	2.21
Chlorobenzene	100-200	A,C	3.49	5.87
2-Chlorophenol	300-350	A,B,C	1.39	1.59
4-Chlorophenol	300-350	A,B,C	2.07	2.80
1,2-Dichlorobenzene	25-200	A,B,C	2.54	3.74
1,3-Dichlorobenzene	25-200	A,B,C	2.58	3.83
Di-n-Octylphthalate	300-500	A,B,C	2.65	3.97
Diphenylamine	100-250	A,B	2.20	3.05
Methyl Methacrylate	300-500	A	-	-
Naphthalene	100-250	A,B,C	1.65	2.02
4-Nitrophenol	300-500	A,B,C	22.33	80.84
Phenacetin	300-500	A,C	3.22	5.22
2,4,6-Trichlorophenol	300-350	A,B,C	1.41	1.63
2,4,5-Trichlorophenol	300-350	A,B,C	7.30	16.64
8330				
Thiourea	300-500	-	-	-
N-Phenylthiourea	100-500	B	-	-
1-Acetyl-2-Thiourea	100-500	A	-	-
8240 Combined, based on average variance			2.31	3.27
8270 Combined, excluding 4-nitrophenol, 2,4,5 TCP			2.40	3.46
8330 Combined			-	-

(10) $S_d = \sqrt{\frac{1}{M} \Sigma \log(X_{ij}) - \log(X_{i'j'})^2}$

where the summation is over

- samples containing the compound
- laboratories i and i' = A, B, C with i=i'
- triplicates j, j' = 1, 2, 3

and M is the number of pairs (X_{ij}, $X_{i'j'}$) of nonzero values by independent laboratories on equivalent samples.

Equation (10) is equivalent to the estimate of $[2(\sigma^2 + \sigma 2^2)]^{1/2}$ derivable using standard analysis of variance techniques when no values are missing. Here σ^2, and $\sigma 2^2$ are respectively components of between and within laboratory variance of log (concentration found). S^2d is an unbiased estimated of $2(\sigma^2 + \sigma 2^2)$ regardless of the imbalance caused by missing values.

For three 8240 compounds, one 8270 compound and all 8330 compounds, no laboratory or only one laboratory reported values on any sample. Therefore, no estimates for between laboratory variability could be derived. The single value for methyl ethyl ketone from Laboratory B was excluded from the analysis. Two unusually low values for diphenylamine on different samples by Laboratory B were also excluded as outliers.

The interlaboratory variability (Vu) and repeatability (Ru) factors shown in Table 8 were calculated as follows using equations (11) and (12).

(11) $Ru = \exp(2S_d)$

(12) $Vu = \exp 2S_d/\sqrt{2})$

The Vu and Ru values are used as previously illustrated, but the 95% confidence intervals are for an observation from one laboratory with respect to a second observation at a different laboratory.

Ru values for 8240 and 8270 compounds indicate that, for most compounds analyzed by these methods, results from independent laboratories can be expected to agree to within factors of two to four. Problems with these methods are indicated by the 11 compounds which one or more laboratories failed to detect in any samples and by unusually high Ru values for 4-nitrophenol and 2,4,5-trichlorophenol. These appear to be due to factors influencing all determinations within laboratories and not to isolated bad analyses.

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3.6 False Positive and Negative Observations

Calculations for precision and bias are used to define the quantitative aspects of an analytical method. There are two important properties of an analytical method which are used to assess the qualitative attributes of a method. These are the number of false positive and number of false negative observations that result when a method is used. The experimental design for the CMA study allows for such an assessment since the identity of every compound in every sample was known.

The following summarizes the qualitative properties for the analytical methods with respect to the compounds included in the CMA study and for the groundwater matrix used for all samples.

3.6.1 Method 8240 (Volatiles)

Only four compounds were spiked into Sample 1; however, two of the laboratories found a total of seven additional compounds in one or more of the blind triplicates (6, 7, 10). These are all false positive observations. This is a particular problem since Sample 1 was prepared to represent an up-gradient well that was supposed to establish background contamination levels both quantitatively and qualitatively. Review of the data reported in Appendix A, Table A-1 also indicates that one gets a different picture for background contamination depending upon which laboratory analyzed the sample and what sample from the blind triplicate is used. Methylene chloride was reported to be present in most samples by all three laboratories. For Sample 1 (6, 7, 10), Table A-2 in Appendix A shows the level of methylene chloride exceeded the total of all other (four) compounds spiked into the sample. It would be reasonable to assume that methylene chloride was present as a contaminant even though the compound was not spiked into the sample. Methylene chloride would not be considered a false positive observation. The compound was not included in the precision and bias calculations since the true concentration was not known.

There were a number of false negative observations with Method 8240. None of the laboratories were able to detect the presence of isobutyl alcohol in any of 12 actual samples (4 samples in triplicate) at concentrations that ranged from 50 to 250 μg/ℓ. The same was true for 1,4-dioxane in six samples where the concentration range was 200-400 μg/ℓ. Two of the laboratories did not detect the presence (6 samples) of acetone (200-400 μg/ℓ), acetonitrile (200-400 μg/ℓ), methyl ethyl ketone (200-400 μg/ℓ), and 1,1,1-trichloroethane (25 μg/ℓ). This represents a serious problem of false negative results. Of the 17 compounds spiked into two or more samples, there were 7 cases of false negative observations for one or more laboratories. This indicates that the list of compounds reported by EPA to be amenable for analysis by Method 8240 is somewhat less than reported.

3.6.2. Method 8270 (Semi-volatiles)

There were a total of eight false positive observations reported by one or more laboratories. In one instance, two laboratories reported the same false positive observation for N-nitrosodiphenylamine and reported it present in the same six samples analyzed. This observation is an interesting situation when two independent laboratories concur on the presence of a component not actually present in samples.

There were 16 compounds spiked into the various samples. Sample 4 contained all 16; Sample 3 contained 8; and, Sample 2 contained 8. Only one laboratory was able to detect and quantify all compounds in all samples. Two of the laboratories did not detect the presence of methyl methacrylate (300-500 μg/ℓ) in any of six samples, nor acetophenone (25-250 μg/ℓ) in five of six samples. Additional false negative observations included diphenylamine (100-250 μg/ℓ), phenacetin (300-500 μg/ℓ), and chlorobenzene (100-200 μg/ℓ). These false negative observations indicate the list of compounds reported by EPA to be amenable for analysis by Method 8270 is somewhat less than reported.

3.6.3. Method 8330 (HPLC, Sulfur Compounds)

False positive observations were a common problem at all three laboratories when the method was followed. One laboratory used a dual wavelength detector and employed absorbance ratios to reduce the number of false positive observations (interferences); however, even this modification did not solve the problem completely.

None of the laboratories were able to detect the presence of thiourea in any sample. There were some reported values for the other two compounds, but for all practical purposes the quantification was so poor it would be better to consider these observations as false negatives, as well.

4. DISCUSSION

A second objective for the CMA study was to determine how well the resulting data would define groundwater contamination. The results from the study were, therefore, reviewed from the perspective of those using the data for an environmental assessment. Because the identity and true concentration level of every organic contaminant (except methylene chloride) is known for each sample, it is possible to evaluate how well the resulting data defined groundwater contamination for this hypothetical situation. This evaluation is strictly applicable only to groundwater samples resembling those used in the study; however, the evaluation provides the basis for what might be expected in other similar situations where the true concentration levels and identity are not known and other available information are factored into the overall assessment. In most situations, the qualitative and quantitative quality of the data base will be much poorer since one does not usually have blind triplicate analyses done at three laboratories.

In Table 9, analytical results for all the spiked compounds for all four samples are classified. For each compound spiked, a laboratory either failed to detect its presence (generated a false negative result) or detected the compound. Detections are further classified according to whether the reported value is within a factor of two of the spiked amount, our minimal standard for quantitative accuracy.

Of the background compounds (also EPA priority pollutants), benzene and toluene were quantified in all samples by all laboratories, 1,1,1-trichloroethane by most, and isobutyl alcohol (Appendix VIII compound) by no one. Methods 8240 and 8270 detected and quantified non-background compounds not containing chlorine in about half of the analyses; they detected and quantified compounds containing chlorine in most analyses. Method 8330 for sulfur-containing organic compounds performed poorly on the three spiked compounds; no reported values fell within a factor of two of the true value.

The user of these data is less concerned with how well the analytical method performs on compound which may or may not be present as groundwater contaminants than with the validity of the particular list of compound names and concentrations reported for his sample. From this point of view, reported results for the simulated down gradient samples (Samples 2, 3, and 4) have been tabulated in Table 10.

A total of 531 analytical detections other than background species or methylene chloride are reported for these samples. 63% (336 of 531) are for species actually spiked at between half and double the reported amount. An additional 19% of reported values represent compounds present but at levels not within a factor of 2 (non-quantitative) of those reported. Most of these reported values are underestimates. 18% of the reported values are for compounds not spiked into the samples. Table 10 also is broken down by analytical method as well as chlorined and non-chlorinated compounds. Using methods 8240 and 8270, results for chlorinated compounds are generally trustworthy, while results for non-chlorinated compounds include 16% and 25% false detections. Three quarters of detections by method 8330 are false positives.

An evaluation of the degree of agreement between independent analyses is also of interest to a user of the data. Table 11 was prepared by examining all possible pairs of samples of the same type submitted to different laboratories. The number of such pairs is 162 (9 x 3 = 27 choices for the first analysis, 2 x 3 = 6 for the second) For each set of values reported by the first laboratory, results of the second laboratory were classified as either (1) not confirming the presence of the compounds, (2) confirming the presence of the compound by an amount differing by a factor of 2 or more from the original amount, or (3) confirming with quantitative agreement.

Based on the tabulation in Table 11, the submitter of samples to two laboratories can expect that results reported from the first laboratory will be confirmed quantitatively (to within a factor of 2) by the second

TABLE 9

PERFORMANCE OF METHODS 8240 8270 AND 8330 ON COMPOUNDS SPIKED INTO GROUNDWATER, BY COMPOUND

COMPOUND (levels in ppb)	% OF SAMPLES IN WHICH COMPOUND NOT DETECTED (FALSE NEGATIVES)	DETECTED BUT NOT QUANTITATED TO WITHIN X2	QUANTITATED WITH FACTOR OF 2
BACKGROUND			
Benzene (50,100)	0	0	100
Toluene (25,75)	0	0	100
Isobutyle Alcohol (50,250)	100	0	0
1,1,1 trichloroethane (25,375)	19	4	78
8240 VOLATILES			
Acetone (200,400)	44	17	39
Acetonitrile (200,400)	83	0	17
1,4 Dioxane (200,400)	83	17	0
Ethyl benzene (200)	6	11	83
Methyl Ethyl Ketone (220,400)	28	28	44
Carbon tetrachloride (200,300)	33	17	50
Chloroform (25,50)	0	0	100
1,1-dichloroethane (100,200)	6	6	89
1,2-dichloroethane (100,200)	0	0	100
1,2-dichloropropane (100,200)	17	0	83
tetrachloroethylene (200,300)	0	0	100
trichloroethylene (200,300)	0	0	100
1,1,2,2, tetrachloroethane (200,300)	0	11	89

TABLE 9 CONTINUED

8270 SEMI-VOLATILES			
Acetophenone (25,250)	56	6	39
Aniline (25,250)	28	17	56
Diphenylamine (100,250)	33	17	50
Di-n-octylphthalate (300,500)	6	11	83
Methyl methacrylate (300,500)	67	0	33
Naphthalene (100,250)	0	22	78
4-nitrophenol (300,500)	0	56	44
Phenacetin (300,500)	33	28	39
Chlorobenzene (100,200)	33	50	17
P-chloro-m-cresol (100,200)	0	28	72
2-chlorophenol (300,350)	0	6	94
4-chlorophenol (300,350)	11	28	61
1,2-dichlorobenzene (25,200)	0	28	72
1,3-dichlorobenzene	0	33	67
2,4,6-trichlorophenol (300,350)	0	0	100
2,4,5-trichlorophenol (300,350)	0	33	67
8330 SULFUR CONTAINING			
Thiourea	100	0	0
1-acetyl-2-thiourea	78	22	0
n-phenylthiourea	67	33	0

TABLE 10

CLASSIFICATION OF DETECTION (SAMPLES 2, 3, 4, ONLY)

COMPOUND CLASS	% OF DETECTIONS WHICH ARE FALSE POSITIVE	PRESENT BUT NOT WITHIN FACTOR OF X2	PRESENT WITHIN X2	(#DETECTION)
8240 Nonchlorinated*	16%	24%	60%	(55)
Chlorine containing**	0%	4%	96%	(134)
8270 Nonchlorinated	25%	20%	55%	(138)
Chlorine containing	4%	26%	70%	(142)
8330 Sulfur containing	76%	24%	None	(62)
Combined performance	18%	19%	63%	(531)

* Benzene, tolune, isobutyl alcohol background compounds not included

** 1,1,1 trichloroethane not included. Methylene chloride false detections not included.

TABLE 11

AGREEMENT BETWEEN ANALYSES RUN BY TWO LABORATORIES
(SAMPLES 2,3,4. ALL PAIRS WITH SAME SAMPLE, DIFFERENT LABORATORY)

COMPOUND CLASS*	% OF COMPOUNDS DETECTED BY THE FIRST LABORATORY WHICH WILL BE NOT DETECTED BY SECOND LAB	FOUND BUT NOT IN QUANTITATIVE AGREEMENT	FOUND, IN AMOUNT WITHIN A FACTOR OF X2 OF ORIGINAL DETERNINATION
8240 Nonchlorinated	49	21	30
Chlorine containing	8	16	76
8270 Nonchlorinated	42	27	31
Chlorine containing	10	22	68
8330 Sulfur containing	88	11*	1**
Combined performance	31%	20%	49%

* Notes under Table 10 apply. Thus, background and Methylene chloride data are excluded.
** Confirmed findings were, in fact, false positives.

laboratory for 49% of the compounds, confirmed qualitatively for an additional 20%, and not confirmed at all for 31%. One in 200 quantitative confirmations and one in 11 qualitative confirmations (with quantitative disagreement) will be for compounds not actually present in the sample.

5. CONCLUSIONS

The results from the CMA SW-846 Assessment Study revealed that even the best (GC/MS) of analytical procedures contained in SW-846 are somewhat inadequate for the analysis of compounds reportedly (EPA) amenable to Methods 8240 and 8270 from the Appendix VIII list. Method 8330 was found to be completely inadequate for the three compounds included in the study. This observation was confirmed by all three laboratories. The statement that SW-846 contains the analytical methods for all (375) Appendix VIII compounds (excluding exotics and water reactive compounds) simply is not true.

Review of results from the assessment of bias (accuracy, recovery) indicated that the three laboratories were not able to meet the EPA criteria to demonstrate one's ability to generate "acceptable accuracy and precision" for a large percentage of compounds included in the study. This observation was unexpected since three prominent and experienced laboratories took part in the study, and the samples contained only pure compounds in a rather simple groundwater matrix.

There were a large number of false positive and negative observations by all laboratories to varying degrees. This observation is of particular concern since GC/MS methodology was employed for rather simple samples in a rather simple groundwater matrix. With less specific detectors such as FID (GC) or UV (HPLC), the problem with false positive and negative observations would be expected to be even more severe. The resulting data for Method 8330 supports this conclusion.

The calculated Vu and Ru values, which express intra- and interlaboratory precision, revealed precision varies from laboratory to laboratory, for different methods, and for specific compounds. These facts must be considered when analytical data are being interpreted to assess an environmental situation.

It is reasonable to conclude that the numerous problems observed for Methods 8240, 8270, and 8330 for the samples included in the CMA SW-846 Assessment Study may be expected for other methods in SW-846 not evaluated. SW-846 certainly has not reached the level of development whereby its use should be mandated by law. Until <u>all</u> the methods contained in SW-846 are adequately validated, SW-846 is nothing more than a collection of methods that may or may not work, and is only suitable as a reference document. Even as a reference source, SW-846 has limitations.

If one reviews the resulting data from the CMA study from a data user's perspective, one would conclude that the analytical community could indeed identify groundwater contamination, but not very well both qualitatively

and quantitatively. The problem with false positive and negative observation would be of major concern in determining immediate corrective action, as well as the potential for future problems.

6. RECOMMENDATIONS

The EPA should not promulgate the mandatory use of SW-846 for testing for Appendix VIII compounds under Subtitle C of RCRA. The methods have only been validated for a few of the Appendix VIII list, and further validation is required before promulgation.

The EPA should concentrate its efforts initially to the validation and development of GC/MS Methods 8240 and 8270. The list of Appendix VIII compounds amenable to these procedures needs to be established, along with supporting precision and bias data.

For Appendix VIII compounds not amenable to Methods 8240 and 8270, EPA needs to validate other methods in SW-846 to confirm they actually work for some matrix with a reasonable expectation that they may work in other matrices.

Multilaboratory studies of all methods similar to the CMA SW-846 Assessment Study would be advisable prior to including a method into a manual such as SW-846 and prior to considering promulgation of a method for mandatory compliance testing. Results from such studies should be subjected to the analytical peer review process and appropriate statistical evaluation, both within and outside the Agency.

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Sharon H. Kneiss, Staff Executive, and
Becky Wilson, Administrative Assistant

7. REFERENCES

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2. Radian Corporation, 1983, "Evaluation of the Feasibility of Analyzing Groundwater For the Appendix VIII Hazardous Constituents", Report to American Petroleum Institute, Washington, D.C.

RLL8506605

8. APPENDIX A

Summary of Data for CMA SW-846
Assessment Study

RLL8506605

TABLE A-1

CMA SW-846 ASSESSMENT STUDY BACKGROUND COMPOUNDS

Compound	Spike Level µg/l	Sample 1			Sample 2			Sample 3			Sample 4		
		Sample No. 6	Sample No. 7	Sample No. 10	Sample No. 2	Sample No. 8	Sample No. 11	Sample No. 1	Sample No. 5	Sample No. 9	Sample No. 3	Sample No. 4	Sample No. 12
Laboratory A													
Benzene	50	64	51	39	61	48	53	46	43	43	(100) 100	110	100
Toluene	25	28	22	18	23	17	21	20	19	18	(75) 60	58	61
Isobutyl Alcohol	50	ND	ND	ND	ND	ND	ND	(250) ND	ND	ND	(250) ND	ND	ND
1,1,1-Trichloroethane	25	24	18	14	20	15	17	16	17	18	(325) 270	280	300
Methylene Chloride [a]	0	120	98	73	96	73	82	81	83	83	ND	ND	ND
Laboratory B													
Benzene	50	59	52	54	53	57	60	53	56	51	(100) 104	127	126
Toluene	25	27	24	26	22	23	24	25	27	23	(75) 66	80	76
Isobutyl Alcohol	50	ND	ND	ND	ND	ND	ND	(250) ND	ND	ND	(250) ND	ND	ND
1,1,1-Trichloroethane	25	31	27	30	27	24	29	30	28	25	(325) 327	418	379
Methylene Chloride [a]	0	92	78	84	67	71	75	68	69	69	62	69	74
Laboratory C													
Benzene	50	32	61	81	50	77	67	50	46	86	(100) 86	150	140
Toluene	25	20	26	38	20	23	23	14	18	27	(75) 44	67	66
Isobutyl Alcohol	50	ND	ND	ND	ND	ND	ND	(250) ND	ND	ND	(250) ND	ND	ND
1,1,1-Trichloroethane	25	13	ND	ND	ND	ND	ND	ND	ND	20	(325) 140	200	240
Methylene Chloride [a]	0	180	100	370	130	100	93	64	160	110	150	84	90

a - Not spiked into samples
ND - Not detected
( ) - Spike levels changed to indicated values

TABLE A-2

CMA SW-846 ASSESSMENT STUDY SAMPLE 1

Methods	Compounds	Spike Level µg/l	Laboratory A Sample No. 6	Laboratory A Sample No. 7	Laboratory A Sample No. 10	Laboratory B Sample No. 6	Laboratory B Sample No. 7	Laboratory B Sample No. 10	Laboratory C Sample No. 6	Laboratory C Sample No. 7	Laboratory C Sample No. 10
8240	Volatiles										
	Benzene	50	64	51	39	59	52	54	32	61	81
	Toluene	25	28	22	18	27	24	26	20	26	38
	Isobutyl Alcohol	50	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1,1,1-Trichloroethane	25	24	18	14	31	27	30	13	ND	ND
	Methylene Chloride[a]	0	120	98	73	92	78	84	180	100	370
	Acetone[a]	0	ND	ND	ND	ND	ND	ND	38	93	46
	2-Butanone[a]	0	ND	ND	ND	ND	ND	ND	13	190	ND
	C_4-Alkylbenzene[a]	0	NS	NS	ND	ND	44	ND	ND	ND	ND
	Aromatic Ketone[a]	0	ND	ND	ND	ND	21	ND	ND	ND	ND
	Alkane[a]	0	ND	ND	ND	ND	74	ND	ND	ND	ND
	Alkene[a]	0	ND	ND	ND	31	ND	ND	ND	ND	ND
8270	Base/Neutrals/Acids										
	Bis(2-Ethylhexyl)Phthalate[a]	0	ND	ND	ND	ND	ND	12	ND	ND	ND
8330	Sulfur Containing										
	1-(o-Chlorophenyl)-2-Thiourea[a]	0	6	7	6	<1	<1	<1	ND	ND	ND
	Epinephrine[a]	0	ND	ND	ND	ND	ND	ND	830	1,290	161

a - Not spiked into sample
ND - Not detected

CMA SW-846 ASSESSMENT STUDY SAMPLE 2

Methods	Compounds	Spike Level µg/l	Laboratory A Sample No. 2	Laboratory A Sample No. 8	Laboratory A Sample No. 11	Laboratory B Sample No. 2	Laboratory B Sample No. 8	Laboratory B Sample No. 11	Laboratory C Sample No. 2	Laboratory C Sample No. 8	Laboratory C Sample No. 11
8240	Volatiles										
	Acetone	200	270	230	250	ND	ND	ND	80	ND	110
	Acetonitrile	200	140	110	140	ND	ND	ND	ND	ND	ND
	Benzene	50	61	48	53	53	57	60	50	77	67
	Chloroform	25	30	21	21	26	26	27	17	19	16
	1,1-Dichloroethane	25	28	20	19	28	29	32	10	ND	17
	1,2-Dichloroethane	100	150	110	110	141	150	145	53	110	100
	1,2-Dichlroopropane	100	91	75	82	110	124	123	87	150	120
	Methylene Chloride [a]	0	96	73	82	67	71	75	130	100	93
	1,1,1-Trichloroethane	25	20	15	17	27	24	29	ND	ND	ND
	Toluene	25	23	17	21	22	23	24	20	23	23
	Methyl Ethyl Ketone	200	190	160	150	ND	20	ND	77	46	210
	Isobutyl Alcohol	50	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1,4-Dioxane	200	ND	ND	ND	ND	ND	ND	6[b]	10[b]	ND[b]
	Alkane [a]	0	ND	ND	ND	ND	ND	130	ND	ND	ND
	Octadecanal [a]	0	ND	ND	ND	ND	42	ND	ND	ND	ND
	1-Propene,1-Methoxy [a]	0	ND	ND	ND	ND	ND	19	ND	ND	ND
8270	Base/Neutrals/Acids										
	Acetophenone	25	16	17	17	ND	46	ND	ND	ND	ND
	Aniline	25	8	7	13	12	ND	ND	ND	ND	ND
	p-Chloro-m-Cresol	100	41	51	45	51	47	55	35	64	53
	1,2-Dichlorobenzene	25	16	12	18	32	20	24	7	12	11
	1,3-Dichlorobenzene	25	17	12	17	28	19	21	6	10	10
	Diphenylamine	100	94	96	80	17	210	130	ND	ND	ND
	Naphthalene	100	58	54	77	69	61	33	33	55	48
	Chlorobenzene	100	46	33	32	ND	ND	ND	15	20	18
	N-Nitrosodiphenylamine	0[a]	ND	ND	ND	410	BMDL	BMDL	90	170	150
	Bis(2-Ethylhexyl)Phthalate	0[a]	ND	ND	7	BMDL	BMDL	BMDL	ND	ND	ND
	Chloromethyl Phenol	0[a]	ND	ND	ND	52	33	19	ND	ND	ND
	Aliphatic Alcohol	0[a]	ND	ND	ND	ND	30	ND	ND	ND	ND
8330	Sulfur Containing										
	Thiourea [a]	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	N-Phenylthiourea	100	ND	ND	ND	10	3	4	ND	ND	ND
	1-Acetyl-2-Thiourea	100	ND	ND	ND	ND	5	ND	ND	ND	ND
	1-(O-Chlorophenyl)Thiourea [a]	0	ND	ND	ND	ND	ND	1	ND	ND	ND
	Ethylene Thiourea [a]	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Diethyl Stilbesterol [a]	0	ND	ND	ND	ND	ND	ND	248	510	3400
	Thioacetamide [a]	0	ND	ND	ND	ND	ND	ND	556	390	ND
	Epinephrine [a]	0	ND	ND	ND	ND	ND	ND	ND	1120	606
	1-Naphthyl-2-Thiourea [a]	0	6	6	7	ND	ND	ND	ND	ND	ND

a - Not spiked into samples.
b - Reported as Base/Neutral semivolatile
ND - Not detected
BMDL - Detected but below quantification level

TABLE A-4

CMA SW-846 ASSESSMENT STUDY SAMPLE 3

Methods	Compound	Spike Level µg/l	Laboratory A Sample No. 1	Laboratory A Sample No. 5	Laboratory A Sample No. 9	Laboratory B Sample No. 1	Laboratory B Sample No. 5	Laboratory B Sample No. 9	Laboratory C Sample No. 1	Laboratory C Sample No. 5	Laboratory C Sample No. 9
8240	**Volatiles**										
	Benzene	50	46	43	43	53	56	51	50	46	86
	Toluene	25	20	19	18	25	27	23	14	18	27
	Isobutyl Alcohol	250	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1,1,1-Trichloroethane	25	16	17	18	30	28	25	ND	ND	20
	Methylene Chloride [a]	0	81	83	83	68	69	69	64	160	110
	Carbon Tetrachloride	200	180	190	200	425	413	350	ND	ND	ND
	Ethyl Benzene	200	110	140	170	225	205	205	100	ND	68
	Tetrachloroethene	200	160	160	170	228	234	215	200	120	170
	Trichloroethene	200	190	180	180	269	272	248	180	160	150
	1,1,2,2-Tetrachloroethane	200	120	130	130	343	251	308	120	190	62
	Methyl Ethyl Ketone [a]	0	ND	ND	ND	ND	ND	ND	15	120	100
	Unsaturated Hydrocarbon [a]	0	ND	ND	ND	ND	33	ND	ND	ND	ND
	Acetone [a]	0	ND	ND	ND	ND	ND	ND	11	95	ND
8270	**Base/Neutral/Acids**										
	Methyl Methacrylate	300	190	220	230	ND	ND	ND	ND	ND	ND
	2-Chlorophenol	300	200	230	270	251	257	222	240	200	220
	Di-n-Octylphthalate	300	210	230	290	186	ND	168	380	250	560
	4-Nitrophenol	300	65	56	40	479	765	310	11	150	140
	Phenacetin	300	250	200	240	ND	ND	ND	140	120	140
	2,4,6-Trichlorophenol	300	210	210	270	245	280	229	190	240	310
	2,4,5-Trichlorophenol	300	310	280	350	71 [b]	75 [b]	110 [b]	250	230	280
	4-Chlorophenol [a]	300	86	100	160	ND [a]	170 [c]	170 [c]	260 [c]	210 [c]	220 [c]
	Bis(2-Ethylhexyl)Phthalate [a]	0	ND	18	17	ND	ND	ND	ND	ND	ND
	1,4-Dioxane [a]	0	ND	ND	ND	ND	ND	ND	14	ND	ND
	Phenyl Isothiocyanate [a]	0	ND	ND	ND	4	9	14	ND	ND	ND
	Acetamide, N-(4-Ethoxyphenyl) [a]	0	ND	ND	ND	10	93	120	ND	ND	ND
	Isothiocyanotobenzene a)	0	15	13	42	ND	ND	ND	ND	ND	ND
8330	**Sulfur Containing**										
	Thiourea	300	ND	ND	ND	ND	ND	ND	ND	ND	ND
	N-Phenylthiourea	300	ND	ND	ND	12	19	10	ND	ND	ND
	1-Acetyl-2-Thiourea	300	5	ND	8	ND	ND	ND	ND	ND	ND
	1-(o-Chlorophenyl)Thiourea [a]	0	6	7	6	ND	ND	44	1820	ND	ND
	1-Naphthyl-2-Thiourea [a]	0	10	17	19	ND	ND	ND	ND	ND	ND
	Diethyl Stilbesterol [a]	0	ND	ND	ND	ND	ND	ND	1390	ND	5460
	Thioacetamide [a]	0	ND	ND	ND	ND	ND	ND	2480	ND	ND
	n-Nitroso-n-Methylurea [a]	0	ND	ND	ND	ND	ND	ND	ND	ND	269
	Epinephrine [a]	0	ND	ND	ND	ND	ND	ND	7880	1490	ND
	Ethylenethiourea [a]	0	ND	ND	ND	ND	ND	ND	ND	ND	854

a - Not spiked into samples b - Reported as trichlorophenol c - Could not distinguish between 3-or-4-chlorophenol ND - Not detected

Methods	Compounds	Spike Level µg/l	Laboratory A Sample No. 3	Laboratory A Sample No. 4	Laboratory A Sample No. 12	Laboratory B Sample No. 3	Laboratory B Sample No. 4	Laboratory B Sample No. 12	Laboratory C Sample No. 3	Laboratory C Sample No. 4	Laboratory C Sample No. 12
8240	**Volatiles**										
	Acetone	400	290	350	250	ND	ND	ND	200	77	85
	Acetonitrile	400	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Benzene	100	100	110	100	104	127	126	86	150	140
	Carbon Tetrachloride	300	290	290	320	435	660	561	ND	ND	ND
	Chloroform	50	46	47	46	49	59	57	46	33	37
	1,1-Dichloroethane	50	40	43	43	56	70	68	49	30	33
	1,2-Dichloroethane	200	240	270	260	260	306	288	100	180	220
	1,2-Dichloropropane	200	160	180	160	232	290	278	ND	ND	ND
	Ethylbenzene	200	170	130	180	204	225	229	43	140	130
	Methylene Chloride [a]	0	ND	ND	ND	62	69	74	150	84	90
	Tetrachloroethane	300	220	210	240	264	355	339	200	330	450
	1,1,1-Trichloroethane	325	270	280	300	327	418	379	140	200	240
	Trichloroethene	300	270	280	280	350	437	423	210	250	400
	Toulene	75	60	58	61	66	80	76	44	67	66
	1,1,2,2-Tetrachloroethane	300	180	180	170	474	561	549	130	310	270
	Methyl Ethyl Ketone	400	230	260	200	ND	ND	ND	200	150	170
	Isobutyl Alcohol	250	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1,4-Dioxane	400	ND	ND	ND	ND	ND	ND	ND	7 [b]	ND
8270	**Base/Neutrals/Acids**										
	Aniline	250	130	150	200	160	350	170	140	150	160
	p-Chloro-m-Cresol	200	190	240	160	110	111	106	83	130	150
	2-Chlorophenol	350	260	280	240	250	277	284	150	220	250
	1,2-Dichlorobenzene	200	150	160	160	170	178	184	61	100	100
	1,3-Dichlorobenzene	200	160	160	160	173	164	181	62	96	100
	Di-n-Octylphthalate	500	300	350	380	166	474	258	120	680	530
	Diphenylamine	250	260	280	240	13	470	280	ND	ND	ND
	Methylmethacrylate	500	350	330	280	ND	ND	ND	ND	ND	ND
	Naphthalene	250	170	160	160	153	175	174	97	150	140
	4-Nitrophenol	500	12	46	82	900	802	794	140	250	260
	Phenacetin	500	390	440	500	ND	ND	ND	150	260	120
	2,4,6-Trichlorophenol	350	240	280	260	327	279	286	200	310	410
	2,4,5-Trichlorophenol	350	330	380	390	130 [d]	62 [d]	15 [d]	180	280	380
	4-Chlorophenol	350	210	240	120	140 [e]	ND [e]	200 [e]	170 [e]	250 [e]	290 [e]
	Chlorobenzene	200	120	120	100	ND	ND	ND	31	53	49
	Acetophenone	250	190	190	120	ND	440	ND	ND	ND	ND
	n-Nitrosodiphenylamine [a]	0	ND	ND	ND	13	329	143	290	540	600
	Isothiocyanaobenzene [a][d]	0	71	95	26	ND	ND	ND	ND	ND	ND
	n-(4-Ethoxyphenyl) Acetamide [a]	0	ND	ND	ND	120	200	100	ND	ND	ND
	Chloromethylphenol [a]	0	ND	ND	ND	110	170	48	ND	ND	ND
	Phenyl Isothiocyanate [a]	0	ND	ND	ND	ND	42	23	ND	ND	ND
	n-(1-Phenylethylidene) Methanamine [a]	0	ND	ND	ND	ND	15	17	ND	ND	ND
8330	**Sulfur Containing**										
	Thiourea	500	ND	ND	ND	ND	ND	ND	ND	ND	ND
	N-Phenylthiourea	500	ND	ND	ND	23/180 [e]	40	25/270 [e]	ND	ND	ND
	1-Acetyl-2-Thiourea	500	13	11	16	ND	ND	ND	ND	ND	ND
	1-(o-Chlorophenyl)-2-Thiourea [a]	0	9	6	9	ND	ND	63	248	93	3010
	1-Naphthyl-2-Thiourea [a]	0	31	33	34	ND	ND	ND	ND	ND	ND
	Diethyl Stilbesterol [a]	0	ND	ND	ND	ND	ND	ND	1140	670	1230
	Thioacetamide [a]	0	ND	ND	ND	ND	ND	ND	569	1300	4060
	1-Phenyl-2-Thiourea [a]	0	ND	ND	ND	ND	ND	ND	76	14	ND
	Epinephrine [a]	0	ND	ND	ND	ND	ND	ND	1190	11800	14300

a - Not spiked into samples
b - Reported as semivolatile (8270)
[illegible]

d - Reported as trichlorophenol
e - Could not distinguish between 3-or-4-chlorophenol
[illegible]

MR. TELLIARD: This morning we have a number of speakers. The way we're going to work this program to get you out of here relatively on time is two very important things. One, we have a boat break and the boat break will take precedence over your other break. The boat break happens to be that we found out last night that the USS Iowa is coming up the channel this morning, and they're ain't many of us who have ever seen a real battleship, so we will take our coffee break when the battleship comes up the channel. In between times, if you have other needs, you're on your own. We can't train you to do everything.

MR. TELLIARD: Our first speaker this morning is yesterday's speaker. Judith is going to talk to us a little bit about the analysis that EPRI has been working on in their round robin on metals analysis. Judith.

JUDITH SCOTT

TRW ENERGY DEVELOPMENT GROUP

UTILITY ROUND ROBIN RESULTS FOR THE DETERMINATION OF ARSENIC AND SELENIUM BY GRAPHITE FURNACE AAS

MRS. SCOTT: Good morning. Neither Winston Chow, Program Manager at EPRI, nor Ray Maddalone, our Program Manager at TRW, could be here today. Ray was hoping as late as last Friday that he'd be able to make it, but he's being detained in Los Angeles against his will. He is foreman of a jury on a major trial which has kept him more than a month longer on jury duty than anyone ever dreamed.

My name is Judy Scott and I have been with the project team since the beginning of the Analytical Methods Qualification Project. Contrary to rumor, I did not bribe a juror to remain a holdout, but I am happy to be here.

EPRI is sponsoring the Analytical Methods Qualification Project at TRW to examine the methods available to determine priority pollutants in utility discharge streams. We are concentrating primarily on the trace metals at this point.

There has been a trend in recent years toward basing discharge limits on water quality criteria, and there is concern over whether or not the methods at hand are capable of determining these low levels in utility matrices. Therefore, we are working right now on a round robin using real world spiked and split samples to measure precision and bias of the analytical methods in utility streams.

The Analytical Methods Qualification Project has four phases. We are now in the first phase which has two rounds, and we have just completed the first round. In the first round we are determining arsenic and selenium by graphite furnace atomic absorption. In the second round, we will be looking at the determination of copper, nickel, chromium and lead by graphite furnace. Today, I will be giving you a progress report on round one.

FIGURE 1

Our title was prepared at a time when we were very optimistic about the length of time it would take for the participating utilities to return their results. Unfortunately, we were overly optimistic and the results are just now coming in and being collated. Although I will not have any hard and fast precision and bias data to give

you, I will present an overview of our activities to date.

FIGURE 2

Initially we sent out 198 requests to utilities to participate in the project and as their responses came back, we screened them. We were looking for utilities which would be representative of the steam electric industry. We also were looking for utilities that had positive experiences with DMR-QA samples in the determination of these elements. In addition, we wanted utilities that routinely use graphite furnace analytical techniques.

Our goal was to have two groups of 30 labs each, one group being fresh water matrices primarily, and one group being saltwater. We were successful on the freshwater labs. However, on the saltwater side, we did not have as many labs responding. In addition, we found that the saltwater labs are using a greater diversity of methods to handle their problems. Therefore, in round one for the arsenic and selenium determination, we had laboratories using three different methods: graphite furnace with matrix modification or matrix matching, graphite furnace with Zeeman background correction, and gaseous hydride. We realize that for the

seawater labs, we'll have at best a qualitative comparison when the results are all in.

Just a brief word about the matrices. You'll notice here, the first two matrices on the freshwater side, river water and ashpond overflow, represent an influent and effluent at a coal-fired utility. Similarly on the seawater side, the first two matrices, seawater and then seawater plus fireside wash, represent also influent and effluent at an oil-fired utility on the west coast.

The next matrix, treated chemical metal cleaning waste (TCMCW), was selected because it's a matrix with disposal problems and a matrix which has typically been difficult to analyze.

The reagent grade water is included so that we can compare the results of this study with those of previous studies and it also is a measure of what utilities can do in an interference-free matrix.

Then, finally, the QA/QC sample. We were fortunate in obtaining vials of concentrate from EPA/EMSL, Cincinnati, which will be used as a measure of absolute bias.

FIGURE 3

The activities have fallen into the categories shown. Once we decided on the matrices and found

the laboratories, we then collected the test samples. The first two samples, riverwater and ashpond overflow, were acquired at an east coast utility, sampled by utility personnel in bottles that we had pre-cleaned and sent to the utility and then they returned to us at TRW. The other three matrices listed were acquired by TRW personnel at a utility on the west coast.

For the spiking and disbursement activity, we used a churn splitter, which was an original design of the USGS scaled up to meet the project needs. We acquired six different churn splitters to handle each of the matrices.

Finally, in the process of anticipating the kind of data we would want from this study, we devised a laboratory data reporting form, which I'll be showing you in a few minutes, as well as a QA/QC questionnaire to help us develop a laboratory profile so that we would have some way, perhaps, when we discovered outliers, of examining laboratory practices in effect during analysis of these samples.

Finally, the analysis of the data will follow the ASTM 2777-77 and we will be deriving estimates of limits of detection and limits of quantitation.

FIGURE 4

This is a picture of the 25 liter polyethylene carboys used to acquire the samples and the shipping crate used. The cleaning that was used for the carboys is the same as that which was used for the bottles on the project; 10 percent hydrochloric acid, soak for 48 hours; 10 percent nitric acid, soak for 48 hours. Then the bottles were filled with reagent grade water until just before use. This is similar to an NBS procedure which we had established would be the best for our purposes during an earlier literature survey. The cleaning method is efficient in removing any trace element contamination, and at the same time, doesn't overly sensitize the surface so that later on you get adsorption of the trace elements that you're trying to add.

FIGURE 5

This is a picture of our disassembled churn splitter which we used on the project. You see the container, the churn disc and then the cover with a hole for the shaft. You'll notice that the spigot is at the bottom. It's modeled after the USGS 14 liter design. The one you're looking at here is 120 liters. We had them fabricated by Bell Arts.

The mixer is extremely efficient. You get a strong suction downward and then upwelling, and USGS knew that the 14 liter size mixing was very efficient. Our next step was to determine whether or not our scaled-up model would be just as efficient.

FIGURE 6

The next slide shows you the result of a test to determine mixing efficiency. We used a water matrix and we spiked into it a manganese solution which we knew would have an ultimate mixed concentration of five parts per million. The churn handle was operated at about seven inches a second. The first minute, samples were withdrawn every 10 seconds and then after that, every 30 seconds until the end of the test. Initially, you can see that there's actually a very strong downward surge, because remember, we're withdrawing the samples at the bottom of the churn mixer. Mixing is essentially complete within two to three minutes. We selected five minutes as the mix time for use on the project.

FIGURE 7

Once the mixing efficiency was verified, we then proceeded to the spiking and disbursement of actual samples. This is a picture of the churn

splitter on the digital scale, which is accurate to a tenth of a kilogram. We added the contents of the carboys to the churn splitter, mixed them for five minutes, and then withdrew samples for density measurements. By measuring both weight and density very accurately, we were able to determine volume at any given point within a few tenths of a percent.

FIGURE 8

This slide shows the volumetric flasks used for measuring density in triplicate.

FIGURE 9

This is a copy of our spiking data worksheet. On this we kept all the relevant data in one place, including the density data and target spiking levels. The target spiking levels were chosen based on the background levels in the samples as well as the methods' recommended use range. We sent each participating laboratory four samples of each matrix: the background, plus three spike levels to cover the recommended use range. At the bottom of the sheet are the formulas used for calculating the amounts of subsequent spike additions.

FIGURE 10

The next slide shows the actual preparation of

the spiking solution. The spikes themselves were 1,000 ppm certified standards which we in turn checked against an independent standard. We used a syringe to deliver the approximate spiking solution that we desired, anywhere from say 2 to 10 milliliters, and then we made an accurate weighing of that amount. We had determined earlier that in the short time spent on the balance, evaporation was not a problem. Then by knowing the weight of the solution and the density, we were able to calculate the exact amount which had been added.

For each sample, we added all six test elements--copper, nickel, chromium and lead and the arsenic and selenium. This was done so that the two rounds would be consistent in terms of any potential interelemental interferences which could bias the results.

FIGURE 11

The standards that were prepared were then combined in a teflon beaker for addition to the churn mixer. You see here the teflon beaker and top of the disc. The mixing action tips it and rinses it and disperses the spike throughout the sample. The churn was operated five minutes before any samples were withdrawn and then continuously during withdrawal of the samples.

FIGURE 12

The next slide is our churning team in action. Sample preparation takes place in an isolated laboratory off-limits to non-project personnel. The bottles being filled with samples are 500 millimeter low-density polyethylene which, as I mentioned, were stored with reagent grade water and emptied just prior to filling. I should mention that the churn master you see on the right reported a marked improvement in his golf game after this activity, undoubtedly due to the large number of bottles that we filled.

We filled approximately 640 bottles for shipping to participating utilities. We have another couple hundred for spares and QA purpose, such as the measurements being made to determine that indeed the samples are stable in the concentrations that we prepared.

FIGURE 13

The bottles shown here have what we call the pretest labels. These labels were designed to minimize the amount of clerical work needed during the filling operation. When you're talking about this many bottles, you want to streamline the filling activity as much as possible. The labels,

which were prepared in advance, contain a code indicating the matrix, the concentration level and the filling order for later traceability. Bottles were removed from storage in groups, a particular spiking level filled, and then the bottles returned to their places on the shelves as a group. They were then stored in locked cabinets until preparation of the boxes to go to the laboratories.

FIGURE 14

This is a bottle with the final test label attached. These labels were also prepared in advance with the exception of the fill order number.

FIGURE 15

The code of the label has several parts. I can tell from the label the lab number, the round number, the matrix identification, concentration level, and fill order number.

The bottles were then bagged, boxed in cardboard boxes, and shipped via two day air to the participating utilities. With the bottles went a set of instructions, a copy of the procedures from the 1983 EPA Methods for Chemical Analysis of Water and Wastes, and a lab survey questionnaire designed to determine the overall capability of a participating laboratory.

I should note that we used special packaging for the reference samples. Those come in, I believe, five percent nitric acid which is considered a hazardous material, and EMSL has a waiver from DOT for this kind of shipment. I was told I probably wouldn't live long enough to get a waiver for this project since EMSL had had so much work getting theirs. The vials were shipped separately as hazardous material, and I was pleased to discover that they, too, usually arrived within two to three days.

FIGURE 16

I have here a copy of our laboratory data reporting form which was a four-part carbonless package. There's actually perforation on the top of the first page, which doesn't show here, which allowed us to use this top part as a label for the bottle. The second and third pages went to the utilities for recording results and then one sheet was returned to us; the final page was kept at TRW for our records. You notice here that we have places to record all the requisite analytical data. In addition, we asked for other information about the conditions of the analysis. Again, we're trying to anticipate our data analysis needs

downstream when certain data points turn out to be outliers. Our goal is to be able to examine factors which might have biased the results, so we've requested furnace conditions, temperatures, times, et cetera.

FIGURE 17

This is a copy of the first page of our general laboratory questionnaire in which we ask for information about the equipment used, sources of standards, the level of operator experience, and calibration ranges that were used for the tests.

FIGURE 18

I want to just briefly summarize what our approach to the data analysis will be. Once the data are collated, we will first perform a screening test in which we look for any values which are less than one-fifth or greater than five times the average value calculated by our laboratory. At that point, these values will be flagged and we will contact the participant to investigate the possibility of transcriptional or decimal errors. I was very interested in what Samuel To had to say yesterday when he reported that as much as 50 percent of DMR-QA errors were due to trascriptional or decimal errors.

If we find that an error is due to what was referred to as a data management error, that value will be corrected for purposes of this data analysis and will be included in its corrected form in the data collection. Any data points which we can't reconcile by that analysis will remain in the data base but will be flagged.

The next analysis of the data will be for systematic errors and we will be using the ASTM five percent two-tail ranking, which is called out in D2777-77. The remaining data will then be examined for outliers using a one percent T-test. I believe the 1985 revision of ASTM D-2777 permits one repeat cycle through this test and that will be the maximum that will be used. I think earlier versions did not specify how many times you could go through this kind of an outlier process.

FIGURE 19

After rejection of outliers, we will then calculate both single operator and overall precision and bias for each element and each matrix. For the percent recovery calculation, the accepted background concentration will be the mean of all reported concentrations after outliers are removed. Other concentration levels will then be calculated

from the background plus added spikes.

Precision data will be plotted against concentration and linear regression used to extrapolate back to zero concentration. This value then, will enable us to develop an estimate of the limit of detection using the standard definition of three times the precision at zero concentration. In the real world, the precision tends to flatten out as you approach zero so, if anything, we'll be coming up with a bit of an optimistic estimate of the method's capability. Finally, any observed biases will be tested for significance at the one percent level.

Currently the data that we've collected are being entered in a dBase III program so that we can spin out appropriate files as needed for the data analysis. The summary of the results from round one will be reviewed with EPRI in the beginning of May, and if all goes well, we will be preparing samples for round two in May. The summary report of these two rounds will be prepared and issued during the August/September time frame.

A brief word about phase two for the Analytical Methods Qualifications Project. We are tentatively scheduled to look at arsenic and

selenium by gaseous hydride, cadmium by graphite furnace, mercury by cold vapor, and iron by flame AAS. Again, I apologize for not having hard data for this presentation, but I thank you for your attention.

MR. TELLIARD: Any questions? Thank you.

Analytical Methods
Qualification

Utility Round Robin Results for the Determination of Arsenic and Selenium by Graphite Furnace AAS

Raymond F. Maddalone, Ph.D (TRW Inc.)
Judith W. Scott (TRW Inc.)
Winston Chow, P.E. (EPRI)

Utility Round Robin Results for the Determination of Arsenic and Selenium by Graphite Furnace AAS

Figure 1

Raymond F. Maddalone, Ph.D (TRW Inc.)
Judith W. Scott (TRW Inc.)
Winston Chow, P.E. (EPRI)

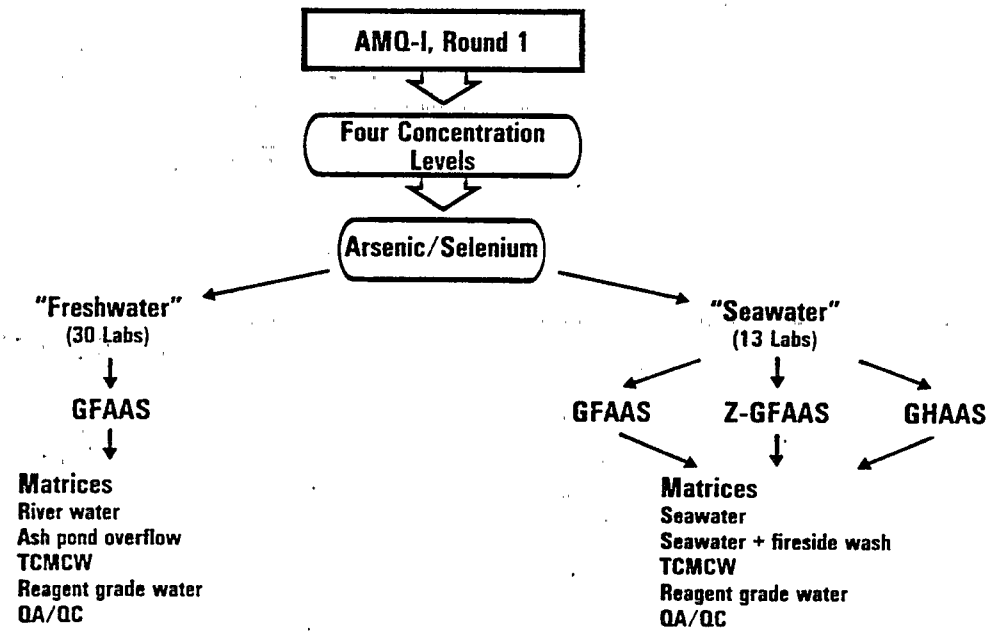

Figure 2

Analytical Methods Qualification

AMQ-I Round 1 Effort

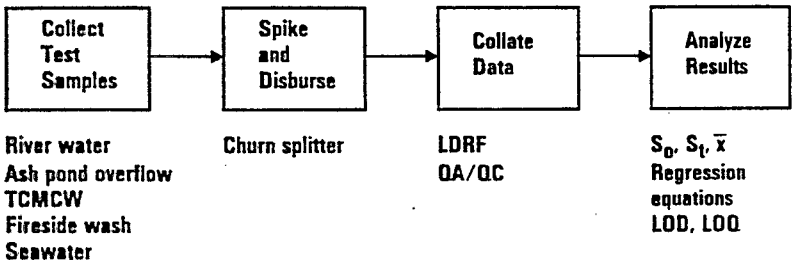

Figure 3

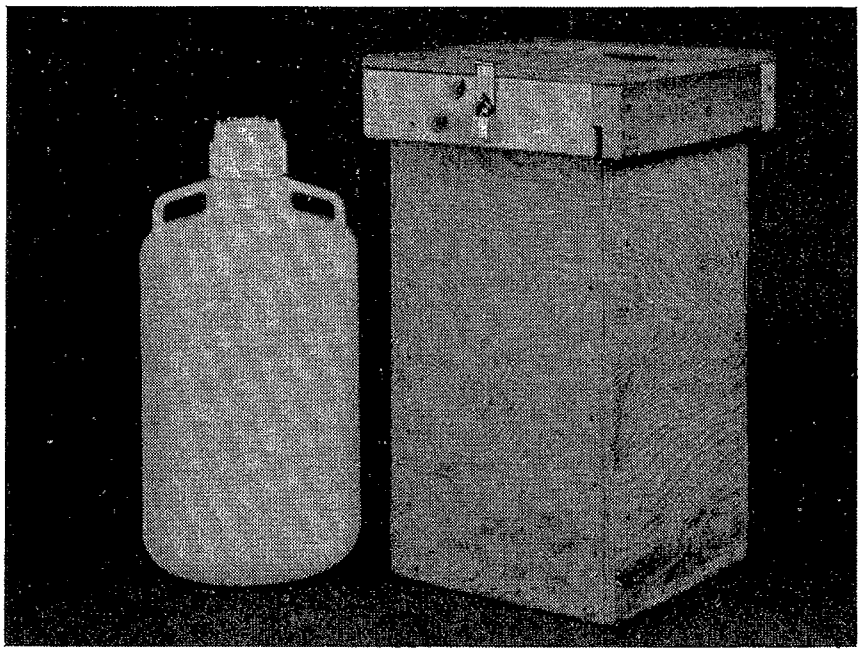

Figure 4

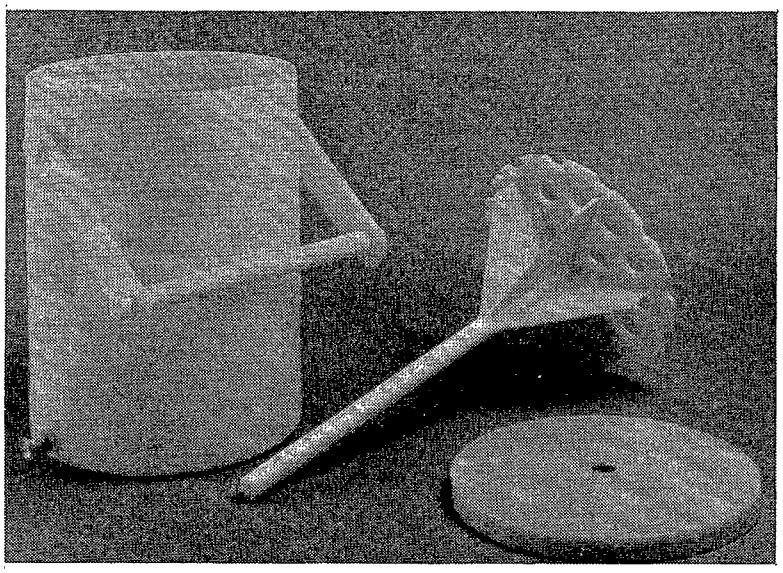

Figure 5

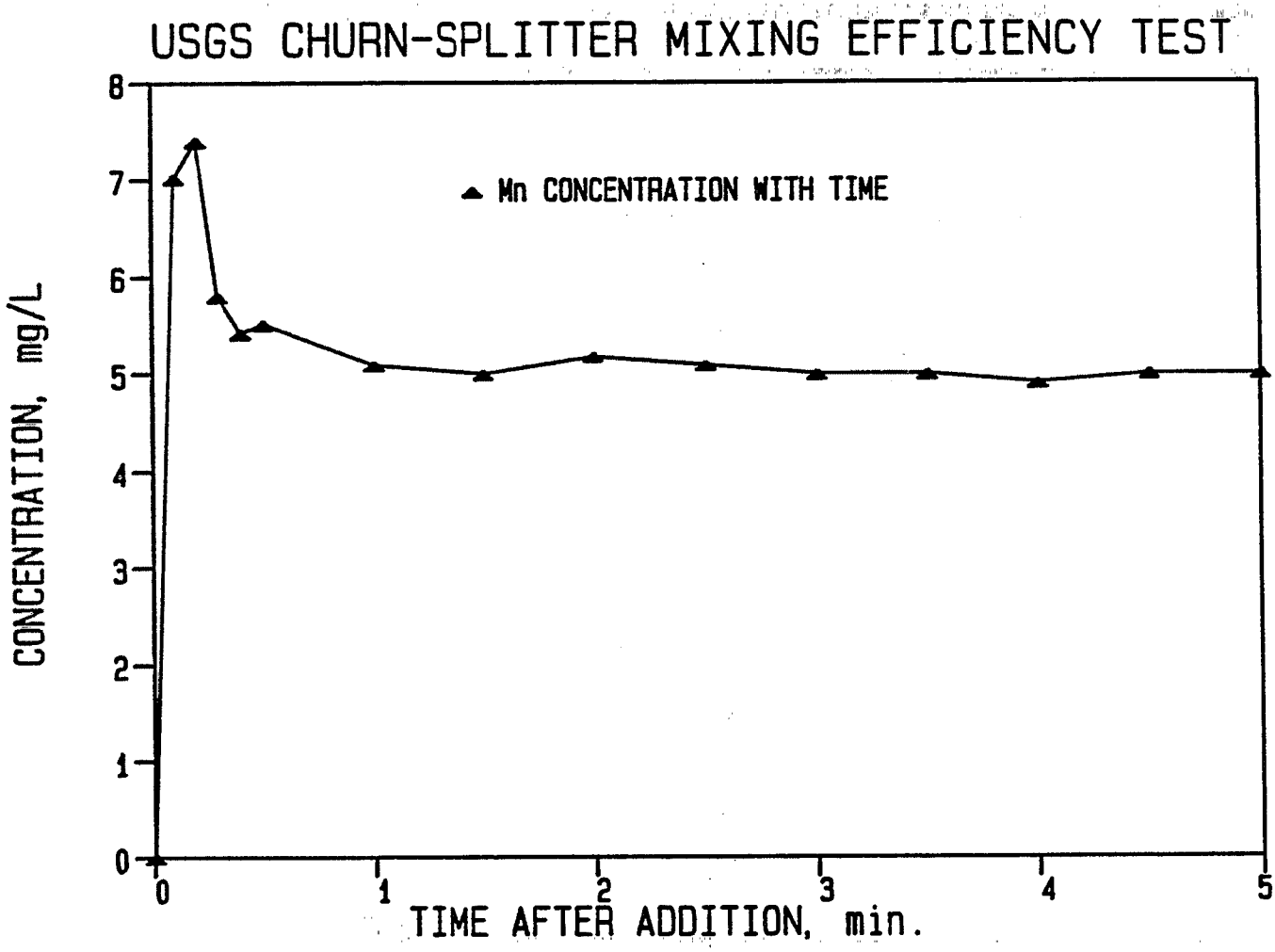
USGS CHURN-SPLITTER MIXING EFFICIENCY TEST
Mn CONCENTRATION WITH TIME
CONCENTRATION, mg/L
0
1
2
3
4
5
6
7
8
0
1
2
3
4
5
TIME AFTER ADDITION, min.

Figure 6

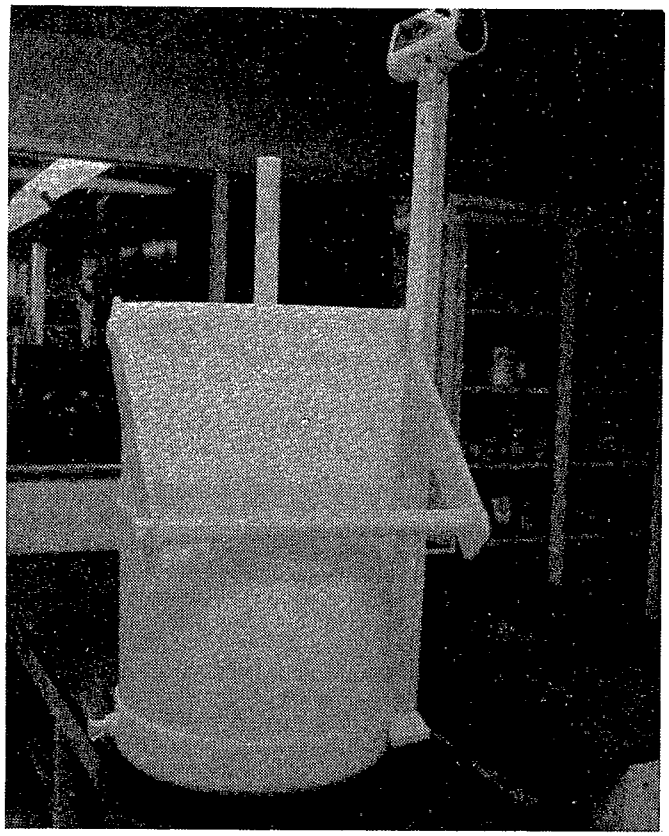

Figure 7

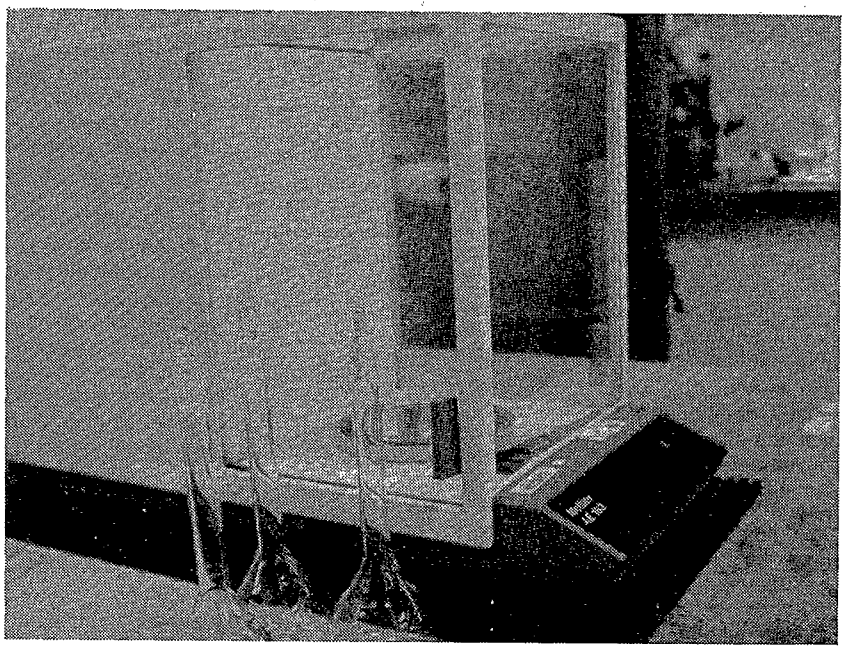

Figure 8

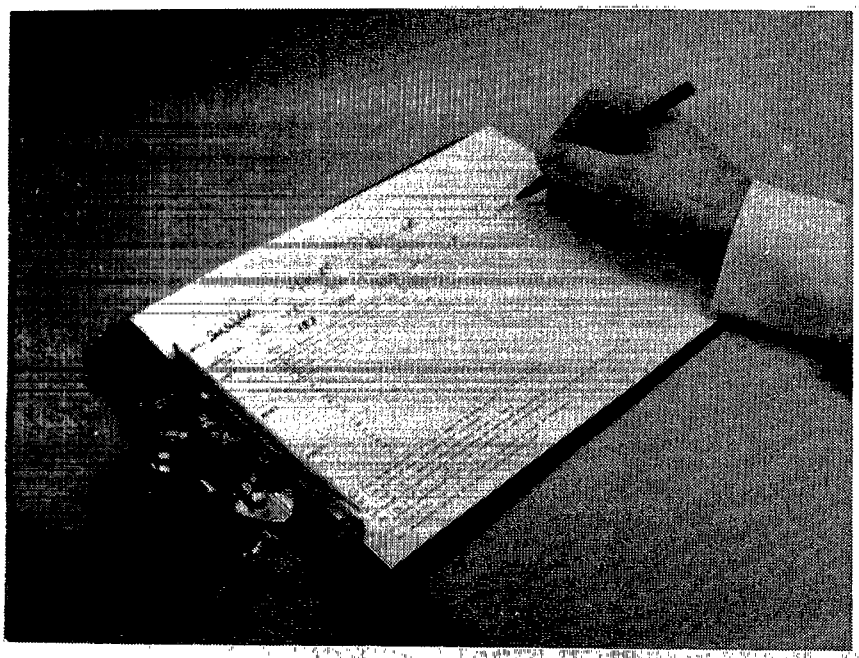

Figure 9

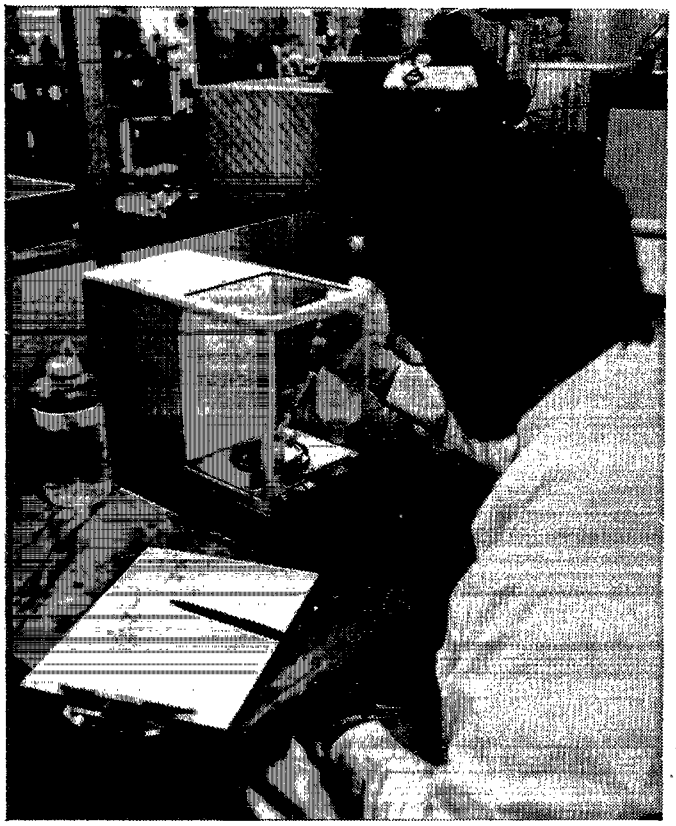

Figure 10

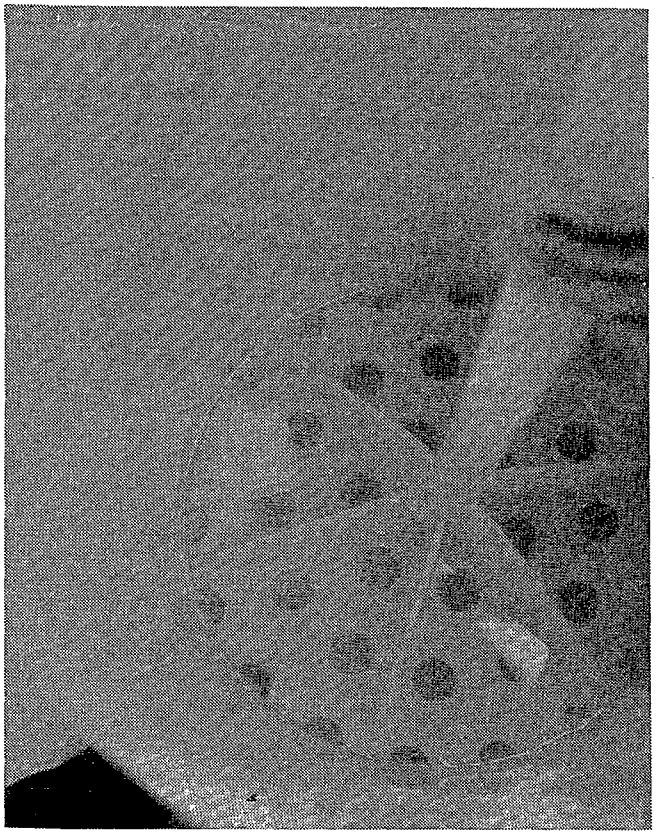

Figure 11

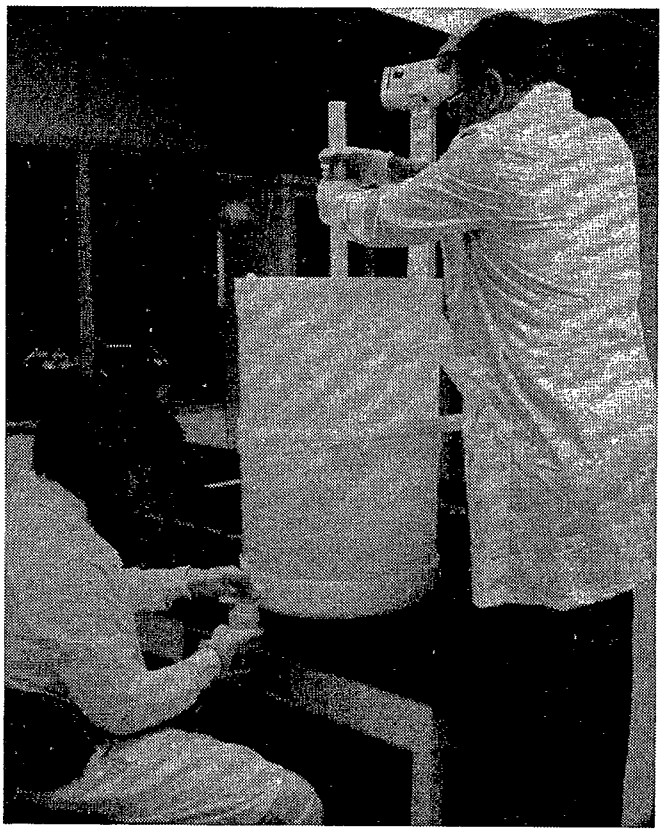

Figure 12

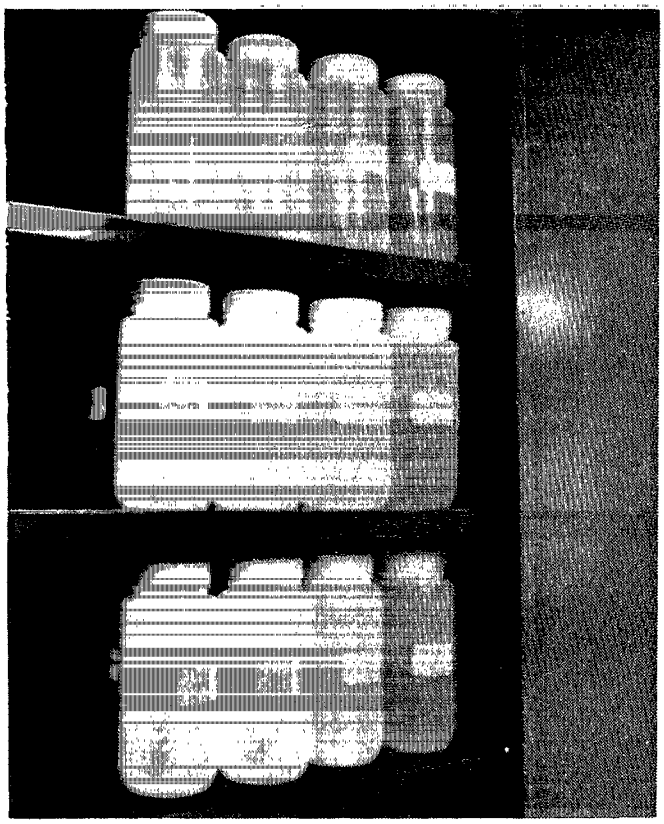

Figure 13

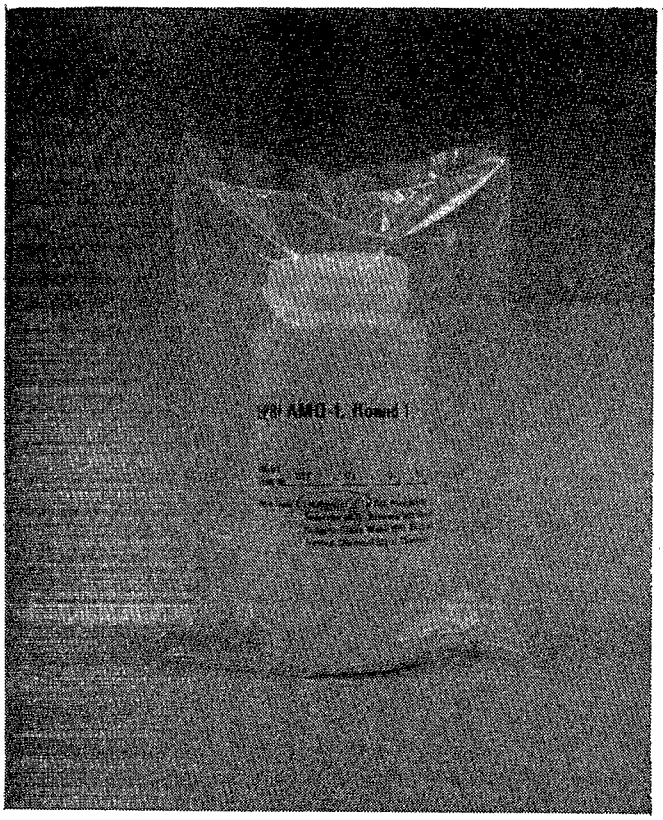

Figure 14

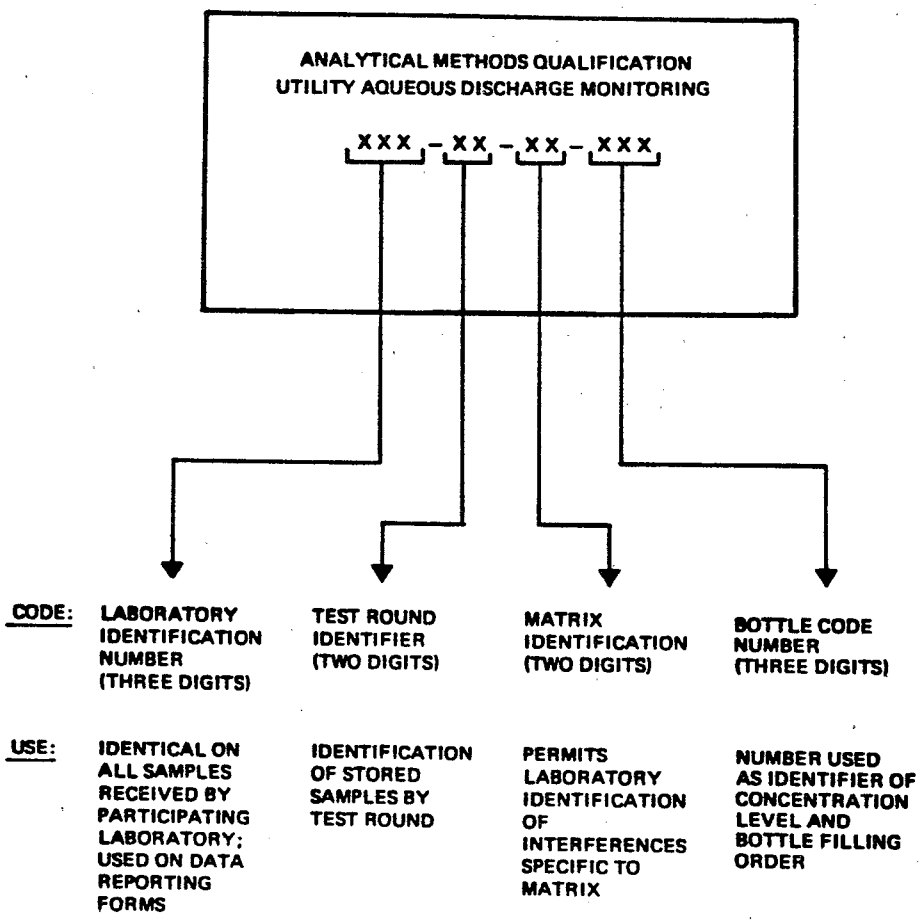
ANALYTICAL METHODS QUALIFICATION
UTILITY AQUEOUS DISCHARGE MONITORING
XXX - XX - XX - XXX
CODE:
LABORATORY IDENTIFICATION NUMBER (THREE DIGITS)
TEST ROUND IDENTIFIER (TWO DIGITS)
MATRIX IDENTIFICATION (TWO DIGITS)
BOTTLE CODE NUMBER (THREE DIGITS)
USE:
IDENTICAL ON ALL SAMPLES RECEIVED BY PARTICIPATING LABORATORY; USED ON DATA REPORTING FORMS
IDENTIFICATION OF STORED SAMPLES BY TEST ROUND
PERMITS LABORATORY IDENTIFICATION OF INTERFERENCES SPECIFIC TO MATRIX
NUMBER USED AS IDENTIFIER OF CONCENTRATION LEVEL AND BOTTLE FILLING ORDER

Figure 15

EPRI AMQ-I, Round 1

AMQ-I/1
Sample ID: _____-_____-_____-_____

Matrix Type: Freshwater (01), Ash Pond Overflow (02), Seawater (03), Seawater with Metal/Wastes (04), Reagent Grade Water (05), QA/QC Ampule (06), Treated Chemical Metal Cleaning Wastes (07)

Element	Analyst	pH at Time of Prep.	Date Prep.	Date Anal.	Was Standard Addition Used?	Furnace Conditions, °C, Sec: Drying Temp	Drying Time	Char Temp	Char Time	Atomization Temp	Atomization Time
As	_____	_____	___	___	_____	_____	_____	_____	_____	_____	_____
Se	_____	_____	___	___	_____	_____	_____	_____	_____	_____	_____

Element	Replicate	Measured Aliquot Conc (μg/L)	Dilution Factor	Calculated Concentration of Orig. Sample (μg/L)
As	1	_____	_____	_____
	2	_____	_____	_____
	3	_____	_____	_____
Se	1	_____	_____	_____
	2	_____	_____	_____
	3	_____	_____	_____

Sample Condition on Arrival: _____

Comments/Problems: _____

White - Sample Label Yellow - TRW's Copy Blue - Participating Laboratory's Copy Buff Card - Disbursement Copy

Figure 16

GENERAL LABORATORY EQUIPMENT AND PRACTICE SURVEY

LABORATORY _____

KEY CONTACT/PHONE: _____

MATRICES ANALYZED: _____

Apparatus

1. Atomic Absorption Spectrometer (AAS):

   Instrumentation Laboratory _____
   Perkin Elmer _____
   Varian _____
   Jarrell-Ash _____
   Fisher Scientific _____
   Beckman _____
   Hitachi _____
   Sargent-Welch _____
   Other: _____
   Model Number: _____

2. Type of AAS:

   Single beam _____
   Double beam _____

3. Type of background correction used during AMQ project:

   None _____
   Deuterium arc _____
   Smith-Hefitje _____
   Secondary line _____
   Zeeman _____
   Other _____

4. Graphite Furnace manufactured by:

   Instrumentation Laboratory _____
   Baird Atomic _____
   Perkin-Elmer _____
   Varian _____
   Jarrell-Ash _____
   Beckman _____
   Sargent-Welch _____
   Other _____
   Model No. _____

Figure 17

Analytical Methods
Qualification

Highlights of Data Analysis Approach

Follows ASTM D2777-77 (1985 version)

Screens for transcriptional errors

- Does not remove data unless it is a reporting error

Ranks laboratories (5% two tail limit)

Tests for individual outliers (1% t-test)

- Permits one repeat of t-test

Figure 18

Analytical Methods
Qualification

Expected Data Outputs

By matrix for each element

- Mean, S_0, S_t, RSD (S_0, S_t)
  - Distribution plots
- % recovery
  - % recovery versus concentration
- Regression (linear) analysis
  - $\bar{x}$ versus S_0
  - $\bar{x}$ versus S_t
- Bias (tested for significance at 1% level)

Figure 19

MR. TELLIARD: Our next speaker is going to talk a little bit about one of our favorite subject, pesticides. Many of you here are living off of them. I understand Mr. Whitlock's story where he has this little can and goes around every Thursday and spots a small piece of ground and then calls for the contamination.

I forgot to announce earlier that there are some limited copies of Paul Britton's talk in the back on the table with the other publications. If you'd like one, feel free to pick one up.

MITCHELL D. ERICKSON, Ph.D.
MIDWEST RESEARCH INSTITUTE
KANSAS CITY, MISSOURI

COMPARISON OF METHODS FOR ANALYSIS OF PCBs

MR. ERICKSON: I would like to present my talk in three segments this morning. The first segment reviews why we are concerned about PCBs. Second, I will talk about philosophy of methods that one would use for analysis. Finally, I will present one answer which is what we've been doing which we think has many advantages over some of the other methods that are available. I'm going to focus more on the broader scope of method development and less on the data, although I should emphasize that there is a substantial body of data backing up all of the work that we've done. I'm going to be comparing different methods and talking about them today.

SLIDE 1 & 2

Just as a quick review, that's a PCB. There are 209 PCBs. The group as a whole, or the individual members, are called congeners. If you refer to them in terms of the degrees of chlorination, you

have 10 different homologs. For instance, tetrachlorobiphenyl is one homolog. Within each homolog group, there are anywhere from 1 to 46 isomers.

SLIDE 3

The commercial production of PCBs. Through 1976, 90 percent of the world's PCBs were manufactured by Monsanto, sold under the trade name of Aroclor, with a number associated with it, 1221 through 1260. The percentage composition of these aroclors is listed by homolog. The point to be made here is that these are complex mixtures. They range from 30 to 60 individual congeners per mixture. That really is what separates PCBs from most other analytes.

PCBs are treated generally as a class, they are regulated as a class, and analysts are asked to do an analysis and then report on a class of compounds rather than on a specific compound. It would be much analogous to being asked to do an ICP analysis and then report total metals, or to report total PAH's. PCBs are complex mixtures in the environment and in virtually any sample. This creates all sorts of analytical problems, which is why PCBs are a little bit different in terms of the approach to a method.

SLIDE 4

The reason that we are concerned about PCBs is that they are present in virtually any environmental compartment. The one sample that I can think of right off hand that I can be reasonably sure that would not have PCBs in it, would be the Hope Diamond, but anything else--you, me, anyone else--has PCBs in them.

SLIDE 5

As I have summarized here, PCBs are stable compounds, and they do biomagnify through the environment, so they are of concern from that respect. They are ubiquitous; they are found at least 5,000 meters depth in the ocean; they are found in the upper atmosphere; they are found in the arctic snow. They are found everywhere. They are toxic, according to some of the old data that prompted their inclusion in TSCA, yet there's a lot of evidence that they're nontoxic. It's a very controversial area. Regardless, TSCA essentially banned their production use, manufacture, et cetera, in 1976.

However, EPA has seen fit for a number of uses to continue and so we are still, at this date, faced with a lot of analytical problems. There are a lot of samples to be analyzed because

of disposal by the utilities, because of environmental contamination, because of spills from PCB capacitors and transformers in your backyard that rupture, and we're also faced, as I mentioned earlier, with a complex analysis.

SLIDE 6

Basically, when one develops a method, one has to keep in mind what the objectives of the various aspects are. For instance, with the extraction, one wants to remove the PCBs from the sample matrix into a solvent. There are several general considerations in selecting an extraction technique. Likewise, with cleanup, you're removing interferences. Another way to look at it is that you are trying to enrich the PCB content at the expense of the other things and there are several different things that one wants to consider. There are many excellent cleanup techniques out there that probably would remove the PCBs. You need to have good recovery.

SLIDE 7

There are three categories of cleanup. There's a gross interference removal. For example, removing fat. One example of a way to do that is with GPC. Removing trace interferences like organochlorine pesticides can be done with column chromatography.

Some of them can also be selectively chemically degraded with chromium trioxide. The last category is not widely applied by people who are simply looking for total PCBs in the sample, but it is very important for people who are trying to do congener-specific analysis. In this category, one fractionates the PCB groups by some of their subtle functional differences. You can use carbon columns to separate these compounds according to the number of ortho-chlorines. So, there are different levels of cleanup.

SLIDE 8

These are the three major instrumental techniques that are used to determine PCB content of a sample: packed column GC/ECD, high resolution GC/ECD, and high resolution GC/EIMS. There are many, many others, of course. Quantitation is where we get into the real difference between regular pesticide analysis and PCB analysis. The difference is that we're trying to get a single number out of a very complex chromatographic profile. It depends on which instrumentation you're using how complex that profile gets, but the Webb-McCall and the total areas and the Aroclor are the standard ways of doing packed column quantitation.

The Aroclor quantitation is, to my way of thinking, the worst choice of all because you're trying to say that the sample contains a commercial product and you're trying to say that it contains no other PCBs and that in fact the product hasn't changed. That works okay if you've got a fresh transformer oil, but it falls down very rapidly when you start having weathering of the samples. There's a lot of data that indicates that you can get as much as an order of magnitude error by trying to quantitate as a specific Aroclor and ignore peaks that might be from another Aroclor or might be from other PCB sources or where you don't take into account the degradation.

Webb-McCall is tried and true. It's been studied in many round robins and proven time and again to give very decent data. With GCY EIMS, one wants to utilize the information available. For instance, one can report the data by homologs. One can also do congener-specific analysis if there is a need to know, for instance, the presence or absence of say 3,3',4,4'-tetrachlorobiphenyl, which is purportedly the most toxic of the PCBs. So there are a variety of ways to quantitate.

SLIDE 9

Another thing that one has to consider when they're doing a method development is what kinds of QC is going to be required. This is just a list of topic headings, any one of which has a dozen different items to consider when planning QC. For instance, under method execution, one wants to have provisions in there for blanks, duplicates, maybe standard addition. One wants to have proper data recording in notebooks and so forth. All of those kinds of things would be under just that one heading of method execution.

SLIDE 10

Now, given that background, we see that there are many methods available for PCBs. In fact, this is only a partial listing. I have a table with 45 different methods. One might call them standard methods. In some of the cases, that's a pretty far stretch of the imagination, since the methods have not been endorsed by any standard-issuing organization. Buried in this list and not explicitly brought out are several methods that are near and dear to this group's heart. For instance, in the water category, there are the methods that we've been talking about off and on here, 608, 625. Under the

solid waste category are 8080, 8250, 8270. Under the sludge category, one of them is 625S. So there are a number of methods that this group is familiar with, but others probably not. The methods differ widely in terms of the specificity. Some of them are highly specific in terms of what options are allowed; some of them are quite general. With some of them, the PCBs are piggy-backed with say, priority pollutants or organo-chlorine pesticides. In other cases, the PCBs are the only analyte that's addressed. Some of these have been highly tested and validated with round robins and so forth, and others basically just got written up and have no validation data behind them.

So that is the background. The overall assessment at this point is that there are a lot of methods out there for PCBs; not enough to do every matrix, though. There are several matrices that are not covered and time and again I've seen people trying to do an analysis and shoehorn in a method that doesn't exactly fit the matrix and was not validated for that matrix. That's part of where we were coming from and why we developed our method.

SLIDE 11

Stepping back for a second, I want to talk a

little bit about the philosophy of writing a method. There are two general approaches that one can take. One is to specify all of the procedures throughout the method. An example of that is the EPA 600 series where there is a fairly explicit set of directions. One of the positive aspects of this is that it gives you an off-the-shelf method. You can go to the manual, grab it, send somebody to the lab and they can do the analysis. Some of the negative aspects are shown there. Basically it means that if you have a method that's validated for a given matrix and a new matrix comes along, you have to make revisions and so forth. Also, it doesn't allow a lot of flexibility for laboratory preferences.

The other option is to specify that the method meets certain performance criteria. An example of that is the method I'll show you in a minute. There are other methods out there that also utilize this general approach to a greater or lesser extent. The options allowed can be numerous and you can cover more analytes in some cases. You can certainly cover more matrices. The key thing is that when you specify that the method must meet certain performance criteria on a per-sample basis, you end up with known data quality on each sample. The downside

of this option is that when you go through the list of options that might be presented, you have to write an in-house protocol and that does require some up-front work on the part of an individual laboratory. So those are the two extremes of method philosophy.

SLIDE 12

Now, changing to what we've done. We've got in draft form at this point, a method that's titled, "Analysis of PCBs in Liquids and Solids." The analytes that can be measured are any mixture of PCBs. The matrices include water, solids, liquids --you name it. It does utilize GC/EIMS. It has options for packed or capillary column GC. The quantitation is based upon 10 individual congeners, one for each homolog. They were selected after an extensive study of all of the available PCB congeners which determined response factors, retention times. We selected the congeners from each homolog class that were averages. The QC that's involved is that on every sample, or four 1 13C-labeled PCBs are added to the sample and the recoveries of those PCBs are measured and reported along with the values for the native PCBs. There are also instrumental performance criteria, blanks, and so forth, as with any

other method, say with 625.

SLIDE 13

The four 13C PCBs that were selected are shown in the bottom half of this slide. The asterisks in the center of the ring denote the 13C labeling. The exact isomers that were picked were picked for expediency in synthesis. We were given six weeks to generate a gram of each of these. Incidentally, an interesting story aside, buying 70 grams of 13C benzene turned out to be quite a trick and somewhat expensive also. That constituted the starting material and was the entire U.S. supply at that time.

The rationale for choosing these recovery surrogates is shown at the top. We wanted compounds that are chemically similar so that there would be no real question about the accuracy of the recovery assessment. We also wanted something that could be differentiated from the analyte. Clearly, these compounds are no good for ECD work because they have no different ECD response than the native ECDs, but in mass spectrometry, a separation of 12 mass units gives you a very nice clear channel to work with. They were not commercially available, so we did a custom synthesis. The four compounds

are now available in mixture solutions from EMSL, Cincinnati. I don't have any specifics but I understand that there are at least three commercial labeled isotope manufacturers that are in some stage of preparation of these compounds for sale, so that if for some reason you don't want to avail yourself of the free ones through Cincinnati, you will also be able to purchase them.

SLIDE 14

The general flow scheme of the method is not a lot different than in many methods. One physically homogenizes the sample--breaks up chunks, stirs it, whatever necessary. Then one adds the surrogates before doing any kind of chemical processing which could change the PCB content. At that point, the analyst has a decision to make, which extraction and cleanup should be used. Pretty much any technique that gives decent recoveries is allowed. We have provided some examples, and I'll get to those in a minute.

After the sample is ready for GC/EIMS, you add an internal standard. The original internal standard was d5-tetrachlorobiphenyl, but that's no longer commercially available, so we've also added d12-chrysene and iodonaphthylene as options, depending

upon laboratory preference. Conduct the GC/EIMS analysis, do qualitative and quantitative data reduction and report as is required by the client, either by homologs or by individual peaks, or just the total number. There is flexibility in the reporting.

SLIDE 15

I mentioned that we allowed a number of extraction techniques. These are just the general categories that are mentioned. Literature references are supplied. The choice of the technique depends upon the sample. For instance, with a chlorinated organic still bottom, your only option might be dilution. In another case, evaporative concentration may be appropriate. If your sample is a volatile solvent and you're trying to find the PCB content, you can simply put it on a rotary evaporator or Kuderna-Danish and achieve say a 8500:1 concentration.

Liquid-liquid extraction is applicable for water samples and many other samples. Liquid-solid extraction is used for soils, et cetera, and others. Matrix destruction might be utilized for a commercial product such as an acid chloride where you hydrolize it to form the acid and then rinse away the acid with water.

SLIDE 16

We've also recommended the specific extraction techniques listed here for a variety of matrices. Most of these should be familiar. The reference to Watts for the blood and the adipose is the old HERL pesticide manual which was formally authored by Randy Watts. "MOG" is Mills Onley Gaither from the classic JAOAC publication in 1963. The AOAC refers to their Official Methods of Analysis manual. FDA refers to the FDA Pesticide Analytical Manual. Those methods are given as specific options. If people want to use those, that's fine.

SLIDE 17

Cleanup options are listed here. Many of these are taken from the oil method of Bellar and Lichtenberg.

SLIDE 18

Quality control is the heart of the method. There are laboratory QC requirements. One must achieve certain performance criteria. It's not precisely specified in our method, but the laboratory has to demonstrate to the client or the regulatory agency or whatever, that they're achieving performance.

SLIDE 19

GC and MS performance are similar to what's found in 625. In addition, one must perform routine qualitative and quantitative calculation checks to make sure that calculation transcription errors are not occurring, much along the line of what was mentioned yesterday by Samuel To. Sample QC--the recoveries must meet specified percent recoveries. The acceptable recovery is something one sets up with a client before you start up. For instance, you might specify that any recovery between 50 percent and 150 percent is acceptable. There is some guidance on mass spectral data quality and it's also important to see that you have an internal standard response that's consistent from sample to sample. Last, but not least, the typical blanks, replicates, and standard addition are all mentioned in the QC section.

SLIDE 20

Now I'd like to present some application slides. This is a fish oil. I believe it's bluefish from this area, the Chesapeake Bay. This is the total ion chromatogram. PCB region is labeled. They don't really stand out. The big peak at the right is labeled just because everybody wants to know

what it is, and it's good old cholesterol.

SLIDE 21

Same fish sample. These are ion plots for tetra, penta, hexa, and heptachlorobiphenyls. You can see that the PCBs are standing out nicely. I don't have a slide, but recoveries of the surrogates were adequate and worked very nicely. This is a typical routine type of sample. People have been determining PCBs in fish since maybe 1969. No big deal.

SLIDE 22

Now, let's move on to something that's a little bit more complex and a little tougher, and that's a by-product PCB problem. By-product PCBs are regulated under TSCA and there are a couple of rules out in the Federal Register.

This is what one sees. This is a chlorinated organic still bottom. We ran it; several other labs ran the same sample in a CMA round robin. The top two traces are the M+2 and the M+4 for octa-chlorobiphenyl, and you see we have a good match for six isomers. The third trace is the 13C octa-surrogate that had, I believe, in this case, 67 percent recovery. This is an extremely complex sample. There's everything from mono- through

deca-chlorobiphenyls in it; somewhere on the order of 80 congeners total in the sample; somewhere around 300 to 500 parts per million aggregate PCBs in the sample. Very, very complex. There are lots of other chlorinated compounds. An ECD chromatogram of this sample just totally obscured the PCBs. In fact, you can see from the RIC the complexity of this particular sample. A tough sample.

SLIDE 23

We have some performance data. This is a table that's in the method writeup. I really don't want to direct your attention so much to the top--that's a tabulation of the possible range of LOQs. Right here we have some intralaboratory data that we generated in our lab. We feel that with good samples and good technique, you should be getting recoveries close to 90 percent. Depending upon the type of sample, we've seen recoveries as low as 22 percent. Those are worst cases. Typically, though, we tend to see 70 to 130. Some of that error is from the MS, which has its own errors associated with it.

An interlaboratory study was conducted about a year and a half ago. The recoveries observed by the different laboratories ranged quite widely, as

you can see. There were some very, very bad data in there. As was mentioned yesterday, some of this was clerical errors. Some of it we patched up. For example, when decimal places were wrong, and so forth, we fixed. In the worst case column, with the 690 percent recovery, one laboratory failed to correctly identify the internal standard. If you don't identify your internal standard, everything goes downhill rapidly. They were given a chance to change their report and took a look at it and said, "Yes, those are the numbers we believe," so we had to take it.

With throwing out some of those really terrible results, we feel like +/-60 percent precision is achievable, and this is for the very low levels in very complex samples. One of the samples in this sample set was in fact that chlorinated benzene waste that I talked about earlier.

SLIDE 24

In summary, the advantages of this type of a method are presented on this slide. As with isotope dilution, although not quite as all-encompassing, we have data of known quality on each sample. That's important. The surrogates, we feel, can reduce the validation time. The idea is where you

have a matrix that you've not encountered before, you really don't know the precise way to handle it in the extraction and cleanup, you simply try your best guess. If the surrogate recoveries are good, you report it. If they're not good, you know you did something wrong and you can go back and do something else, but you don't have to do a $50,000 validation project before you get started.

We eliminated Aroclor as a calibration mixture. It eliminates the temptation to try to make every sample with PCBs in it be an Aroclor. Most of the PCBs in the environment did start out as Aroclors, but they're weathering and as the years go by, the profiles are going to look less and less like the original Monsanto products. We are quantitating all the way from mono through deca. We have incorporated flexibility that accomodates laboratory preferences.

SLIDE 25

It's not a perfect method. The surrogates must be incorporated into the matrix. If you've got a hard plastic, for instance, and you throw the PCBs on the top and don't get them down inside, you're going to get good surrogate recoveries, but you really haven't incorporated them into the matrix.

We are extrapolating from 77 to 209 congeners and there's some error associated with that. We have complex matrices, complex analytes and we are having a lot of sources of error. That holds true for PCBs regardless of which method you're talking about. It's always going to have inherently more error than a single analyte method. Finally, keep in mind that any method is only as good as the person who's performing the work.

Thank you. Are there questions?

COMPARISON OF METHODS OF ANALYSIS FOR PCBs

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Kansas City, Missouri 64110

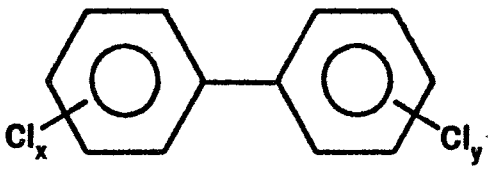

Cl$_x$
Cl$_y$

Table 2-I. PCB Nomenclature Categories

Category	Number of Individual Compounds[a]
Congener	209
Homolog	10
Isomers/Homolog	1-46

a Natural isotopic abundance only.

Table 2-V. Average Molecular Composition (wt.%) of Some Aroclors

Homolog (Chlorines)	Aroclor						
	1221	1232[a]	1016	1242	1248	1254	1260
0	10						
1	50	26	2	1			
2	35	29	19	13	1		
3	4	24	57	45	2	1	
4	1	15	22	31	49	15	
5				10	27	53	12
6					2	26	42
7						4	38
8							7
9							1

a Five percent unidentified (biphenyl?).

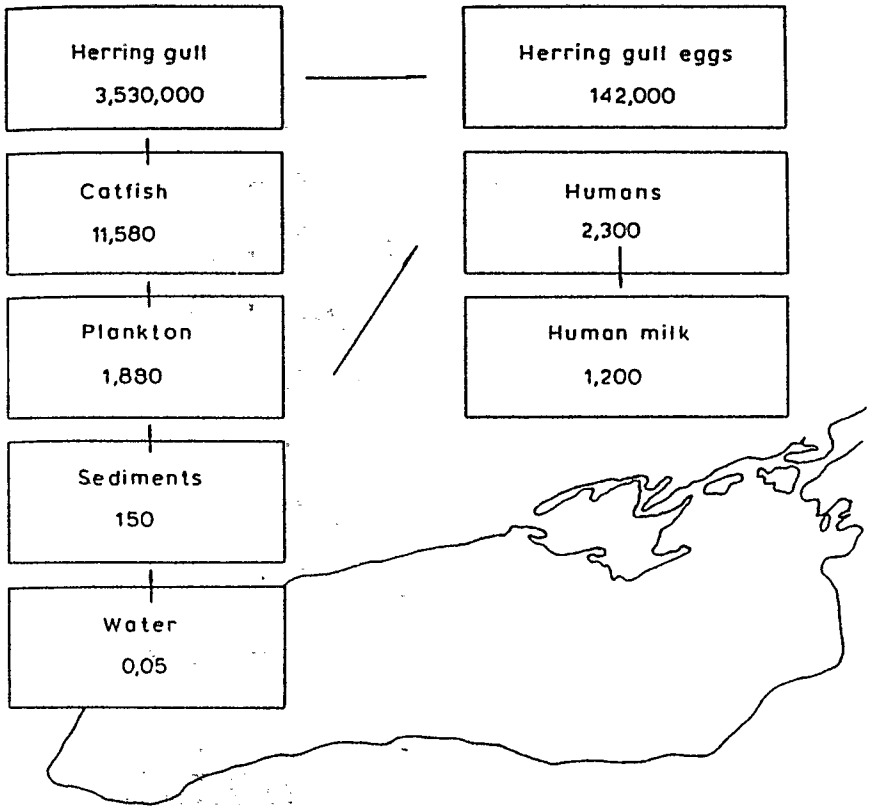
Source: Brinkman and DeKok, 1980. Reproduced with permission, copyright 1980, Elsevier Biomedical Press BV.

TYPICAL ENVIRONMENTAL LEVELS

Location	Air ng/m^3	Water/Rain ng/L
Remote	0.02 - 0.5	0.03 - 10
Great Lakes	0.1 - 5	10 - 150
Rural	0.1 - 2	1 - 50
Urban	0.5 - 30	10 - 250
Indoor	<10 - 620	
Indoor (Ballast)	5,900	
Stack Gas	<10 - 60,000	
Occupational	10^5 - 10^7	

UNIQUE

- Stable
- Biomagnification
- Ubiquitous
- Toxic
- Nontoxic
- Banned 1976
- Still Used
- Complex Analysis



Biomagnification of PCB in the lake Ontario basin (PPB)

EXTRACTION

OBJECTIVE:

Remove PCBs from Sample Matrix to Solvent

GENERAL CONSIDERATIONS:

1. Good Partition
2. Good Contact
3. Removal of Major Interferences (''Cleanup'')
4. Solvent Compatible with Rest of Analysis
5. Cost (Labor, Supplies, Equipment)

CLEANUP

Objective

Remove interferences (enrichment)

General Considerations

1. Remove specific interferences (DDT)
2. Remove general background (oil)
3. Keep PCBs
4. Dependent on Determination Step
5. Cost (labor, supplies, equipment)

LEVELS OF CLEANUP

Level	Example	Technique
1. Gross Interferences	fat	GPC
2. Trace Interferences	OC Pesticides	Silica Column CrO_3
3. PCB Fractionation	o,o′ Substitution	Carbon

SOLVENT EVAPORATION

Kuderna – Danish

Rotary Evaporator

N_2 Blowdown

(Skill and Care = Success)

DETERMINATION	QUANTITATION
PGC/ECD	Webb-McCall Total Areas Aroclor
HRGC/ECD	Congener-Specific (Total Areas) (Aroclor)
HRGC/EIMS	Homologs Congener-Specific (Aroclor) (Total Areas)

QUALITY CONTROL CONSIDERATIONS IN PCB ANALYSIS

Method Validation

Sampling

Method Execution

Sample Preparation and Analysis

Data Reduction and Reporting

Confirmation

Networks of Multiple Laboratories

STANDARD PROCEDURES

Procedure	Matrix	GC Detector
ANSI	Water, sediment, soil	ECD
ASTM	Capacitor askarels	FID
ASTM	Water (2), soil, sediment	ECD
EPA (3)	Sludge	ECD
EPA (2)	Solid waste	ECD; EIMS
EPA	Soil, sediment	ECD
EPA (4)	Water	ECD, HECD, or EIMS
EPA	Transformer fluids or waste oils	HECD, ECD, or EIMS
EPA	Air near hazardous waste sites collected on PUF	ECD
EPA	"spills"	ECD
EPA (by-products)	Products or wastes	EIMS
Monsanto	Water, sediment	ECD

STANDARD METHOD PHILOSOPHY

	+	-
Procedural Specification (e.g. 600 Series)	Historical Interlab Consistency "Off the Shelf"	Few Options Allowed Single Matrix Requires Updates
Performance Specification (e.g. OTS Homolog)	Options Multiple Analytes Many Matrices Known Data Quality	Protocol Required

ANALYSIS OF PCBs IN LIQUIDS AND SOLIDS
(Erickson et al, 1985)

Analytes: Any Mixture of PCBs

Matrices: Virtually any Solid or Liquid

Determination: GC/EIMS

Quantitation: 10 Congeners

QC: Four-^{13}C-PCBs (Sample Performance)
Instrumental Performance
Blanks, etc.

RECOVERY SURROGATES

Criteria

1. Chemical similarity (identical liquid chromatography, identical behavior under acid cleanup)
2. Able to be differentiated from analyte

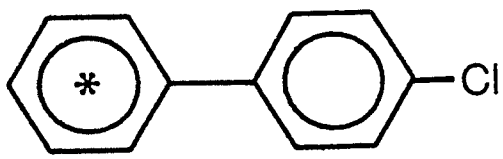
Best Choice

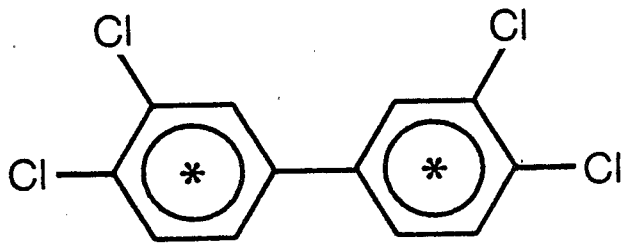
^{13}C labeled PCB

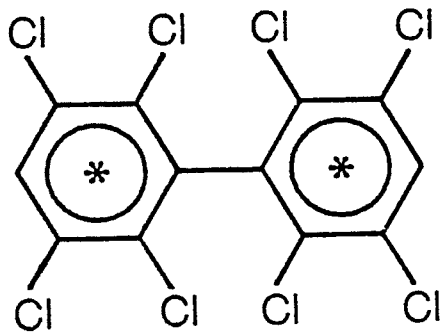
But: Not commercially available

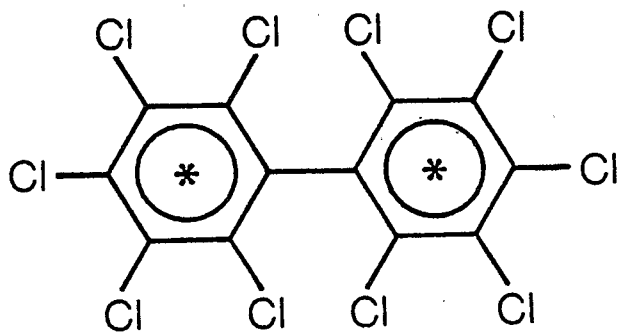
Solution: Custom Synthesis

(Enough for thousands of analyses)

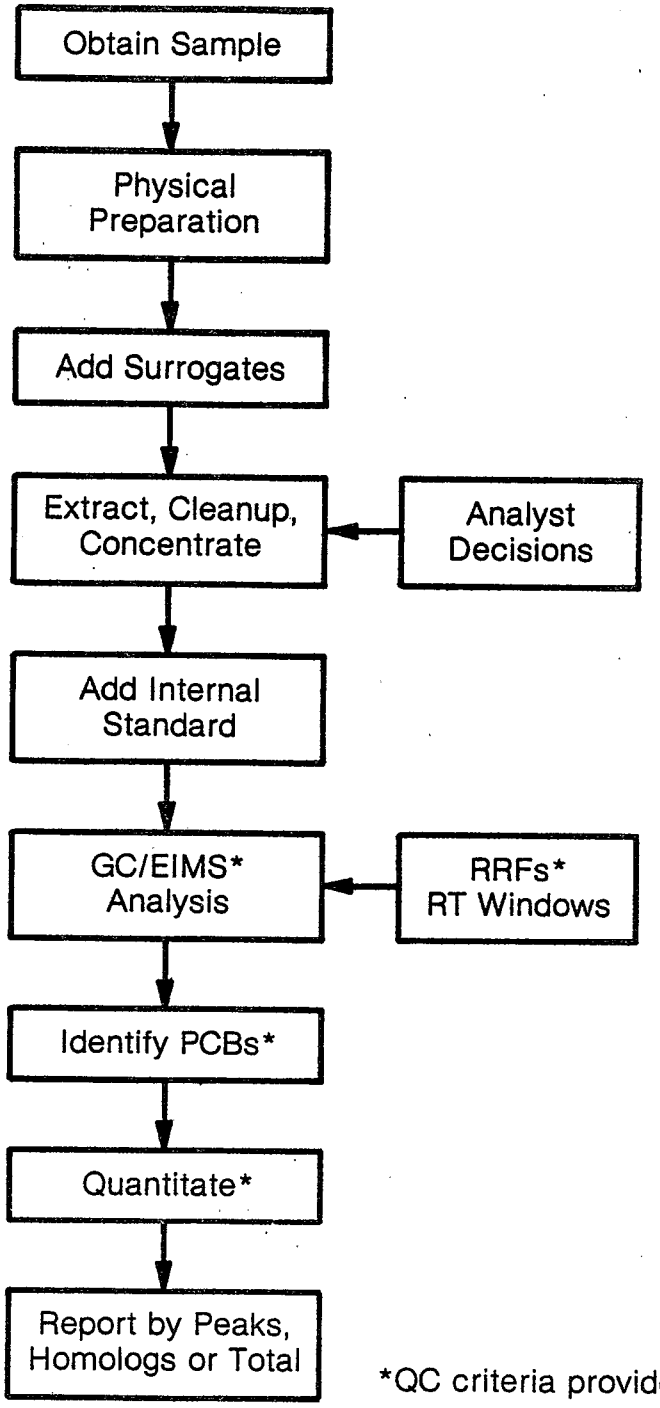








ANALYTICAL METHOD



*QC criteria provided

EXTRACTION TECHNIQUES

- Dilution
- Direct Injection
- Evaporative Concentration
- Liquid-Liquid Extraction
- Liquid-Solid Extraction
- Sorbent Column Extraction
- Thermal Desorption
- Matrix Destruction

POTENTIAL EXTRACTION TECHNIQUES FOR SOME MATRICES

Water (608, 625)

Sewage, Sludge, Hazardous Waste (EPA 'Sludge')

Sediment and Soil (ASTM)

Blood (Watts)

Adipose (MOG, Watts)

Fish (AOAC)

Food and Plant Tissue (MOG, FDA)

Paper (AOAC)

Oil (Bellar and Lichtenberg)

CLEANUP OPTIONS

Acid

Florisil Column

Alumina Column

Silica Gel Column

Gel Permeation Chromatography

Acetonitrile Partition

Florisil Slurry

Base (Saponification)

HPLC

QUALITY CONTROL

Lab QC

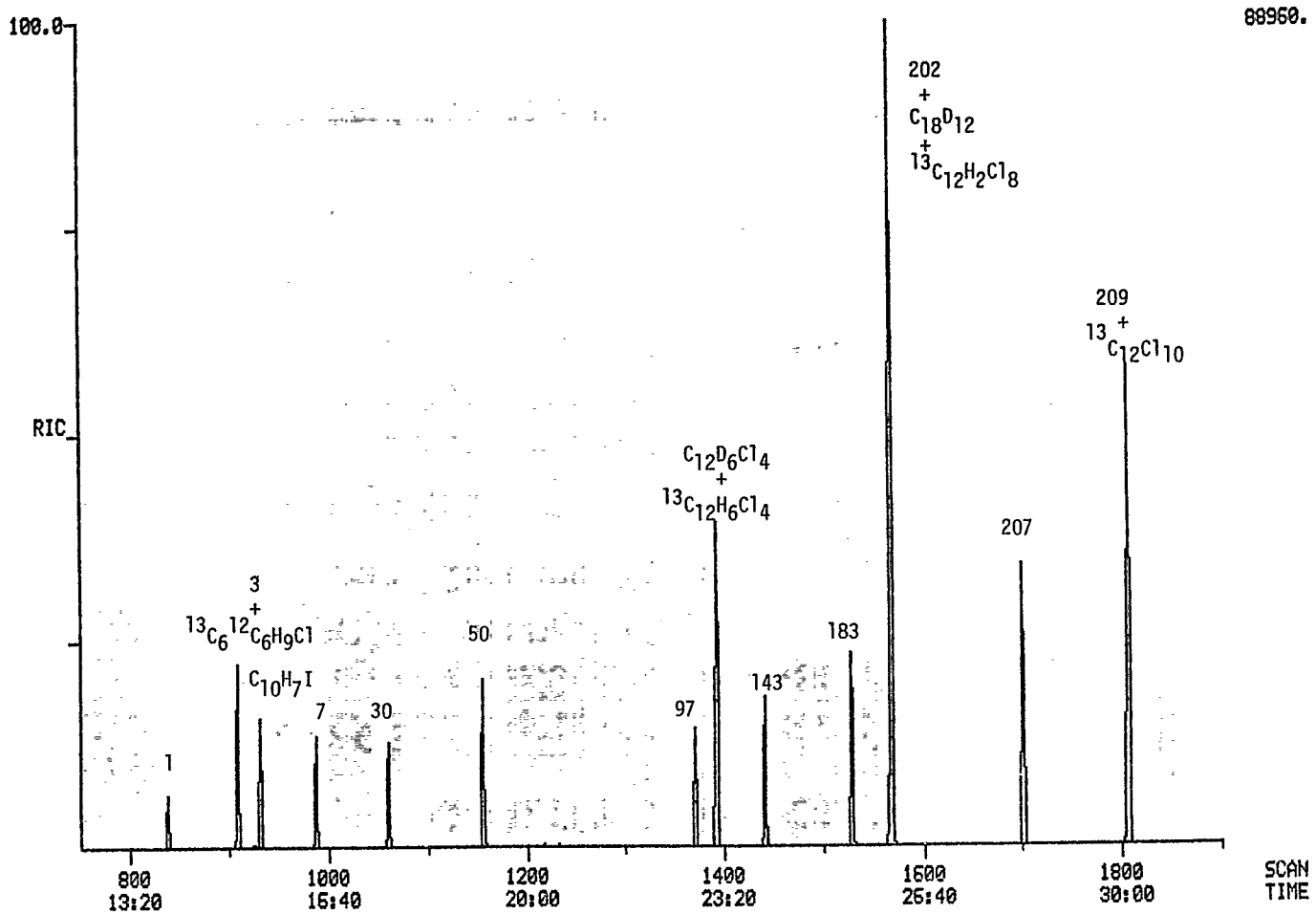
Performance Certification—Stable RFs
GC and MS Performance
Check Qual. and Quant.

Sample QC

Surrogate Recoveries (e.g. 50-150%)
Mass Spectral Quality
Consistent Internal Standard Response

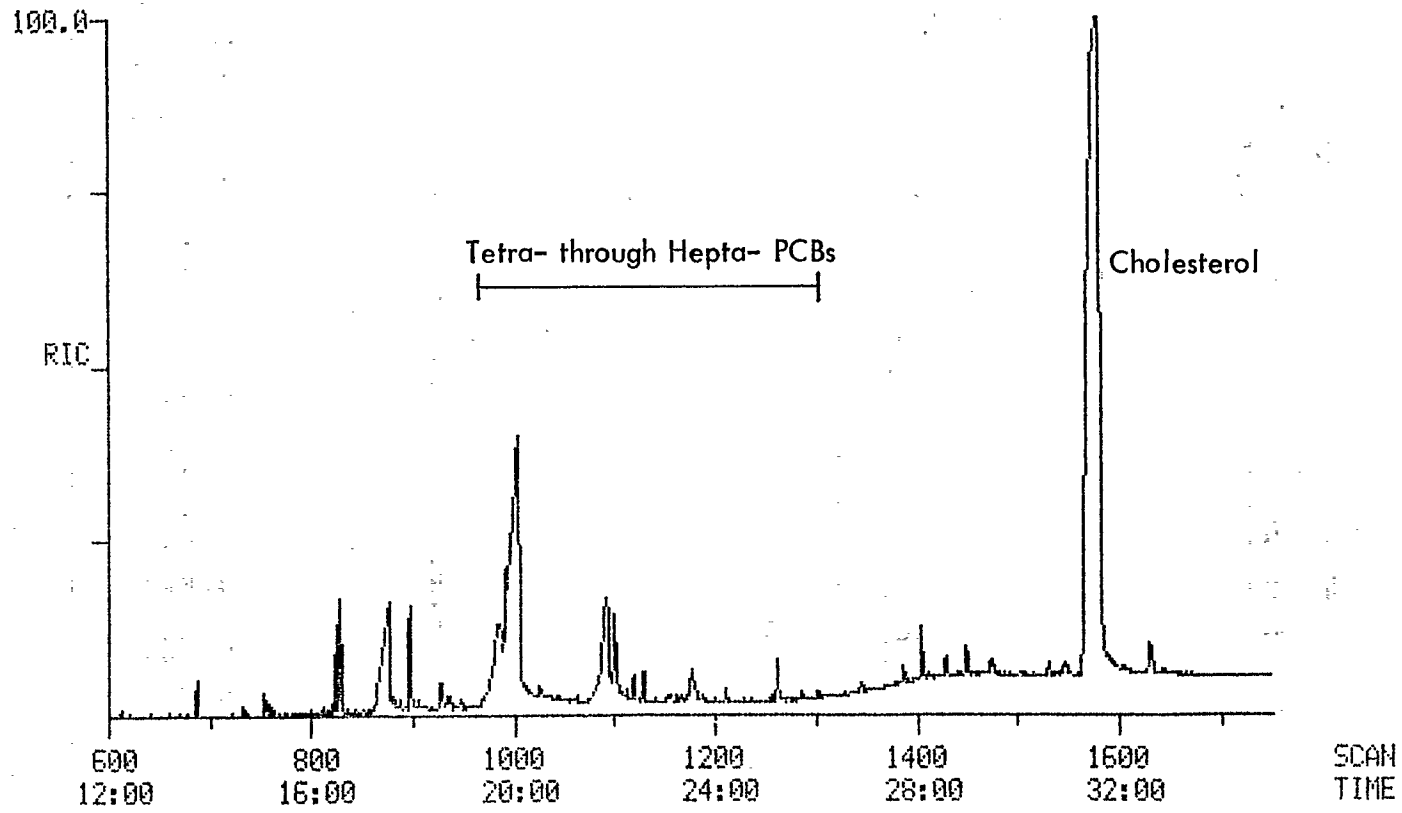
Sample Set QC

Blanks
Replicates
Standard Addition


88960.
100.0
RIC
1
3
+
$^{13}C_6{}^{12}C_6H_9Cl$
$C_{10}H_7I$
7
30
50
97
$C_{12}D_6Cl_4$
+
$^{13}C_{12}H_6Cl_4$
143
183
202
+
$C_{18}D_{12}$
+
$^{13}C_{12}H_2Cl_8$
207
209
+
$^{13}C_{12}Cl_{10}$
800
13:20
1000
16:40
1200
20:00
1400
23:20
1600
26:40
1800
30:00
SCAN
TIME

METHOD PERFORMANCE PARAMETERS

Measurement	Best Case	Worst Case	Typical Value
Instrumental LOQ (ng/μL)			
Full Scan	0.5	10	1
Limited Mass Scan	0.1	0.5	0.05
Selected Ion Monitoring	0.005	0.5	0.025
Sample Concentration Factor	1,000 → 1	1 → 100	–
Matrix Interference Level (ng/μL)	0.01	1,000	–
Sample Injection Volume (μL)	10	0.1	1
Method LOQ (μg/g)	10^{-5}	500	1
Intralaboratory			
Recovery (%)	90 + 15	< 50, > 200	70-130
Precision (%)	–	–	+ 34%
Accuracy	–	–	–
Interlaboratory			
Recovery (%)	–	22-690	–
Precision (%)	–	–	± 60
Accuracy	–	–	± 62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RIC
Tetra- through Hepta- PCBs
Cholesterol
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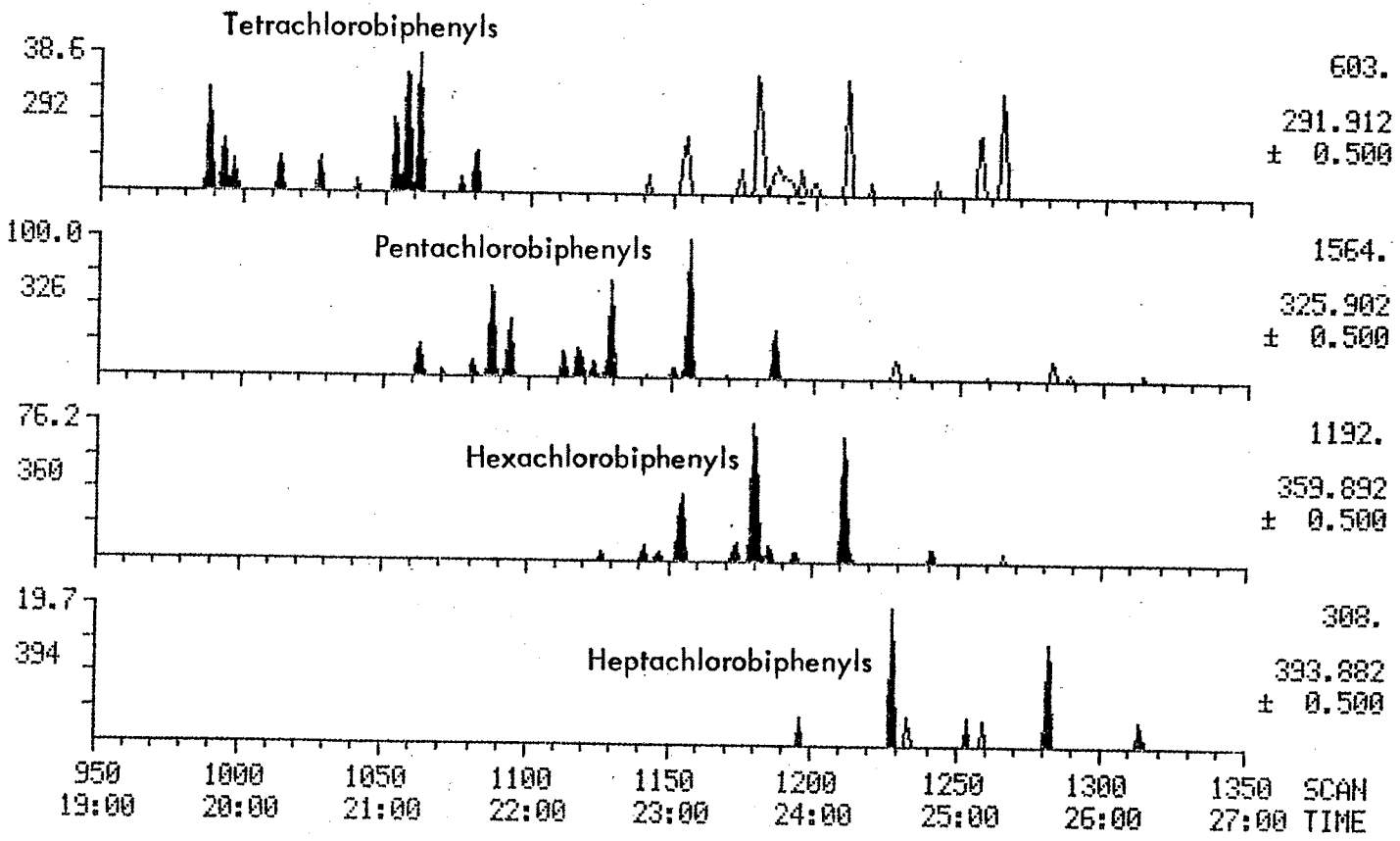
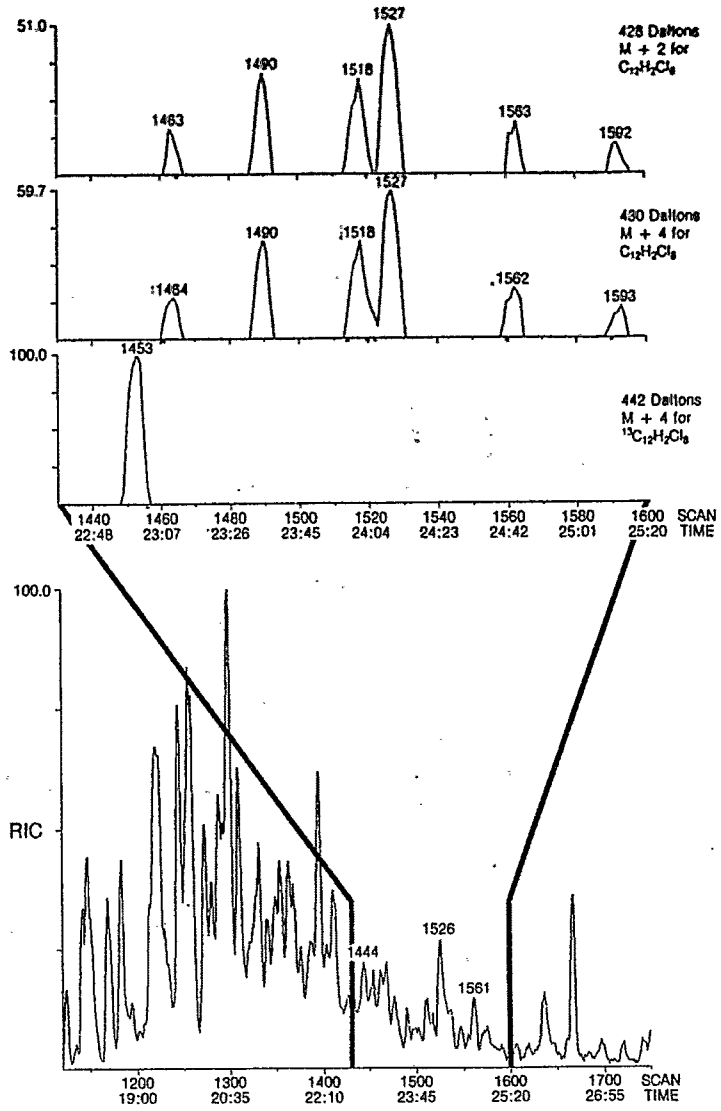
800
16:00
1000
20:00
1200
24:00
1400
28:00
1600
32:00
SCAN
TIME


Tetrachlorobiphenyls
38.6
292
603.
291.912
± 0.500
Pentachlorobiphenyls
100.0
326
1564.
325.902
± 0.500
Hexachlorobiphenyls
76.2
360
1192.
359.892
± 0.500
Heptachlorobiphenyls
19.7
394
308.
393.882
± 0.500
950 1000 1050 1100 1150 1200 1250 1300 1350 SCAN
19:00 20:00 21:00 22:00 23:00 24:00 25:00 26:00 27:00 TIME


51.0
1463
1490
1518
1527
1563
1592
428 Daltons
M + 2 for
$C_{12}H_2Cl_8$
59.7
1484
1490
1518
1527
1562
1593
430 Daltons
M + 4 for
$C_{12}H_2Cl_8$
100.0
1453
442 Daltons
M + 4 for
$^{13}C_{12}H_2Cl_8$
1440 22:48
1460 23:07
1480 23:26
1500 23:45
1520 24:04
1540 24:23
1560 24:42
1580 25:01
1600 25:20
SCAN TIME
100.0
RIC
1444
1526
1561
1200 19:00
1300 20:35
1400 22:10
1500 23:45
1600 25:20
1700 26:55
SCAN TIME

ADVANTAGES

- Surrogates provide data of *known* quality
- Surrogates can reduce validation time
- Eliminates Aroclor as calibration mixture
- Can quantitate *all* PCBs, not just Aroclor-type mixtures
- Flexibility permits application to different matrices
- Flexibility accommodates cleanup preferences

PROBLEMS

- Surrogate must be incorporated. If not, surrogate recovery ≠ analyte recovery.
- Must extrapolate RRF from 77 of the 209 congeners available for measurement. Measured average RRFs may not be true average.
- Complex matrices and complex analytes yield many sources of data interpretation error.
- Method only as good as the analyst.

QUESTION AND ANSWER SESSION

MR. KROCHTA: Bill Krochta, PPG Industries. Mitch, you mentioned that the PCBs were stable, however, there are reports that some of the congeners are extremely sensitive or degrade with radiation or light. Do you take any precautions or have you noticed this to be a problem?

MR. ERICKSON: Well, there's no question that PCBs do have a half-life. Some of the monos have a very short biological half-life in, say, aquatic systems. The one thing that I've heard where people have worried over the years about degradation is a concentrated sulfuric acid cleanup. There are some reports in the literature that the lower PCBs can be degraded in a sulfuric acid cleanup. Those reports are for hot sulfuric acid, say 50 degrees for 15 minutes. There are also many reports in the literature where people have done sulfuric acid at room temperature with monochloro- and dichlorobiphenyls and had no losses.

That's the one thing I know of where people have actually done some studies. There's some conflicting literature, but basically if you don't

heat it up, it seems like you can get away with sulfuric acid treatment.

With regard to photochemical degradation in the laboratory, I'd say that's a minimal problem. I don't know of any data to support that.

MR. TELLIARD: Any other questions? Thank you.

MR. TELLIARD: Our next speaker is Denis Lin. Denis Lin is from some organization whose letters I can never remember. Denis is going to talk about some volatile analysis.

DENIS C. K. LIN, PH.D.

ENVIRONMENTAL TESTING AND CERTIFICATION CORPORATION

ANALYSIS OF VOLATILE WATER SOLUBLE COMPOUNDS

DR. LIN: Good morning, ladies and gentleman. By all means, get up, get a cup of coffee; you probably need it to hear me speak. Before I start getting into this particular presentation, contemplating what transpired yesterday in terms of the presentations and statistics, et cetera, perhaps I should change my title to, In Search Of Our Experience in a Preliminary Study of Analysis of Volatile Water Soluble Compounds. Before I really start again, I'd like to acknowledge my co-workers, Faith Dees and Dave Lessing, who have worked hard in generating the data and some of these viewgraphs.

As you have heard of, this list called Appendix VIII compounds, we as an analytical service lab, always have some kinds that come in to request for something somewhat different, like dioxin in combat army boots, folate in baby plastic panties and all Appendix VIII compounds in groundwater. We have our approaches to Appendix VIII compounds and

today, I'm not going to talk about all of them. I'm specifically interested in a certain segment of it, which seemingly very simple. You say, how could someone analyze formic acid in water or isobutanol in water. The first thing you think of is, I drink, I worry about alcohol in blood, so you think, how do you analyze ethanol in blood. Clinical tests have shown that when you directly inject the serum, you get very good results. You always heard John McGuire preaching about direct aqueous injection on some of these so-called water soluble compounds. Certainly direct aqueous injection is an approach to doing these kinds of analyses.

However, some of the regulators don't like the detection limits because they are somewhat high. In that respect, there has been some work done through, I believe, and contracted by Athens , the master analytical scheme. There is this animal called heated purge and trap which has been suggested to us to tackle some of these Appendix VIII compounds. So today, what I'm going to talk about is our experience of doing these compounds via that method compared to say, perhaps direct aqueous injection.

The objective is to make sure that heated purge

and trap worked in those compounds of interest, and also, in the same process, we would like to know the linear range of sensitivity and somewhat of an off-the-cuff error range, not a very rigorous winsorized, whatever, statistic approach.

In this particular study, what we have done is, because we want to compare it with direct aqueous injection, we have deliberately chosen columns that can be used for direct aqueous injection. If everything works, the heated purge and trap technique should have a thousand-fold better sensitivity, mainly because in direct aqueous injection normally you inject micrometer quantities and in our case, we inject about 5 microliters. When you do heated purge and trap, you use 5 mil but the absolute amount of analyte is the same. So the concentration factor if one considers it that way, is a thousand-fold.

The data I'm going to show is going to talk about absolute amount. So whenever the heated purge and trap technique works, you can factor in a thousand-fold better sensitivity. You have to compare apples with apples, I guess.

SLIDE 2

As George said, there are 375 compounds listed

in the Appendix VIII list. Some of these are water soluble compounds and here's the list. The approach that we tried to tackle first is either direct aqueous injection or heated purge and trap. The absolute amount range that we looked into is between 5 ng. to 5,000 ng. We don't know what's the right number so we explore the whole range. The column, as stated, is down at the bottom. There's a spelling error in the nitrosopyrolidine.

SLIDE 3

The next list...since we're doing this, we said we might as well take a look at other things because other things are of interest also. These are very commonly found alcohols. Methanol to butanol. If you are in clinical analysis, you know that every time you do blood alcohol you use methanol and isobutyl alcohol just to check up. The column again is at the bottom.

SLIDE 4

The third list we have is a list of Cl-C4 alcule acids. The clinical chemists that have done this for a while...I listen to them and they always seem to have very good results. I'd like to try it and if you look at a catalog, they always also show very good results.

When you get into the nitty-gritty, things don't seem to be as rosy as the literature says. So this is the list of acids we looked at and the column we used, which was recommended by direct aqueous injection for Cl-C8 acids.

SLIDE 5

I'm going to make the corrections on the first one. The rest I'm not going to make any corrections there. Dave Lessy is a young man who's the technician in our group and who made all these slides and I just didn't have the heart to make him go back and do it all over again. The Y axis is actually the absolute area per nanogram, so it is not relative response factor. It is response factor. The black asterisk is the average reponse factor of triplicate analysis. We bootlegged this project. We didn't have the time or the luxury to do 7 out of 10. We used all the data in triplicate. The red marks are basically the standard deviation error range you want to consider that way. At the bottom of the X axis is the absolute amount analyzed. In other words, 5 mL contain 100 ng. of the analyte or 100 ng. of the analyte in 5 mils of the water, assuming that you have 100 percent purge efficiency.

In the ideal situation, if the world is perfect, it should be a straight line across the graph. As I said, this is not, so, there are situations where one thing is better than others, and in this particular case, this is the direct aqueous injection of 1-4 dioxane compared to the next one which is the heated purge and trap technique.

In almost all the cases, the 5,000 ng. absolute amount is just off range as linearity goes. Depends on the compound, for those that are successful, it is somewhere between 50 to 5,000 ng. absolute amount. We did not use any internal standard in this study. I'm sure you are probably thinking what Cl3 internal standard can you use in this study to improve it.

SLIDE 6

We'll move on to the next one that will be fairly repetitive now, in terms of some of these charts. The next one, if I remember correctly, should be ethylcyanide gas. This is the direct aqueous injection of ethylcyanide.

SLIDE 7

The next one is the heated purge and trap. That may be an old photo of that chart. It looks very straight. It looks as though it's

almost the idea case for the heated purge and trap as far as linearity goes, from 50 to about 5,000 ng. absolute amount.

SLIDE 8

This is aereol alcohol. It looks like 50 ng. doesn't seem to be appropriate. It should start somewhere higher for direct aqueous injection. The heated purge and trap of this alcohol seems to be better in terms of linearity compared to the direct aqueous injection.

SLIDE 9

As you remember, George demonstrated amply yesterday, isobutyl alcohol if you use 8240 as a way to test without any modification is a miserable failure. We have experienced that and we do recommend either use direct aqueous injection, which gives a better result, or mix heated purge and trap for isobutyl alcohol.

SLIDE 10

Then we move on to the next series of the compounds as a continuation in terms of alcohol. Methanol. Next one is the heated purge and trap for methanol. I believe that there are couple more alcohols that we have done. This is one.

SLIDE 11

The next one is butynol using heated purge and trap. It's very linear in terms of response factor across the range. The heated purge and trap. I believe the next set is the acids.

SLIDE 12

I started with reversed order, somewhat, for the acids. Acetic acid was the only one that we have some success in using the heated purge and trap technique. The other acids that we look into simply fail. We did not have any response, but that doesn't deter us from looking into the direct aqueous injection technique, because you have to have some way, no matter how poor, in some sense of sensitivity. If you are pushed, you at least can report some detection limit for the acids. The next three or four are all acids, direct aqueous injection. I'm not impressed with the failure, but at least we got a response.

SLIDES 13, 14, 15

This is acetic acid with direct aqueous injection, and there's a couple more. Direct acid, crotonic acid. That's all the acids. If I recall correctly, formic acid is in Appendix VIII list and

our experience with formic acid has been miserable in terms of getting any consistent result. We tried direct aqueous, we tried to extract with ether. Every now and then we get response and then all of a sudden, for no reason you don't have any response at all. So, the formic acid, if you look into the biomedical community where they worry about the acid in urine, there seems to have literature assess that it can be done, and I always thought water is simpler than urine, for whatever reason. Maybe the urine is essential to have successful formic analysis to do something with water.

SLIDE 16

In our opinion, as I said--this is a preliminary study, using the heated purge and trap technique, the range that we think is reasonable is represented in the second column from the right. The percentage standard deviation is calculated basically by forming the so called, the one outside range, an average response factor. Maybe George and Paul can argue the validity on using this. As I said, this is just some estimation how good, bad or indifferent the method is.

The third column, just for interest's sake,

what we did is assume the direct aqueous injection is 100 percent recovery and compare absolute response of the heated purge and trap to the direct aqueous injection.

It looks very easy indeed. The direct aqueous injection represents 100 percent recovery. These are for the Appendix VIII compounds. I don't know why Dave didn't have the chance to calculate out the nitrosalpyrolidine.

SLIDE 17

These are for the alcohols in terms of heated purge and trap. Quickly, go down and we don't know why for two propanol, the purge efficiency is 159 percent, but I believe in reporting what you see. Maybe somebody else may not, maybe John McGuire has a lot of experience in these functions here. So we don't know why it was 159 percent, but if... other than that, things look very normal.

How can we improve this? This is a bootleg project and we still have to look further. There are a number of things. Obviously, if you decide heated purge and trap is a viable way to go, then we should really dig deep into the perhaps master analytical scheme which has a section on heated purge and trap, and try to optimize the material

or the purge and trap conditions. I'm sure addition of internal standard, perhaps the C13 label ones, will improve the precision and accuracy. The chromatographic conditions, as I said, because we want to compare apples with apples, we did not think in terms of...for those series of compounds we are thinking in terms, how can we have comparable results and how can we improve the purging efficiency.

The three items that we have thought of that might just be the change, and I'd welcome any other ones that you can help me on, is solvent Ph adjustment and saponification.

As far as the preliminary study goes, that's all I have to say, and I welcome any suggestions and questions.

MR. TELLIARD: Any questions? You going to let him off?

MR. TELLIARD: Now a completely different subject. We'd like to keep this meeting still somewhat ressembling industrial waste chemistry, so our next speaker is going to talk on one of our...this is for all you old shit-chemists, so many of you people out there with GC/MS don't even know what one of these things is let alone what the letters mean. But our next speaker is going to talk about the basic shit chemistry, commonly called a BOD.

JAMES C. YOUNG

UNIVERSITY OF ARKANSAS
DEPARTMENT OF CIVIL ENGINEERING

DETERMINATION OF FIVE-DAY CARBONACEOUS BOD
IN WASTEWATER

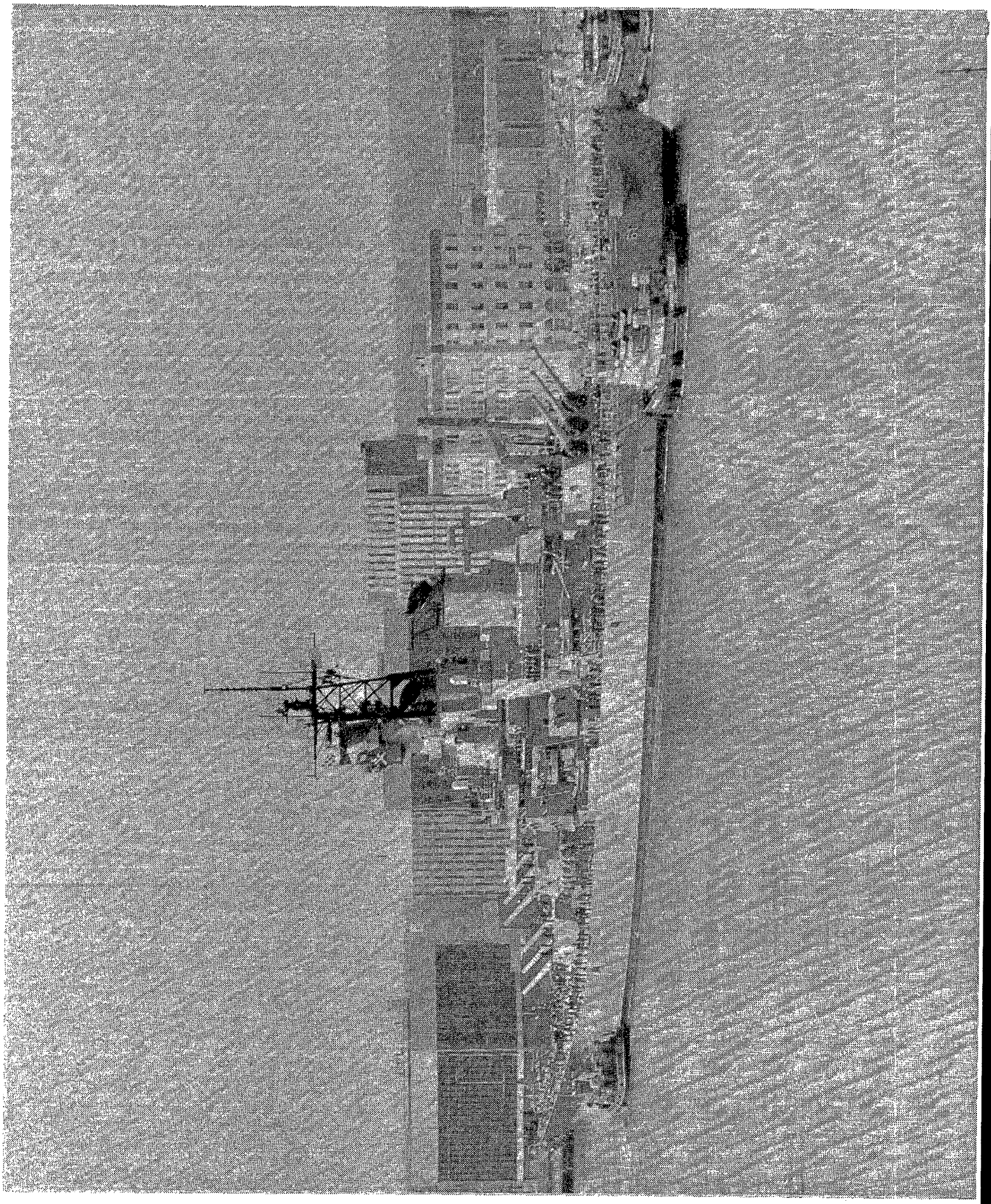
MR. YOUNG: Good morning. I feel a little bit out of place...

VOICE FROM AUDIENCE: The ship is coming!

(WHEREUPON, a 10 minute break was taken to view the battleship.)

MR. YOUNG: This is the first time I've ever been in competition with a battleship. I guess I should have known who'd win. When you stop and think about it, that battleship is probably older than most of us here. I'm not sure when it was first built; it was a few years ago. Any historians here that know? I expect it was built in the late 30's or early 40's.

(Revised presentation submitted.)



BIOCHEMICAL OXYGEN DEMAND:
MEASUREMENT AND INTERPRETATION

By

James C. Young

Professor and Head of Civil Engineering

University of Arkansas, Fayetteville, AR 72701

Prepared for presentation at the

Eighth Annual Analytical Symposium on the

Analysis of Pollutants in the Environment,

Norfolk, VA

April 3-4, 1985

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INTRODUCTION

The Biochemical Oxygen Demand (BOD) test is probably the least understood and most frequently misused of any analytical measurement in the field of water pollution control. Yet the proper measurement and interpretation of BOD data is highly essential to the evaluation of the performance of wastewater treatment plants and to determining the impact of waste loads on receiving streams.

Not only must the wastewater analyst know how to measure BOD properly, but plant operators and others involved in pollution control must know how to use the results of BOD tests. They must have a basic knowledge of the factors affecting the accuracy and precision of the measurement technique and must understand the basic factors causing the test results to deviate from anticipated or normal values.

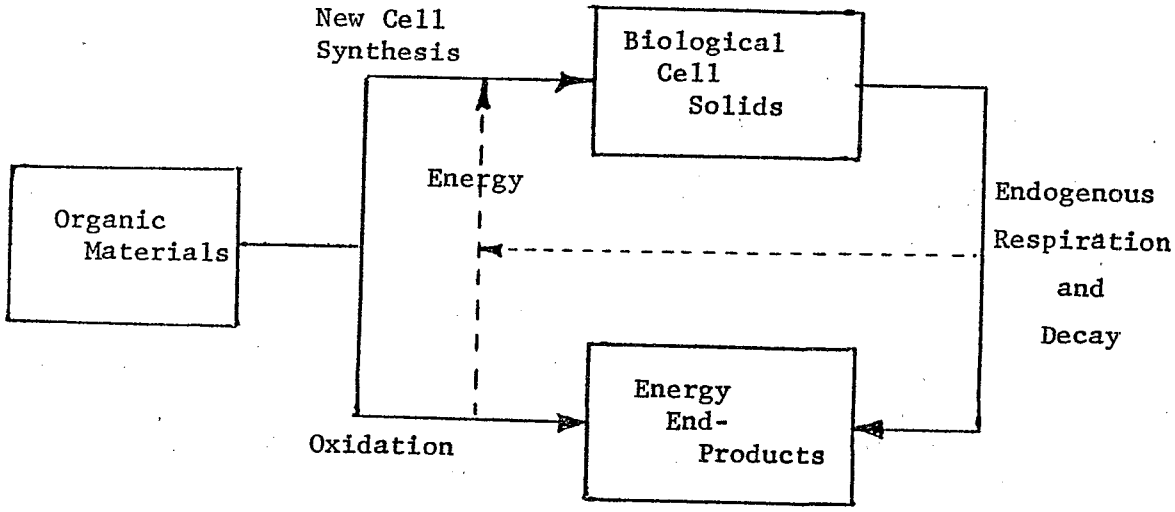
The purpose of this paper is to review the BOD test procedure, to discuss factors affecting its accuracy and precision, and to present recent developments in analytical procedures, specifically nitrification control, that affect the interpretation and use of BOD data.

THE BOD REACTION

The basics of the BOD test are simple: organic materials are decomposed in the presence of oxygen to form carbon dioxide and water (energy and products) and to synthesize new cells (Figure 1). These biological cell solids in turn require oxygen for respiration and can decay and release free organic matter that is recycled through the oxidation/systhesis process. This biological process is termed heterotrophic growth and the resultant oxygen uptake represents Carbonaeous Biochemical Oxygen Demand (CBOD).

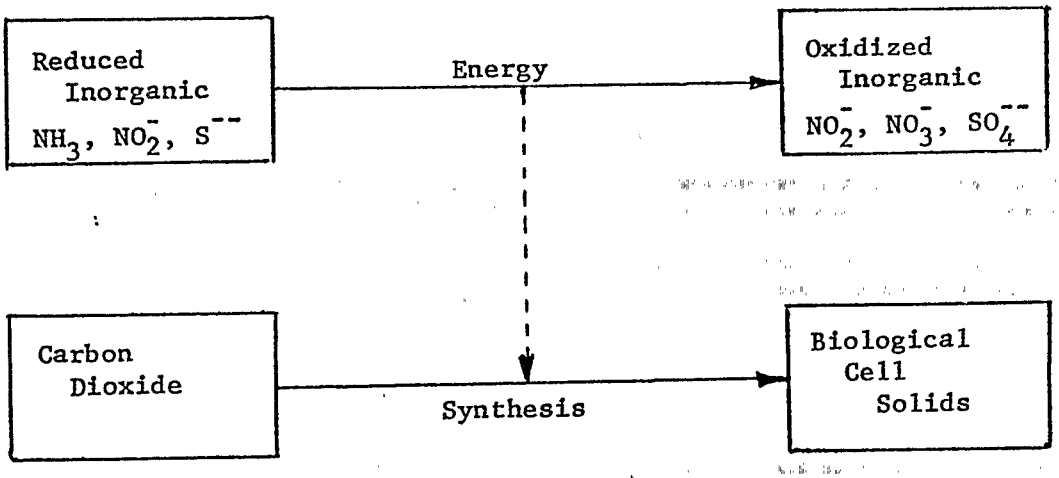
Reduced inorganic--specifically, ammonia (NH_3), nitrite (NO_2), and sulfide ($S^{=}$)--also are oxidized biologically to create energy for synthesis of carbon dioxide to biological cell solids (Figure 2). This latter, or autotrophic, reaction can occur simultaneously with the carbonaceous reaction, separate from the carbonaceous reaction, or may not occur at all in a given sample. The BOD test provides a measure of the amount of oxygen consumed in these biochemical reactions and can, if measured properly, provide a reasonably accurate indication of the amount of biodegradable organic matter and reduced inorganics present in the water sample at the beginning of a test.

The oxygen uptake reaction is time dependent as illustrated in Figure 3 and the accuracy of BOD measurement is a function of the time at which the measurement (reading) is taken and the rate and extent of completion of the two major biological reactions. The challenge of BOD measurements is to control or manage the factors contributing to the rate and amount of oxygen consumed up to the time the oxygen uptake is measured, and to make sure that tests conducted at different times have a common basis of comparison.



$$\text{Organic Material} + O_2 \longrightarrow CO_2 + H_2O + \text{Cell Solids}$$

Figure 1. Schematic diagram and equation expressing components of heterotrophic growth.



a) $NH_3 + 3/2\ O_2 \longrightarrow HNO_2 + H_2O$

b) $HNO_2 + 1/2\ O_2 \longrightarrow HNO_3$

Net: $NH_3 + 2\ O_2 \longrightarrow HNO_3 + H_2O$

Figure 2. Schematic diagram and equations experssing components of autotrophic growth, specifically for the nitrification reaction.

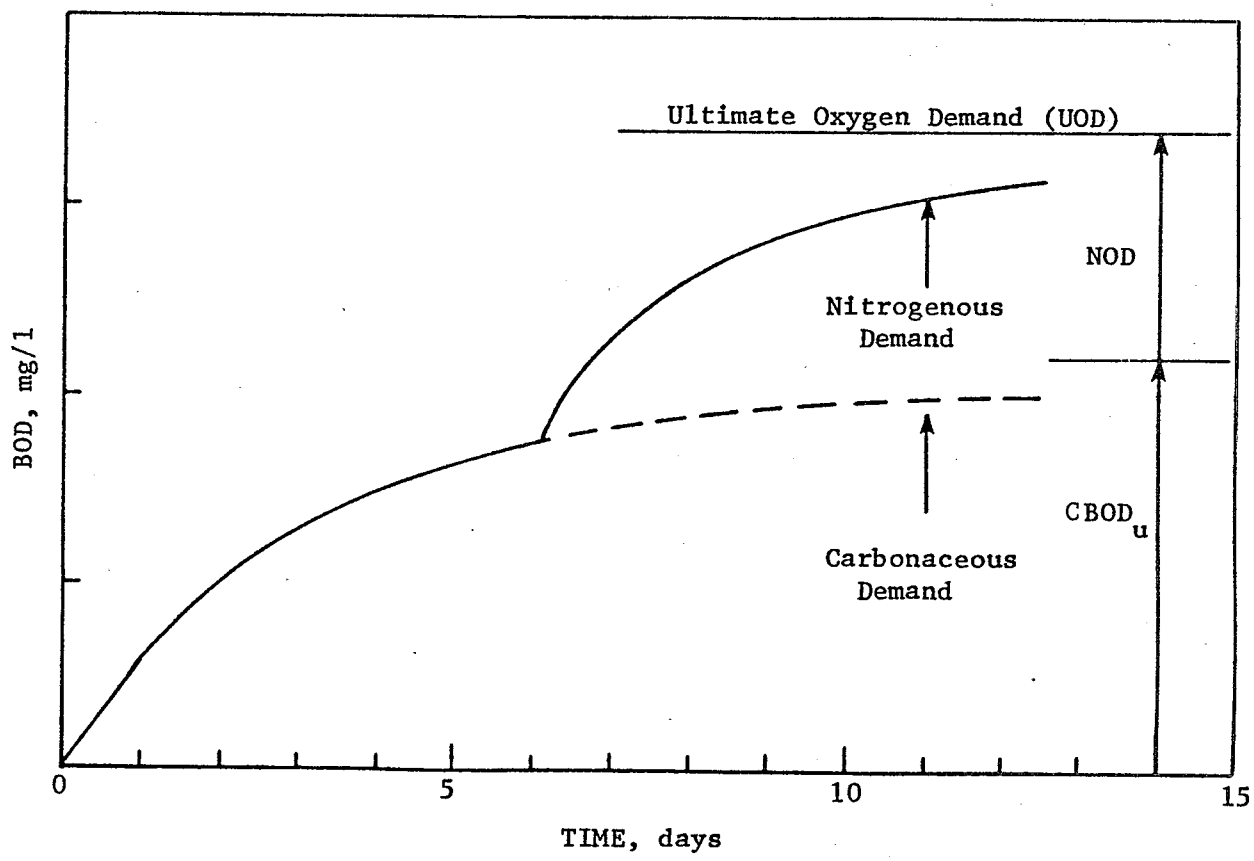


Figure 3. Schematic representation of a typical BOD curve showing carbonaceous and nitrogenous oxygen demand reactions

THE STANDARD BOD TEST

The standard (traditional) procedure for measuring BOD is the dilution method (Standard Methods, 1985). In this method, a sample of wastewater is diluted with a standard mixture of distilled water and known amounts of nutrients and buffering agents. The purpose of dilution is to reduce the total concentration of oxygen demanding material in the diluted sample to below about 10 mg/l so that the amount of dissolved oxygen exceeds the 5-day oxygen uptake capacity of the diluted sample. The diluted sample is then placed in a "standard", usually 300 mL bottle, sealed or capped, and incubated at 20°C in a dark room for five-days. The reduction of dissolved oxygen concentration in the five day-test period is then multiplied by the dilution ratio to give a measure of 5-day biochemical oxygen demand (BOD).

The plague of the BOD test is that the largest sources of variability are not analytical. Almost any experienced analyst can prepare an acceptable dilution water, can make accurate dilutions, can measure dissolved oxygen relatively precisely and accurately, and can multiply by a dilution ratio. The problem is that the major sources of variation are biological and largely uncontrolable by the analyst. And while these variations are natural, that is they represent inherent characteristics of the sample, it is difficult to compare the results of one test to the results of another. The need, then, is to understand the factors contributing to variability so that the resulting test data are more easily understood and provide a more consistent measure of a known biological reaction (see Young, 1984).

The goal of committees assigned to improve the standard BOD test has been to establish procedures that minimize variability and maximize the

uniformity between test procedures. Over the last 40 years (about 1940 to 1980) the Standard Methods BOD Task Group has made relatively minor refinements in the method of preparing dilution water, in the buffer formulation, dilution procedure, the seeding procedure and the precise time of starting and ending a test (Young, McDermott and Jenkins, 1982). However, the largest source of test variability -- the nitrogenous oxygen demand (NOD) reaction -- has been largely ignored. The effect of the nitrification reaction was acknowledged in the 1930's in studies of the impact of waste loads on receiving streams, and its impact on BOD reactions was well documented in the late 1940's (Hurwitz, et al, 1947). Methods such as acidification or pasteurization were even proposed for the inhibition of the nitrogenous oxygen demand reaction in BOD tests (Sawyer and Bradney, 1946).

The impact of nitrification on the variability of test data is illustrated in Figure 4. Shown here is a correlation of 5-day BOD as measured by electrolytic respirometer (EBOD) and 5-day dilution BOD. In Plant A, nitrification was inhibited in both test procedures; at Plant M, nitrification control was not used in either test. A major part of the variability was attributed to the fact that the nitrification reaction was at different stages of completion in each test at the end of the 5-day test period.

Failure to separate carbonaceous and nitrogenous BOD can lead to gross errors in calculating the impact of wastewater discharges to receiving streams. For example, consider Figure 5. Shown here is a BOD reaction exhibiting both carbonaceous and nitrogenous oxygen demands. With nitrification inhibited, the carbonaceous BOD reaction would exhibit a

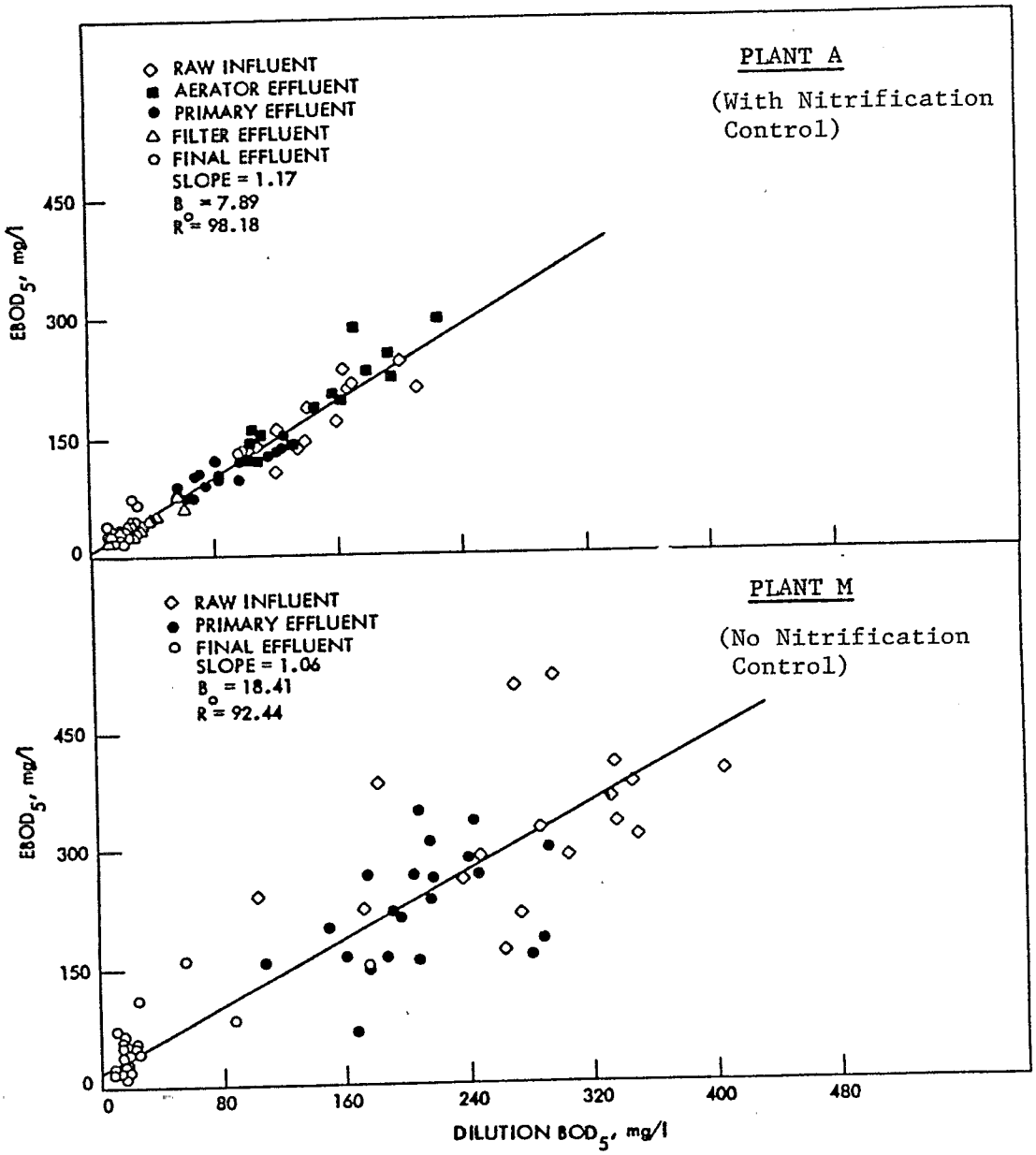


Figure 4. Comparison of electrolytic and dilution BOD measurements showing the effect of nitrification on the variability of test data.

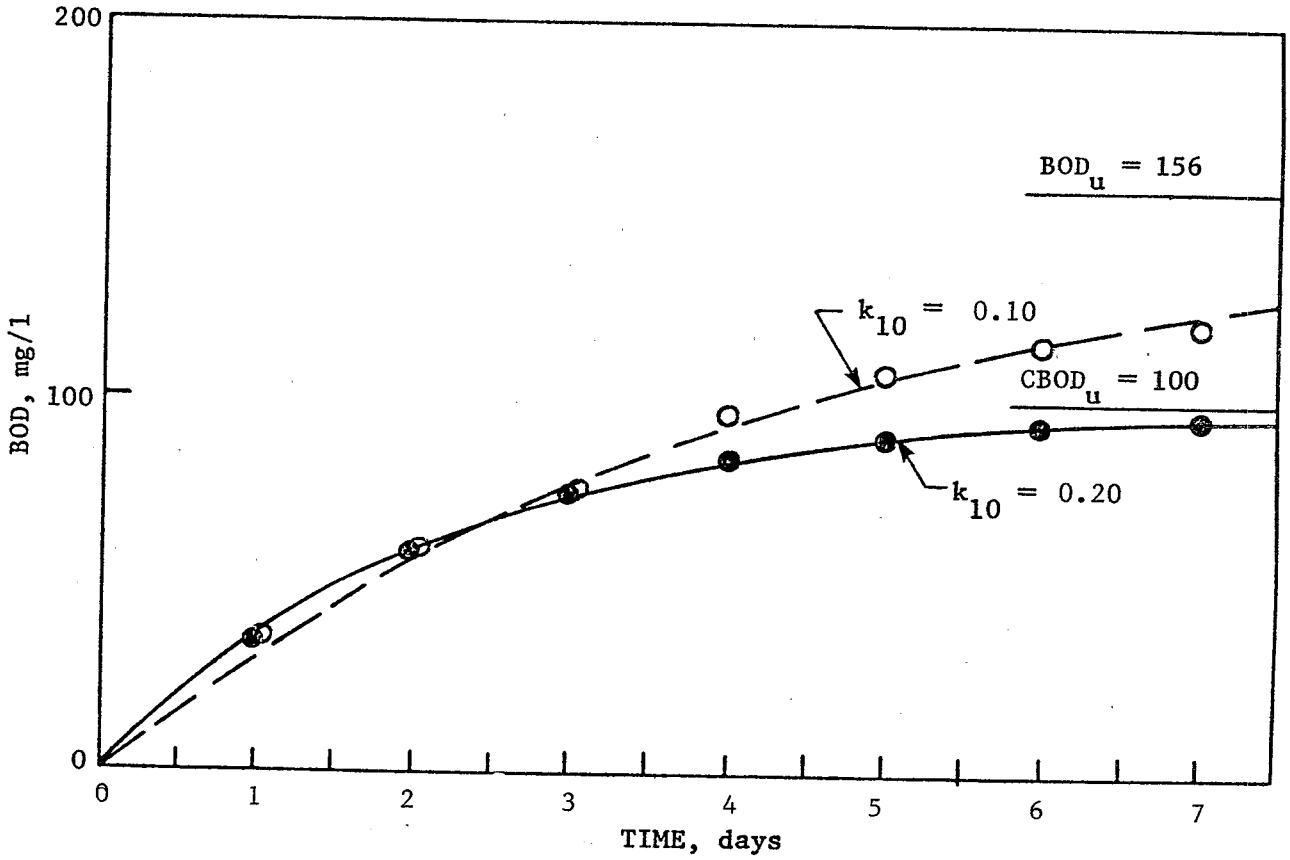


Figure 5. Comparison of measured and calculated BOD curves and reaction coefficients for a wastewater sample with and without nitrification inhibition.

curve represented by the solid line which would have a relatively high first-order rate and a reasonably low ultimate carbonaceous BOD ($CBOD_u$). With nitrification, the best-fit, first-order equation (dashed line) would show an apparent decrease in rate coefficient and an increase in ultimate oxygen demand ($CBOD_u$ + NOD). Obviously the use of these two different sets of reaction coefficients to represent the BOD reaction for the same wastewater would have a dramatic effect on the results of an oxygen depletion model using uptake rate and measured 5-day BOD as input parameters. The impact of nitrification in BOD tests on treatment plant compliance is well-documented by Hall and Foxen (1983).

The reasons for not including nitrification control in BOD tests in the past seem to have been both technical and non-technical. The methods available were cumbersome and did not improve the accuracy of the test since the carbonaceous BOD was adversely affected by pasteurization and acidification, and reseeding was required. This changed both the chemical and biochemical nature of the test sample. But perhaps the largest reason for not separating carbonaceous and nitrogenous BOD in tests is related to the purposes for which BOD was used, especially in treated effluents. Prior to 1972, when major amendments to the Clean Water Act (PL-92-500) were passed, levels of treatment were based largely on process technology. That is, communities were required to have primary or secondary treatment based on size and location of the city, and secondary treatment was defined as a "biological process". Hence, trickling filters, activated sludge, rotating biological contactors, lagoons, etc.--were essentially equivalent and the choice of one over the other was largely based on economics and operability. PL-92-500 led to the definition of secondary treatment in terms of effluent quality, that is, 30 mg/l BOD and suspended solids on a monthly average basis, 45 mg/l for a peak 7-day period. Too few people

realized at the time that so called "secondary processes" could not consistently provide "secondary effluent" quality unless the measure of effluent quality was carbonaceous BOD.

However, the standard method for measuring BOD--as developed by Standard Methods and accepted by the U.S. Environmental Protection Agency--did not allow for separation of the carbonaceous and nitrogenous components of the BOD reaction. Thus began a 10-year debate of the merits of and need for basing treatment performance on carbonaceous BOD alone or carbonaceous plus nitrogenous BOD. The arguments were based largely on the premise that nitrogenous oxygen uptake is in fact biochemical oxygen demand and the fact that the multi-decade base of BOD data did not include only carbonaceous BOD measurements; and decisions for granting discharge permits were based almost entirely on calculations using past BOD records.

After considerable debate, numerous lawsuits, and significant expenditures of funds for construction of treatment plants to provide "secondary" or better quality effluent, EPA has accepted the use of carbonaceous BOD as a measure of permit compliance, although in restricted situations and subject to state approval. Consequently, we now are where we should have been 10 to 12 years ago in BOD measurement and application technology.

The challenge now is to procede as rapidly as possibly to using carbonaceous and nitrogenous BOD as independent performance references for biological treatment plants, thus requiring a thorough understanding of how to conduct BOD tests involving separate measurements of carbonaceous and nitrogenous BOD.

NITRIFICATION CONTROL METHODS

The accepted method for measuring carbonaceous and nitrogenous BOD is to inhibit the nitrogenous oxygen uptake reaction in one set of samples thereby leaving only the measurement of carbonaceous demand. The nitrogenous oxygen demand is measured by setting up a parallel set of samples so that nitrogenous oxygen uptake is the difference between inhibited and uninhibited samples. An alternate method is to measure the amount of reduced nitrogen in a sample—either as ammonia-nitrogen or Total Kjeldhal Nitrogen (TKN)—and calculating the anticipated NOD using the equations stated in Figure 2. This latter method is considered to be as accurate as direct measurement in BOD bottles and much simpler and faster.

The presently accepted method of inhibiting the nitrogenous reaction in BOD tests is to use chemical inhibitors of the nitrification reaction. While pasteurization or acidification have been used sporadically in the past, these methods are cumbersome and are not as reliable as is chemical inhibition. Two chemicals can be used: allythiourea (ATU) or trichloromethyl pyridine (TCMP). Allylthiourea seems to be the favored chemical in Europe, and while effective it does have some disadvantages. First, it is biodegradable and its oxygen demand can be a significant part of the total BOD of samples having large dilution ratios. Secondly, since it is biodegradable, it loses its effectiveness after a few days and must be replenished periodically in long term tests.

The favored chemical in U.S. methods is 2-chloro-6-(trichloromethyl) pyridine. This chemical is highly specific in inhibiting the ammonia to nitrite nitrogen conversion and does not adversely affect carbonaceous demand reactions (Young, 1973, 1983). It is quite stable in water and it is effective for up to 30 days in BOD tests. One disadvantage of TCMP is that it is not soluble in water and must be added to samples as a powder.

This is not a major problem, however, because commercial dispensors are available to provide accurate doses to individual BOD bottles.

The effectiveness of TCMP as a nitrification inhibitor has been demonstrated in both dilution and respirometer tests (Young, 1973, 1983). Typical results are shown in Figure 6, and nitrogen and oxygen balances are shown in Table 1 for a number of test cases. In all cases, TCMP has been shown effective for nitrification control and there is no evidence that it interferes with the carbonaceous BOD reaction.

ACCURACY AND PRECISION OF BOD AND NOD TESTS

One problem facing BOD measurements is that it is not possible to know the stoichiometry or completeness of the reaction at any time so that there is no standard for establishing accuracy. Standard Methods (APHA, 1980) gives a procedure for checking the dilution procedure and for identifying problems with seeding or measurement technique. This involves adding 5 ml of a solution containing 50 mg/l each of glucose and glutamic acid to seeded dilution water and measuring the 5-day BOD. The theoretical oxygen demand of this mixture is 308 mg/l. If the measured BOD_5 falls within a range of 220 + 20 mg/l after seed correction, the analyst can feel with some confidence that his procedure is correct and that there is no toxic substance in the dilution water. This, however, does not establish a method for determining the accuracy of the test.

Test precision reflects the ability to repeat a measurement in a set of replicates or among tests conducted under a given set of conditions. It is important when establishing precision that a common or known basis of reference is used. For eample, it should be made clear whether the analysis of precision includes sampling, seeding, and transfer errors or simply reflects the variability between replicates after samples have been

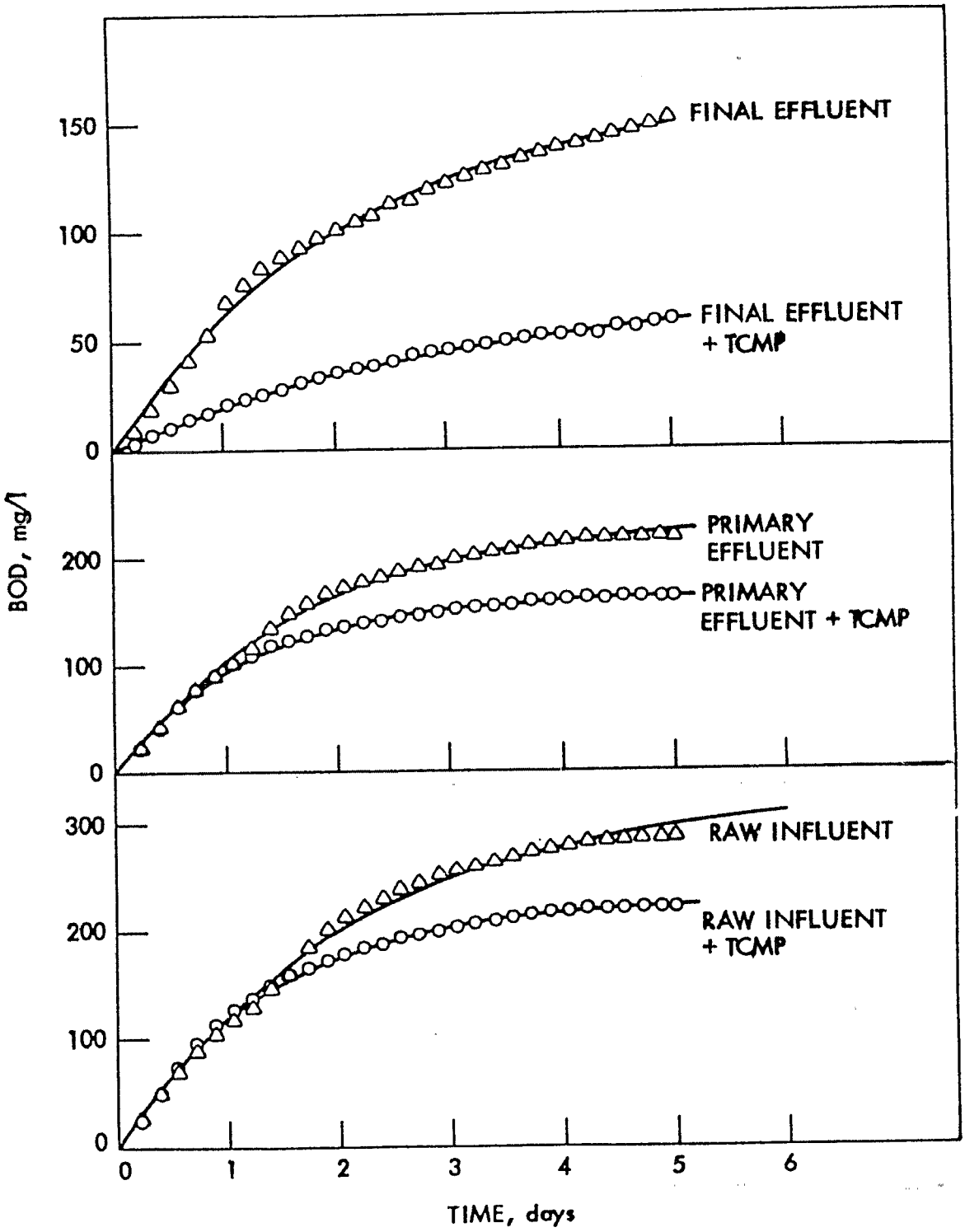


Figure 6. Examples of BOD measurements with and without nitrification inhibition (From Young, 1972)

Table 1. Measured total and carbonaceous BOD and measured and calculated nitrogenous oxygen demand (NOD).
(From Young, 1973).

Sample	BOD, mg/l		NOD, mg/l	
	Total	Carbon-aceous	Measured[a]	Calculated[b]
Primary effluent	328	224	104	96
Trickling filter effluent	80	25	55	56
Activated sludge effluent	75	16	59	56
Trickling filter effluent	297	95	202	224
Trickling filter effluent	100	38	62	58
Trickling filter effluent	135	84	51	51

[a]Measured NOD = Total BOD-Carbonaceous BOD
[b]Calculated NOD = $(NH_3 \rightarrow NO_3^-) \times 4.33 + (NO_2^- \rightarrow NO_3^-) \times 1.11$

transfered, seeded and diluted. Measurement precision for a given analyst can be consistently below 7 percent if sampling, dilution and transfer errors are eliminated and nitrification is controlled. This verification would include natural biological variability between samples plus errors in measuring dissolved oxygen concentration. Another contributing factor is that the error in DO measurement is multiplied by the dilution ratio so that considerable error may be introduced when measuring the BOD of a high-strength sample that requires a large dilution.

A common technique for monitoring BOD test methodology is to conduct interlaboratory analyses of mixtures containing known amounts of organic materials (typically glucose-glutamic acid). Such samples are sent to a number of different analyst who are asked to measure the dilution BOD using their normal analytical procedure at 2, 3, 5, 6-day or similar intervals by using a stated method for seeding. Typical results of this type of analysis are shown in Tables 2 and 3. While interlaboratory testing does not give a good indicator of precision of BOD measurements in a given laboratory or treatment plant environment, it does give an indication of the variability of analyses from plant to plant.

THE FUTURE OF THE BOD TEST

The BOD test has been in use in essentially its present form for about 60 years. Throughout this period, there has been considerable criticism about the soundness of the test procedure and its use as a pollution control measure. So why does the test continue to be used? Basically, the reason is that no acceptable substitute test has been developed that responds to and provides a measure of the amount of biodegradable matter present in a wastewater.

Table 2. Precision of BOD_5 measurements.

Parameter	Reference Source			
	Method Research Study 3, EPA, 1971 (Dilution Test)		Ballinger and Lishka, 1962 (Dilution test)	Young and Baumann, 1976 (Respirometer)
	Low-level	High level		
Theoretical value, mg/L	2.2 (BOD_5)	194 (BOD_5)	308 (THOD)	350 (THOD)
Mean of measured BOD_5 values, mg/L	2.12	175	214	296
Recovery, %	96	90	69	85
Coefficient of Variability, %	33.2	15.0	19.5	5.8
No. of analyses/ No. of laboratories	74/56	73/56	34/34	4/4

Table 3. Interlaboratory analysis of 5-day BOD as conducted by the U.S. Environmental Protection Agency (From Britton, 1985).

Study	Added Glucose/ Glutamic Acid (50/50) in mg/L	EPA & State Laboratories Reporting	Statistics Mean (X, mg/L)	Std. Dev. (S, mg/L)	Coef.[a] of Var.
WP003	37.0	98	25.47	4.299	16.9
	340	102	229.9	37.06	16.1
WP004	6.0	85	4.270	.8710	20.4
	325	85	218.7	33.36	15.3
WP005	4.0	102	2.734	.6672	24.4
	176	102	118.8	17.05	14.4
WP006	101.3	90	68.97	9.785	14.2
	24.2	95	16.81	2.131	12.7
WP007	6.0	94	4.09	1.0662	26.1
	144.0	95	100.0	12.603	12.6
WP008	39.1	68	26.7	3.377	12.6
	89.6	70	61.5	6.609	10.7
WP009	4.0	65	2.91	0.9528	32.7
	231.0	68	146.0	27.257	18.3
WP010	114.0	69	75.4	10.408	13.8
	161.0	69	103.6	17.850	17.2
WP011	5.0	74	3.56	0.5500	15.4
	192.0	75	125.2	14.714	11.8

[a] Coef. of Var. = Std. Dev./X, %

From these data (WP007-WP011) the following linear relationships may be calculated:

$X = 0.665$ (added level) $+ 0.225$; with an $R^2 = 0.99^+$

$S = 0.0998$ (added level) $+ 0.430$; with an $R^2 = 0.99^+$

Consequently, until a better and more acceptable biochemical-based test is developed, we no doubt will have to continue to use the BOD test as one measure of water quality. Our objective, then, should be to learn as much as possible about the factors affecting the BOD test and to learn to control those factors that interfere with proper analysis, interpretation and application. Presently, the factors contributing to nitrification in BOD tests are known and methods for nitrification control are available. We should use this technology!

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PEGGY KNIGHT, PH.D.

WEYERHAEUSER ANALYTICAL TESTING SERVICES

ANALYSIS OF PRIORITY POLLUTANTS BELOW FIVE NANOGRAMS (ON COLUMN) IN MARINE SEDIMENTS BY ISOTOPE DILUTION GC/MS

DR. KNIGHT: I've been spending a lot of time the last few weeks trying to figure out why I should give this talk. When Dale Rushneck called last year to ask if I could give a talk about this project, I was, of course, flattered. I told Dale that there were other labs, much larger labs, out there which had used the method and who had participated in the development of the technique, which we had not. That they also had better precision and accuracy, and I was certain they had better QA/QC practices. Dale asked anyway and I guess I'm here.

I think probably the advantage to my giving this talk is to show that a small lab used to handling a variety of analyses can effectively use the isotope dilution technique for the analysis of priority pollutants in complex matrices.

Our lab is relatively small. Our chromatography group consists of four full time people and one

part time consultant. We have one Finnegan GC/MS and a battery of GCs. Like many industrial labs, we analyze a plethora of compounds in about as many matrices as we can come up with. Seldom do we see very many samples which develop into what might be called routine analysis.

The hundred or so samples in this particular project consisted of marine sediment samples to be analyzed ultimately for the Washington DOE through a contractor for the Port of Tacoma. Our lab was subcontracted for these analyses. The sediments were from the Blair and Milwaukee waterways which impinge on Commencement Bay in the Puget Sound. Commencement Bay, as many of you know, is a Superfund site. Part of the requirements for the analysis were the detection limits should be as low as practical and at least five micrograms compound per extract.

SLIDE 1

The method was based on the EPA isotope dilution method 1925 for priority pollutants. It was modified to use soxhlet extraction of the wet sediment and add few other procedures to clean up the sample extract before analysis. This modication was developed jointly by Tetratech and California

Analytical Laboratories for the Superfund Project analysis of Commencement Bay sediment.

One hundred grams of wet sediment were weighed directly into a soxhlet extraction thimble. The soxhlet had been previously cleaned by detergent washing, extensively rinsing it with water, rinsing it with solvent and then running the thimble and the glassware overnight with the extraction solvent. Five micograms of each label were spiked into the sediment, and the sediment stirred and the extraction started. The sediment was stirred after the first hour and twice more within the next eight hours in attempt to minimize channeling.

The extract was transferred to a separatory funnel and half-saturated aqueous sodium sulfate added. The aqueous phase was acidified and then extracted. The aqueous phase was then made basic and and re-extracted with methylene chloride. We had a great problem here with emulsion formation and it would have been a heck of a lot easier if we had been able to simply ignore the bases, but they wouldn't let us get away with it.

Acid/neutral and base extracts were combined and Kuderna-Danish evaporated with a steam bath. Recoveries of all compounds were good to this stage.

We had taken blank spikes through the procedures to find out where the recovery losses would be.

The sample was shaken with mercury to remove sulfur. We seemed to lose benzidene at this stage at the five microgram level. To get rid of the mercury sulfide fines, which were extensive, the extract was repeatedly centrifuged and decanted. Fines which remained collected at the top of the GPC column.

Gel used for the GPC was Biobeads SX-3 from Biorad. The columns were nitrogen pressurized and manually operated. The gel was optimized for separation of corn oil lipid from phthalate and recovery of pentachlorophenol, and the phthalate. The columns were reused, but if an exceedingly sloppy sample was analyzed, the column was discarded or the column was washed.

Part of the extract was split out for pesticides, but since it's not an isotope dilution technique, I will not be discussing them. I understand now that several of the labelled pesticides are available.

The solvent was exchanged and the extract run through with solvent washings, a disposable C-18 solid phase extraction column.

As some of the higher labeled PAHs fluoresce very well, we watched the fluorescence throughout the entire procedure. This gave an excellent visual check of the progress. By this aid, it seemed that we lost a bit of higher PAHs on the SPE column, however, they were not removed even with methylene chloride. The extract was concentrated again, the injection internal standard, dichlorobiphenyl added, and sample injected on the GC/MS.

The instrument used was a Finnegan 4000 GC/MS with a Nova 3 data system. We used standard columns, DB-5. It could be useful to say that a standard of 4ug/mL of Phenanthrene gave an area of about 50,000. At these instrument settings we found that we sometimes had a lot of problems with disc space. Sometimes the more concentrated samples would run out off of the end of the disc, which would be disconcerting.

Numbers were developed from a program developed by Dale Rushneck and Joel Karnofski, made available modified, from Finnegan which reverse-searches for labels. After finding these, it searches for the corresponding non-labeled compounds using a window around a linear least squares fit of current times compared to reference retention times.

The lower limits were calculated on a compound-by-compound basis for each individual sample using instrument detection limits and adjusting this by the sample weight, recovery of the corresponding label, and the variability of the area of the injection internal standard, DFB.

The calibration curve consisted of five points ranging nominally from 0.5ug/mL to 25 ug/mL. For the phenols, the range was usually slightly higher. I've picked out a few to show. They are fairly typical. Some of them were as straight as strings.

SLIDES 2&3

The precision and accuracy data are based on duplicate, spike pairs; that is, a sample was taken in triplicate, analyzed in duplicate, and and the remaining replicate spiked with five micrograms of the non-labeled materials. It was carried through the entire procedure. This was done on approximately 10 percent of the samples.

SLIDE 4

Again, I've picked out a few to show as a complete table would be absolutely indigestible. These precision and accuracy figures are in percent recovery of the spike. These are fairly typical. In general, the PAHs are a little better and the

phenols, of course, are worse. Butylbenzylphthalate has no label standard to recover.

Somehow, when I proofed the slides, I managed to miss the big blank area there. It doesn't mean that they weren't recovered except for 4-nitrophenol which usually is not. I count it among those compounds which don't really work.

In comparison with Table 8 in Method 1625, most of these figures correspond fairly well. The accuracy figures are usually well within the ranges. The standard deviations sometimes are a bit higher and sometimes within the ranges. The recovery of the labels over on this side usually were within the range. The label recovery figures represent a summary of the data over all samples as well as the duplicates and spikes.

To take a few of these examples, 2-chlorophenol from 1625, the range for accuracy is 79-135 and the standard deviation is +/-13 in 1625. Cabel recovery ranges from 23 to 255 percent. Dichlorobenzene is 63-201, the standard deviation is 43, and a lower limit for the recovery is not specified although it should be present, and up to 550 percent.

To fill in these numbers that I'm missing, our recoveries here for acenaphthene were somewhere

between about 30 and 150 percent. 4-Nitrophenol was not recovered in the sample much of the time. It just happened to be for some reason with our spiked samples. Fluorene ranged from 40 to 180.

Most of the compounds that are analyzed by this method are really very similar to this. If the PAHs seem more highly represented than warrants, it's because those compounds were the compounds with which I was dealing. I should point out that in comparison with the precision and recovery samples in 1625, 1625 figures were developed using what Dale referred to as a "real world reagent water spike," whereas these are actual sediment samples and, of course, have to be corrected for the amount that was in the sample itself.

There were a few compounds for which the method seemed ultimately to fail, failure being determined by non-recovery of the label. As was mentioned earlier, benzidene was lost even on a blank spike at five micrograms at the mercury stage. Other compounds did not seem to survive the procedure, also at this level; 2,4-Dinitrotoluene, 2-Methyl 4,6-Dinitrophenol and 4-Nitrophenol.

Hexachlorocyclopentadiene may or may not survive the procedure. We seemed to have problems

getting that compound through the column. On a fresh column or a regenerated column, there are no difficulties. However, after a few of the sediment extracts have been injected on the column, the response rapidly degrades. A rather similar effect was exhibited with acenaphthene. On a new or regenerated column, the response was twice what it would have been on a column which had been exposed to sediment extracts. Consequently, after the sediment extracts, we reexamined the calibration curve.

SLIDE 6

This is a pictorial representation of the replication of numbers. A two sample plot. The amount at 50 would represent five micrograms of compound. The PAHs are also.

SLIDE 7

This is just blowing up that particular slide. The phenols are, as expected, more scattered.

SLIDE 8

These are the people in the lab that also participated in the study: Ed Barnes, Candy McFaul, Jim Leong and Mike Grove. Two of them have spent a lot of time doing extractions and...well, three spent a lot of time doing extractions and the rest of us were involved in data manipulation and keeping

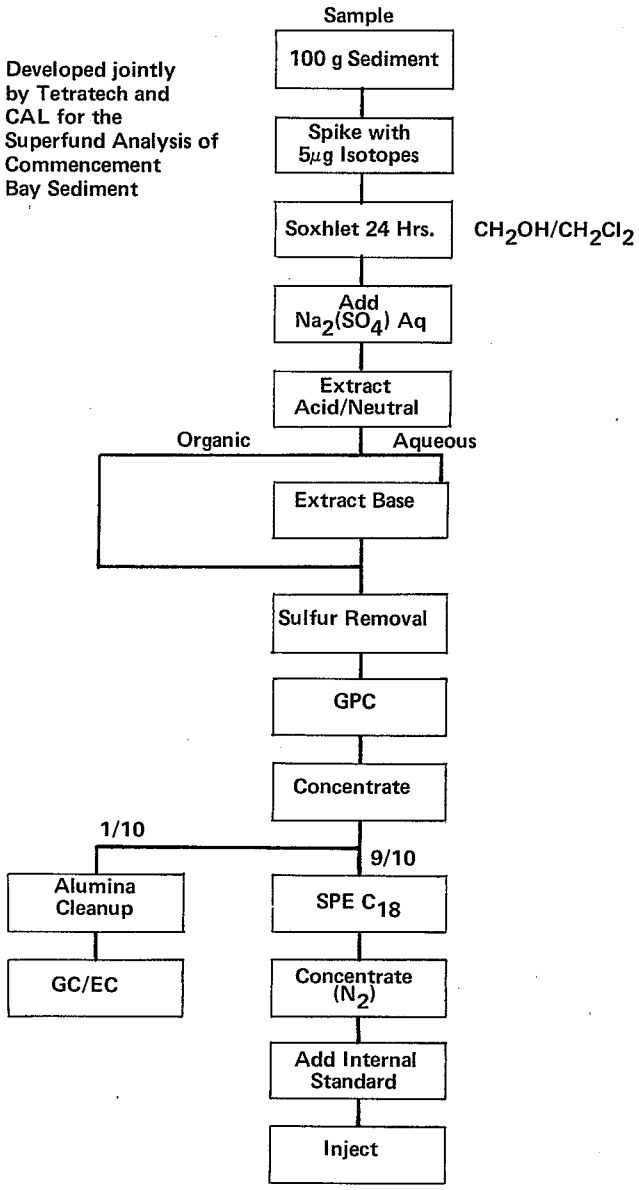
the lab going. You learn a lot about your lab when you have a project like this; how well the group functions as a unit, how well the normal workload is handled with the additional sample load, and where some of the faults in normal practices are.

We had two major faults which came to light during the project, one caused by the other. All the data had to be manually transferred at least twice before the final report. Sometimes this can act as a prompt to double check the data. In our case, we did have transcription errors which developed and it also took so much time to transfer the data that we allowed some false positives through. The compounds which come to mind are benzidene and N-nitrosodiphenylamine. Fortunately, a QA/QC review practice outside caught these errors before the data was entered into the data base.

With a small number of samples, the errors would not have occurred, but with the bulk of work-- the sheer number of numbers-- they did occur. As a result, one of the highest priorities in our laboratory has become the computerized transfer of data, and I might add that no report is issued until it has been doubly rechecked, no matter how loudly the client screams or the lab manager.

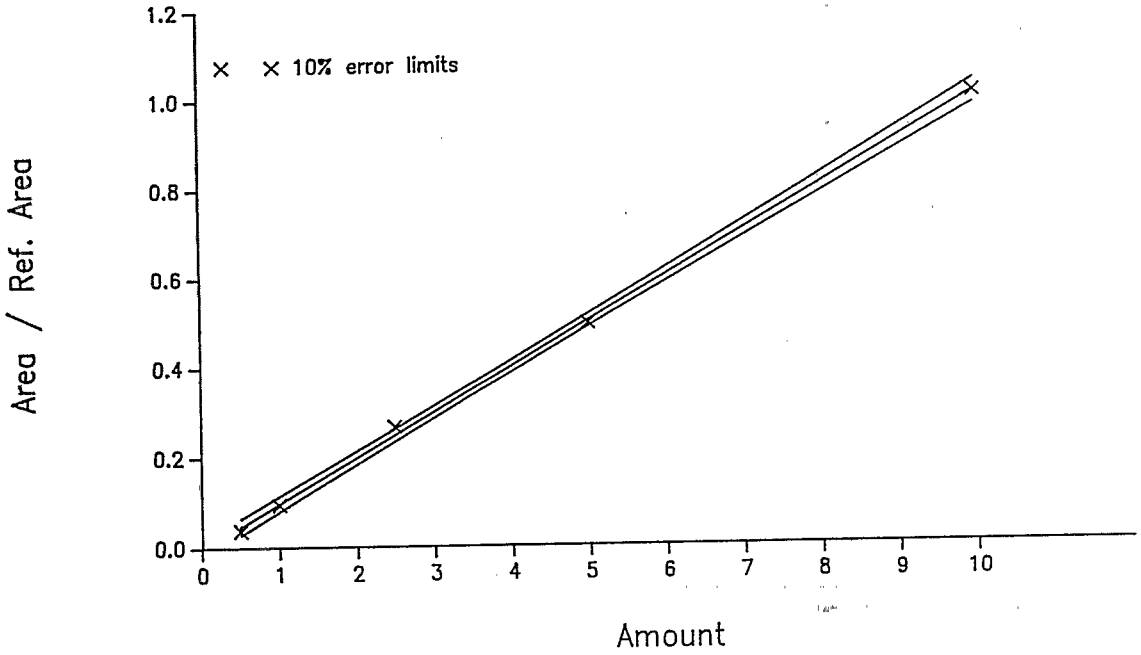
Are there any questions?

Developed jointly by Tetratech and CAL for the Superfund Analysis of Commencement Bay Sediment



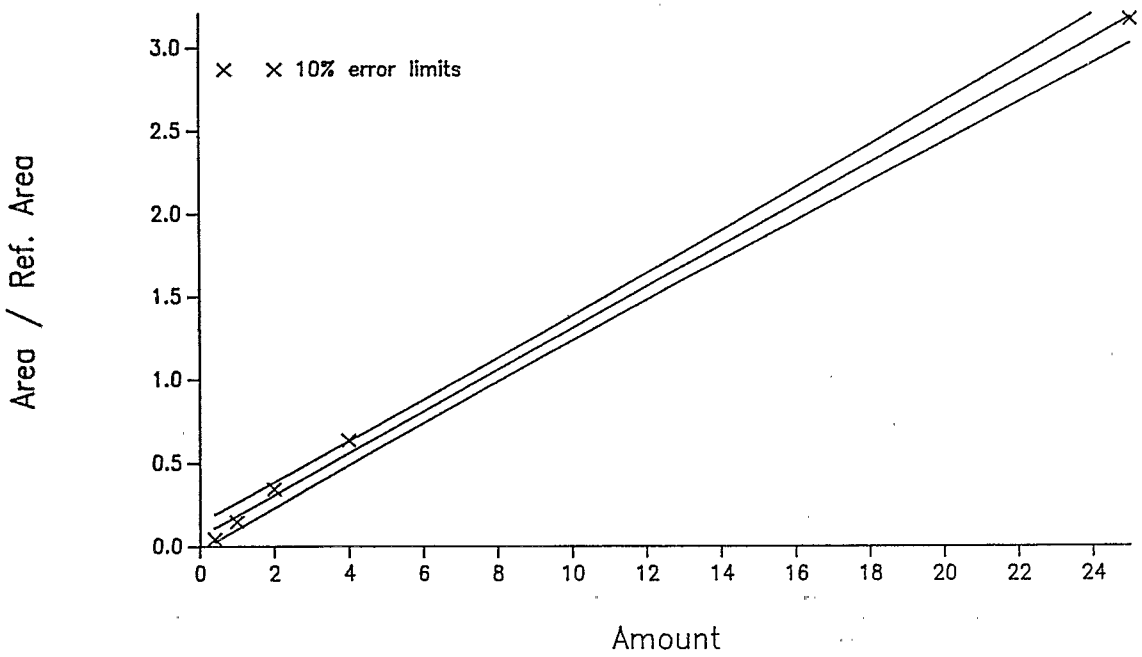
2–chlorophenol

Ref: D4–2–chlorophenol



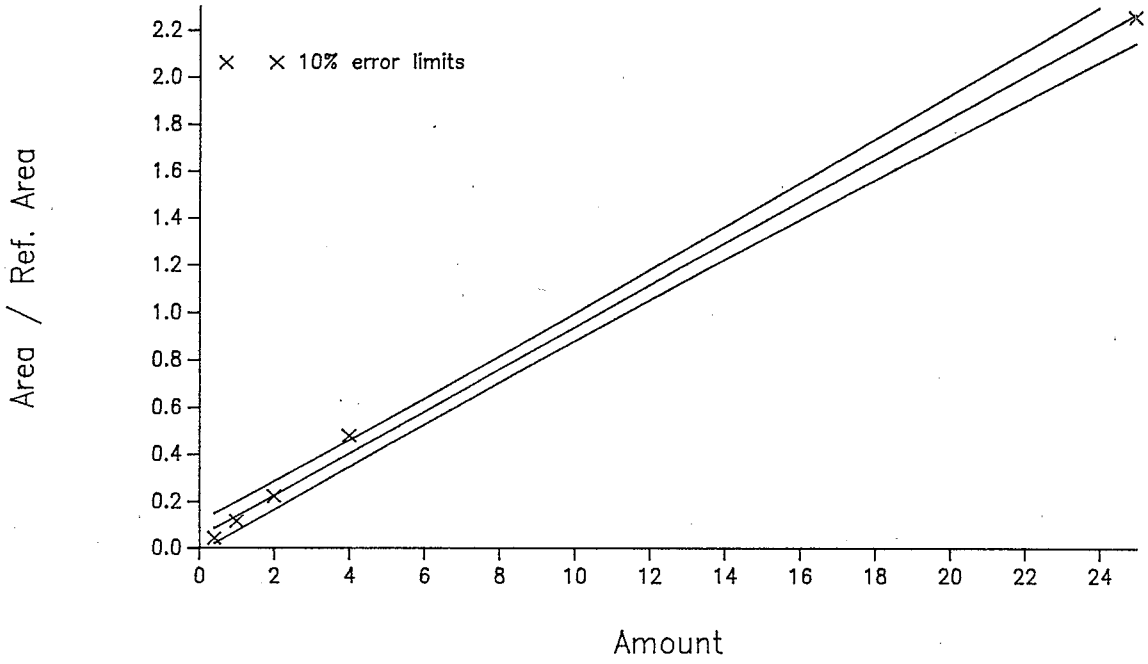
1,3–Dichlorobenzene

Ref: D4–1,3–Dichlorobenzene



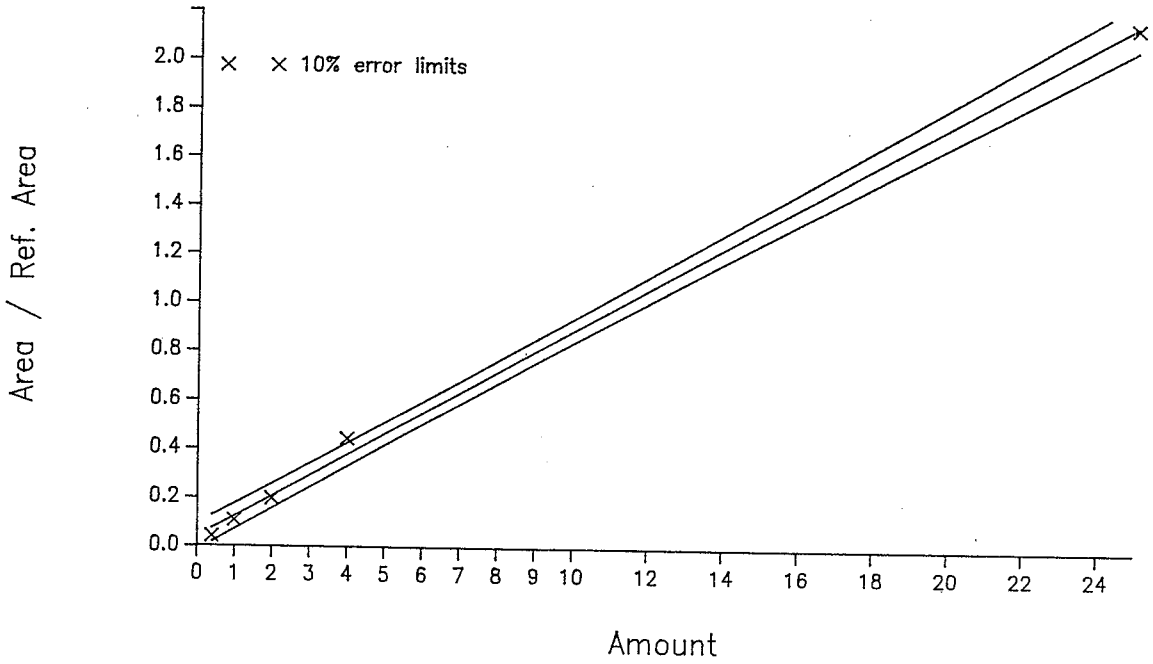
Fluorene

Ref: D10–Fluorene



Benzo(ghi)perylene

Ref: D12–Benzo(ghi)perylene

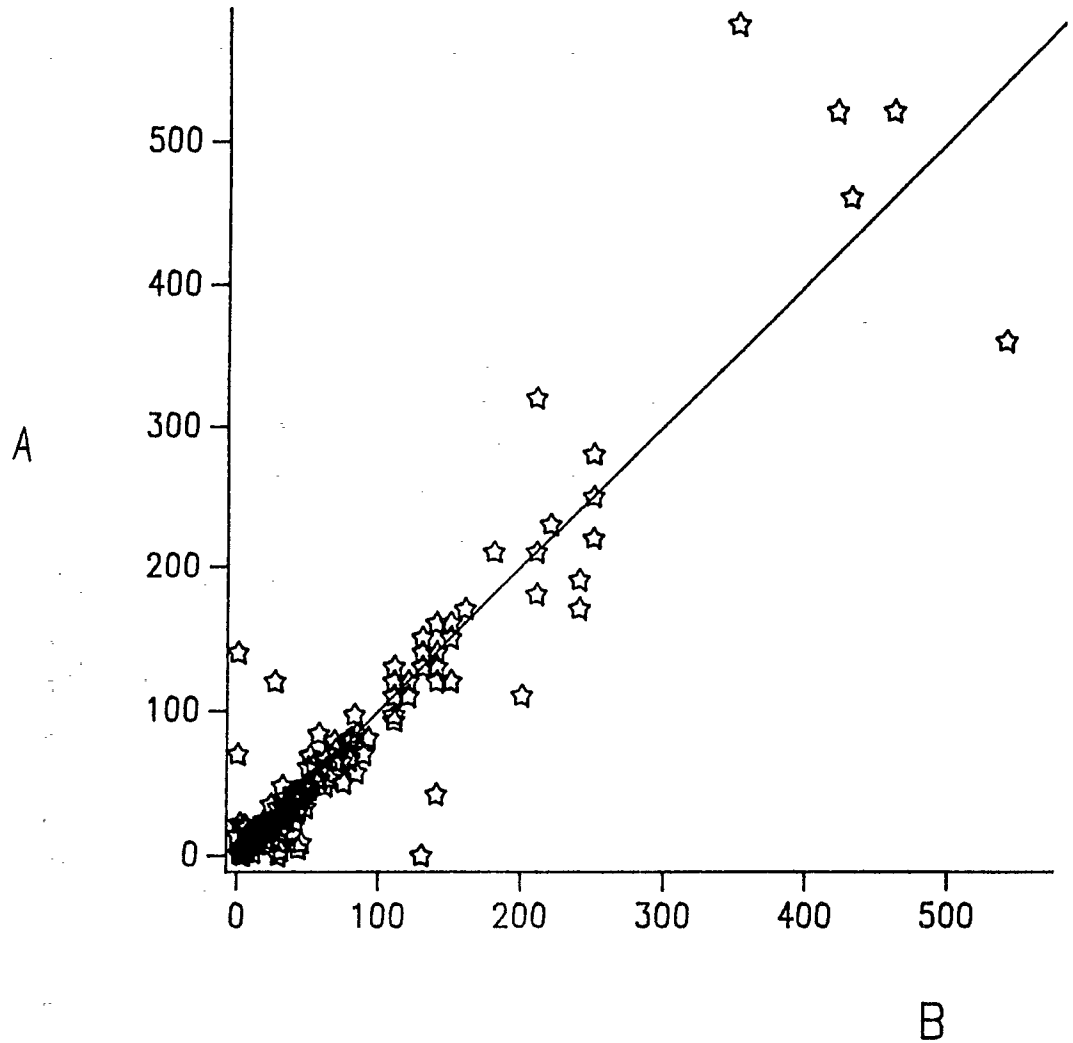


COMPOUND	PRECISION/ACCURACY	LABEL RECOVERY
2-Chlorophenol	94 +/- 9.8	16-75
1,3-Dichlorobenzene	128 +/- 56	2-63
Acenaphthene	104 +/- 20	42-83
4-Nitrophenol	95 +/- 27	0-250
Fluorene	80 +/- 38	47-154
Pentachlorophenol	107 +/- 45	18-180
Phenanthrene	106 +/- 28	13-147
Fluoranthene	105 +/- 26	32-150
Butylbenzylphthalate	80 +/- 15	------
Chrysene	100 +/- 30	40-250
Benzo(a)pyrene	91 +/- 46	12-300
Benzo(ghi)perylene	102 +/- 41	14-400

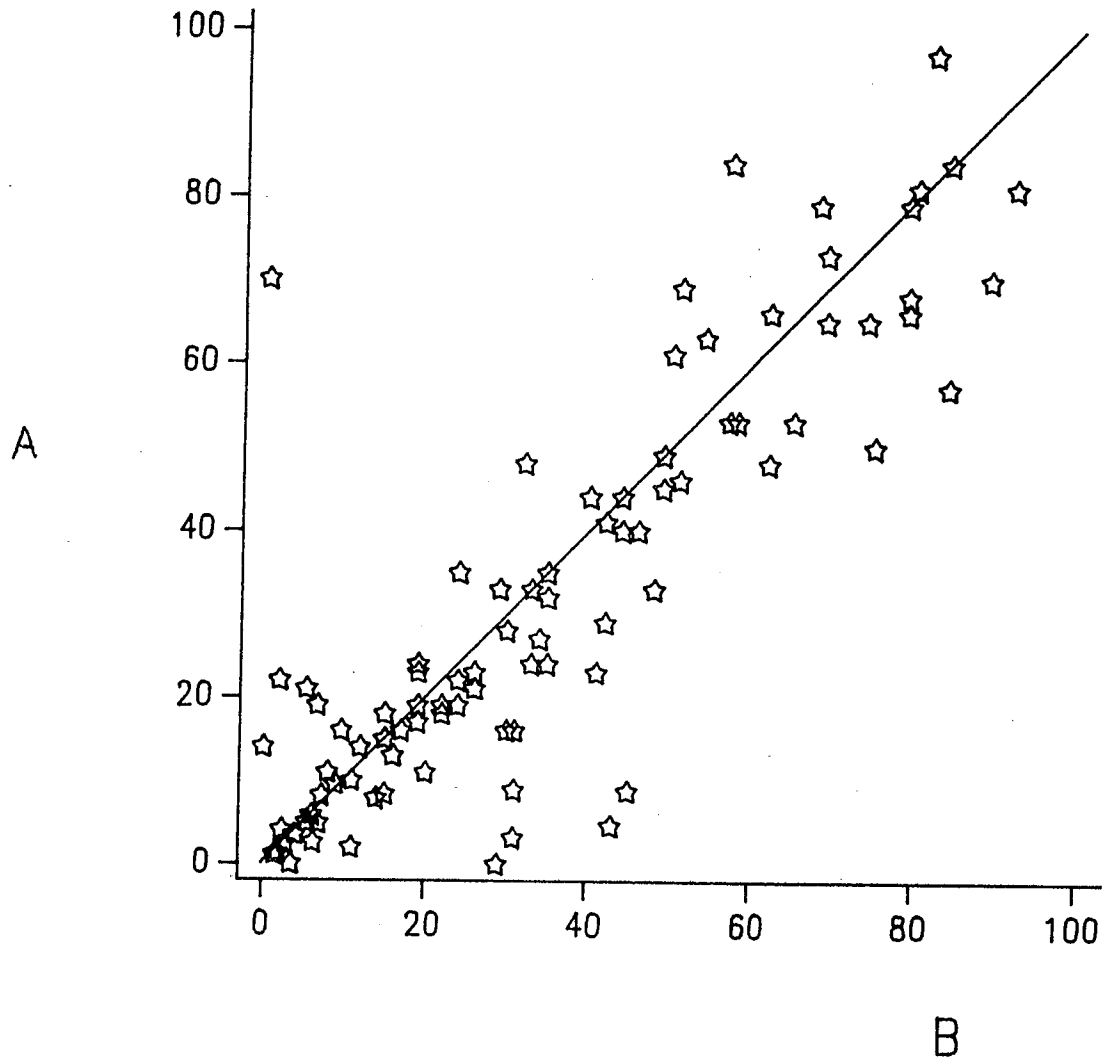
COMPOUNDS GENERALLY NOT RECOVERED

- Benzidene
- *Hexachlorocyclopentadiene
- 2,4 - Dinitrotoluene
- 4 - Nitrophenol
- 2 - Methyl - 4,6 - Dinitrophenol

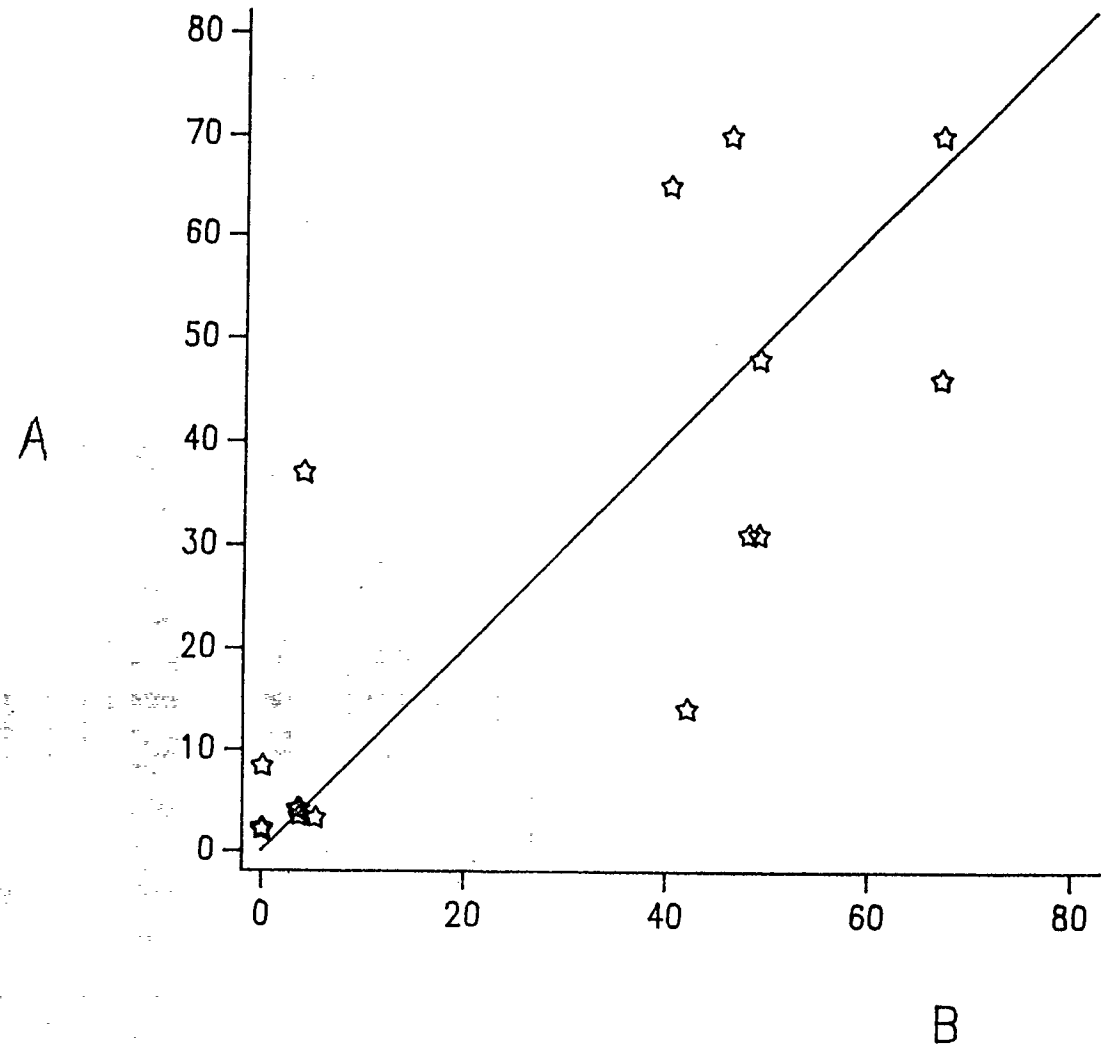
Replicates of PAH



Replicates of PAH



Replicates of Phenols



QUESTION AND ANSWER SESSION

MRS. VOLLMERHAUSEN: Jill Vollmerhausen, Martin Marietta. You said you had a lot of trouble with emulsions. I was wondering what method you used to handle the emulsions?

MRS. KNIGHT: We used centrifugation.

MRS. VOLLMERHAUSEN: Did that usually take care of it?

MRS. KNIGHT: Well, it got us to about 80 percent of recovery of the extract after repeat centrifugation, but it sometimes took a long time.

MRS. VOLLMERHAUSEN: I also notice you use soxhlet and sep funnel extraction.

MRS. KNIGHT: That's right.

MRS. VOLLMERHAUSEN: What was the reason for that?

MRS. KNIGHT: The methods were dictated by a client and previous studies had indicated that this was necessary...

MR. TAYLOR: The answer for that is when you extract 100 grams of wet sediment--and methanols are used in this--you have

a horrendous amount of water which you then have to separate. That's also the reason for the sodium sulfate.

MR. STANKO: George Stanko, Shell Development. In your spiking procedure, you spiked the labelled compounds into the wet sediment?

MRS. KNIGHT: That's right.

MR. STANKO: Did you conduct any experiments on taking the sediment, drying it first and comparing your spiking of labelled compounds onto a dry sediment, then add the water and do your extraction, versus spiking labelled compounds into a wet sediment?

MRS. KNIGHT: No, we didn't undertake that study. The methods, as I said, were totally specified. Whether that study has been done, perhaps Paul would like to comment.

MR. BARRICK: I have an answer for that. The major reason that wet sediments were used is because there have been several studies that show with marine sediments and other kinds of things like this, that if you do a drying procedure on them, unless it's very careful freeze drying, you get development of artifacts, active sites,

things like that. When we were dealing with having to recover acids, bases, neutrals, pesticides, everything all over the place, you simply couldn't afford to do a modification of the matrix through drying, so we did a wet sediment extract and then removal of the water through the separatory technique.

There are so many different phase associations of all these chemicals that you can't expect the isotope dilution spike to mimic the recovery out of the matrix of all those things. There are surface cuttings, things that are engulfed and locked up in matrices and clay lattices and things like this. So the real purpose of this was to get your spikes in there, get them mixed up, get them as equilibrated as possible, and then pull them out and primarily use the isotopes as an analytical recovery spike so you could see what was being lost in the lab and what wasn't.

You were making the tacit assumption that you were starting out with a given amount of extract from your matrix, but they weren't geared in a true sense of isotope dilution to say that it was mimicking the total recovery of every single compound out of every single one of the sum matrices within the samples.

MR. TELLIARD: Bob, would you tell the ladies who you are?

MR. BARRICK: I'm sorry. Bob Barrick, Tetra Tech.

MR. STANKO: I have one final question on that. When you actually calculated the concentrations of the analyte, did you use the isotope dilution calculation or did you use the isotope dilution compounds, or were you spiking only to give you an indication of quality assurance? In other words, how did you use the labelled spiking data? Was it incorporated into calculations?

MRS. KNIGHT: Yes, it was. That was also specified in the method that we were to use.

MR. STANKO: I think I would have some concerns based on the information you just presented.

MRS. LESAGE: Suzanne Lesage, Canada. I would like to know if you had to look for other unknown compounds in those marine sediments, and if so, did the addition of all those labelled compounds make the chromatograms so complicated as you would have a hard time.

MRS. KNIGHT: We did not

have to look for other unknown compounds. It would make the identification of those materials perhaps more complex, but as has been noted before, the masses for the labelled compounds are fairly unique that we're using, and I believe it would have helped quite a bit in making sure that these were the labels. Also, we knew what the labels were supposed to be and what labels were involved and where they occurred.

MRS. LESAGE: Except in coellution problems.

MRS. KNIGHT: Well, coellution problems happen.

MR. TELLIARD: Thank you, Peggy.

MR. TELLIARD: Our next speaker is going to talk about some ion chromatography. Jack is from Cincinnati--that other lab. Jack.

JOHN D. PFAFF

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY

USES OF ION CHROMATOGRAPHY FOR INORGANIC ANALYTES IN WATER

MR. PFAFF: We've heard so many talks throughout the last day and a half on HPLC, I thought maybe what I ought to do is back up a little bit here and tell you what the difference between ion chromatography and HPLC is.

Basically, it's very simple. HPLC came first. IC or ion chromatography was a later addition to HPLC. The original design for ion chromatography came from Dow Chemistry in the early 70's and it was originally designed to do inorganic ions using aqueous solutions and using conductivity detectors. Throughout the years, it has grown up to a certain extent, and now it can be used for some of the smaller organic materials and it certainly is using more different types of eluants than just the aqueous types.

SLIDE 1

What I've tried to do here is give you a very basic schematic of an ion chromatographic system.

There are basically two types of ion chromatography, if you want to call it that, and this is due to patents more than anything else. When Dionex came out with the first commercial type of ion chromatograph, they used what's known as a suppressor--I'll get into that in just a minute--and they patented the use of the term suppressor and the use of a suppressor column following the separator column. Consequently, anyone else who wanted to get into the field, could not use a suppressor column. So now you have what is called a suppressed and non-suppressed, or as some of them like to call it, electronically suppressed pieces of equipment.

If you start at the top of the slide where the eluent is coming down, you get to the injection port. The injection port is a simple straightforward thing except when you try to modify it and put what is called a preconcentrator column in there. In the Dionex instrument, which most of my work has been done on, the injection port goes into a replaceable sample loop so that you can change the sample loop to put in any size from approximately 10 microliters to somewhere in the neighborhood of one milliliter. Of course, you get corresponding peak broadening depending on

the size that you use.

Another way of injecting larger amounts of samples into the instrument is to put in a concentrator column, which is very similar to the separator column that is being used. It is placed behind the sample injection port and up to 100 millimeters can be injected through this concentrator column. The ions of interest are held on the concentrator column while the matrix is allowed to go to waste. The instrument is then switched over and the eluent is passed through the concentrator column and goes through the separation mode. So when you start talking about detection limits, you usually have to specify what size sample loop you're using and/or whether you're using preconcentration of the sample.

The next thing in the line is usually what's called a guard column. The guard column is simply put there to protect the investment that you put into the separator column. You simply don't want to ruin your separator column, so you put something that can be sacrificed at the head of the column. Let me say here that the instrument itself cannot tolerate solids. Depending on whether you're talking about the eluent or the sample, you're

talking about particles in the neighborhood of about 0.02 microns that have to be taken out. They are taken out to a certain extent by the guard column, but then you have to replace this more often. The separator column, obviously, as in any kind of chromatography, is the heart of the system. It does the separation so that you isolate one of your components from the other. I want to discuss a little bit later the advantages of suppressed and non-suppressed, so let's just continue on down the slide.

The dash lines that I've put in here are simply to differentiate between the suppressed and the non-suppressed pieces of equipment. Anything between the dashed lines then, would be suppressed and if you sort of surgically remove that section of the slide, you would have, in essence, a non-suppressed piece of equipment. So in the Dionex equipment, you go through a suppressor.

Now, what's the sense of the suppressor? The suppressor is going to take the eluent, which for anions is usually some ratio of carbonate/bicarbonate solution, and change it into a non-conducting species so that your background is extremely low, and in turn, it changes your anions to the

acid form so you're looking at things like HCL or HF, which are highly conductive.

Now, this does one thing for the Dionex equipment. It does increase the sensitivity levels which you can go down to. Usually a rule of thumb is that you're talking somewhere in the neighborhood of one order of magnitude difference in sensitivity between suppressed and non-suppressed equipment.

So, you go through the suppressor. If you'll notice, you have two outlets on your suppressor. You have a regenerative going in and out as well as an eluent in and out. This is a relatively new piece of equipment. The original suppressors were packed bed suppressors. I don't know how many of you have ever used ion chromatography, particularly with packed bed suppressors. The packed bed suppressors were good for let's say 12 hours of use before they just simply quit on you. This necessitated approximately two hours of regeneration before you could put the instrument back into operation.

The introduction of the fiber suppressors did away with the drawbacks of packed bed suppression completely. The suppressor is not a packed column but rather it's more of a hollow fiber

packed with beads, simply to cut down on the dead volume, and it is constantly being regenerated by a flow of sulfuric acid counter-current to the flow of the sample.

After the suppressor, the sample enters the detector; whichever kind of detector you're using. Probably the most used type of detector is the conductivity detector, but as I'll show you later, you have many different choices now that you can use.

SLIDE 2

This is a separation of the common anions. For those of you who can't read the slide, fluoride chloride nitrate, not total, but orthophosphate, bromate nitrate and sulfate are separated. The eight minute separation time is going to be very dependent on the type of separator column you're using and your eluent. It can vary anywhere from 8 to let's say 20 minutes, depending on the column. This particular column here is the proprietary column for Dionex that is referred to as an AS-4 column. I know it's a matter of choice, however, I don't particularly care for the AS-4 column. It's very easily overloaded and, secondly, if you change any of these ratios of solutions that you see here,

you tend to get a lot of interference. My personal preference is that I would rather stretch it out and have a larger concentration that I can look at.

We did most of our work with what's called the AS-3 column. It's a little older column, it's more loosely packed and consequently, you can put more material on it, but your separation times are going to be at least three times as long. For sulfate, we're talking somewhere in the neighborhood of let's say about 20 minutes for elution. Sulfate is your last elutor from this column.

I mentioned that I would later differentiate between suppressed and non-suppressed pieces of equipment. Sensitivity is better for the suppressed equipment. What are we talking about? As I mentioned, about an order of magnitude. The cost is significantly different. Your non-suppressed pieces of equipment are going to be considerably cheaper than your suppressed pieces of equipment.

Eluents and columns that can be used with the two different kinds of pieces of equipment tend to be more numerous for the non-suppressed pieces of equipment. Why? Number one, you have many more companies actually involved in the production of

non-suppressed equipment. You even have a few people who are producing columns now that really don't produce the equipment themselves and I think this is a healthy situation. It's the way I think gas chromatography went in its infancy. If you do tend to get these people, I think you're going to get away from proprietary columns and there's going to be more competition and I think there's going to be a more successful introduction of columns.

I mentioned the fact that our lab has a Dionex piece of equipment, a 2120, which is the latest in their series. Let me point out one thing here, when you start looking at people's slides. If you notice this negative peak right here, this is what's known as the water dip. This is the thing that causes a lot of gray hair in people who are trying to do anionic separation on ion chromatography. If you see the water dip coming out in your standard anion separation after fluoride, you can sort of guess that the chromatogram that you're looking at was done on a packed bed suppressor. When you get to the fiber suppressor, the negative peak occurs in front of fluoride, and then it starts to cause more and more interferences in the separation of fluoride.

One other thing with packed bed suppressors. The retention times changed drastically as they tended to exhaust themselves, and you really had to watch what you were doing because even the water dip would migrate into an area where it would become an interference.

SLIDE 3

Talking about the migration of retention times, one of the drawbacks of ion chromatography seems to be the fact that the retention times can vary considerably with concentration. This work was some early work that we did. I picked out the worst and best possible cases, obviously. Fluoride is an early eluter, so as you can see, if you jump from 100 ppm to .5 ppm, you're only talking two-hundredths of a minute change, which you might as well forget. There's really no difference there. For nitrate using that same ratio of 100 parts to one-half a part, you'll notice now that you're talking in the neighborhood of two minutes variation with concentration. In sulfate, which is the worst, going from 500 ppm to 1, you're changing in the neighborhood of four minutes in retention time.

I think the one thing here that you've got to say right at the beginning of any kind of a

methodology using ion chromatography is, you've got to do an awful lot of standard injection into the instrument so that you can get some kind of an idea of what it is that you're actually looking at. There's just no way around it.

SLIDE 4

This is a slide that I borrowed as a figure from Small who published a paper in Analytical Chemistry and that paper introduced the hollow fiber suppressor for Dionex. If you're looking at the first one...the only difference between the first and the second is the size of the packed bed suppressor...you'll notice the two dips; number one, the water dip and secondly, the carbonate dip. The second one shows that as the column gets longer, the two peaks seem to change in retention time. The third one actually shows that with the hollow fiber suppressor, they migrate together and you think you only have one peak, but in essence you don't.

Let's treat them separately. The water dip peak, although it is something that gives you a problem, is easily gotten around and that is simply by matching the ionic strength of your sample to the eluent that you're using. That sounds

difficult, but if you take and make up an eluent at 100 times the concentration that you're using in the instrument, and simply add 1 mL of that to 100 mL of your sample, you really haven't changed much in the form of concentration, but your water dip will level out and you don't have to worry about interference from the water dip.

The carbonate dip, although Small showed it as a negative dip, this negative dip shows that you have depletion of the carbonate ion; that it's lower in concentration than the eluent. What happens if you have carbonate present in your sample at a higher level? What happens is you get a positive peak. For a long time with the system that we were using, we were trying to determine fluoride and the reproducibility of it was not good. Eventually we weakened the eluent so much trying to determine it that our fluoride peak in natural water samples evolved into two peaks. What happened was that carbonate elutes at the same time as fluoride and is a severe interference as far as fluoride determination is concerned.

The carbonate is one of the replaceable ions in the separator column. It is the ion that the anion replace throughout the separation. So if

you have a sample that is very high in concentration of total ionic strength--add everything together, you tend to get a rather high carbonate peak due to the separator column, and somehow or another, you can't quite do without that. So you're always going to have it. We originally started off looking at this as hardness, but later on we found out that it has been coming off the column.

How did we get around it? Actually, we got a column made by another company, other than Dionex, and put it into the suppressed equipment. It will actually separate the carbonate from the fluoride, so we're hoping that we can now do this quantitatively.

SLIDE 5

I mentioned the fact that there are other detectors, and so I included this slide so that you could see that you can do things other than the standard anions. What you're looking at, the bottom chromatograph, is the common six anions done by a conductivity detector while the top chromatogram--this is the separation here--is done by an electrochemical detector.

If you'll notice the same sample here, you've got your separation down in here of your standard

anions, but now we're getting sulfite, cyanide and bromate coming out on the electrochemical detector. These detectors can be run in series since neither are destructive, and you can get considerable separation. Since cyanide is one of the materials that is most commonly sought after for separation, we're hoping that this particular separation can be used to do some of the separations for cyanide in place of some of the wet methodology that is used at the current time.

In addition to electrochemical detectors, ultraviolet detectors, fluorescence detectors can be used and there's even an adjunct, if you will, detector known as a post column reactor in which a chemical is pumped into the system to react with the eluent and the anions, or whatever it is you're trying to separate, that will usually give you a reaction that can be determined using ultraviolet detectors.

Getting back to some of the interferences for the common anions, aluminum interferes, obviously, with the fluoride, but that has nothing to do with the ion chromatograph. With fluoride, which is first off the column, you're really interfering with what we call the garbage peak--everything

have trouble in zeroing the baseline. This is part of the reason for this order of magnitude difference.

In way of summary, let me say that I think ion chromatography has a place in the analytical scheme of things. I want to say one other thing, and that's to caution you to run a lot of standards and spikes because of this migration of retention time, in order to be sure of what you're doing. I think it is probably one of the better and more promising techniques to come along, in inorganic chemistry anyway, since the advent of atomic absorption.

Thank you. Any questions?

MR. TELLIARD: Any questions? Thank you.

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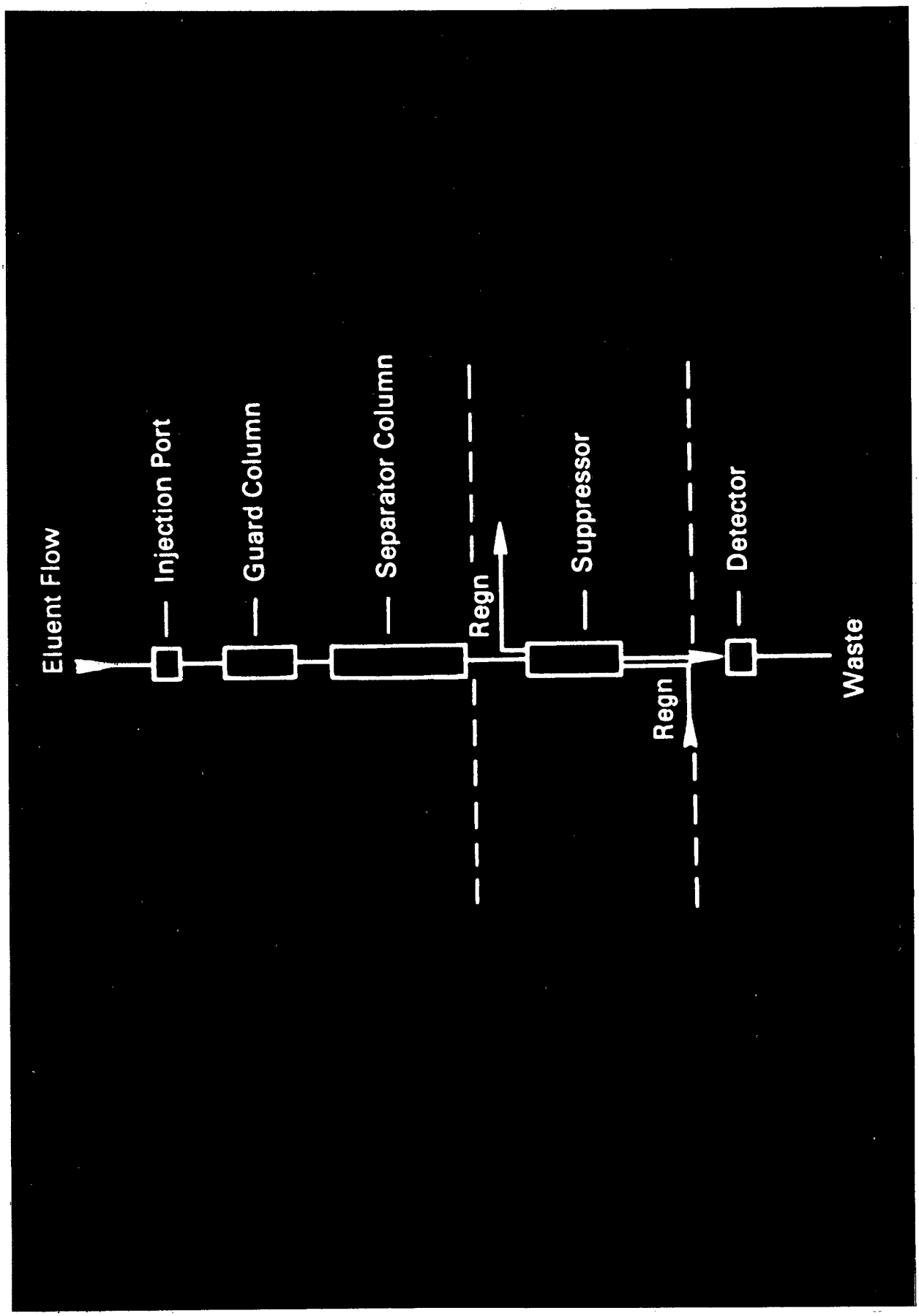
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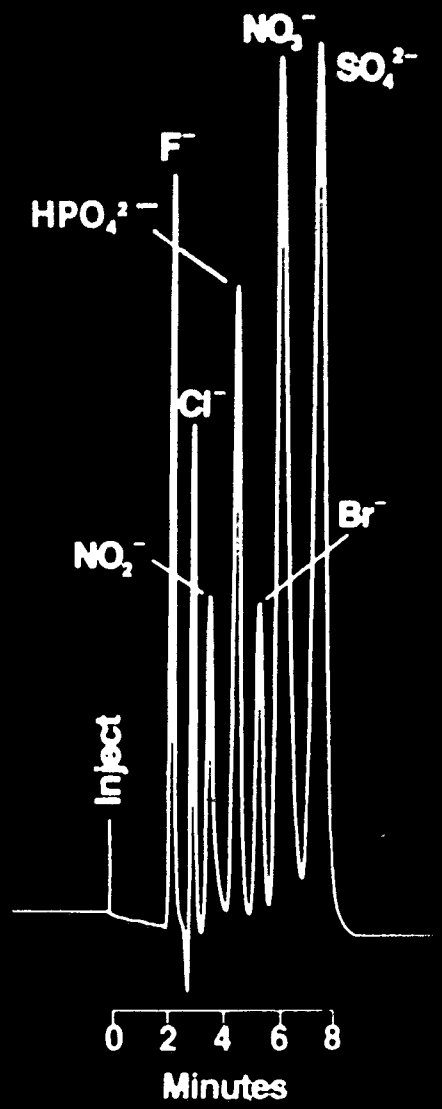
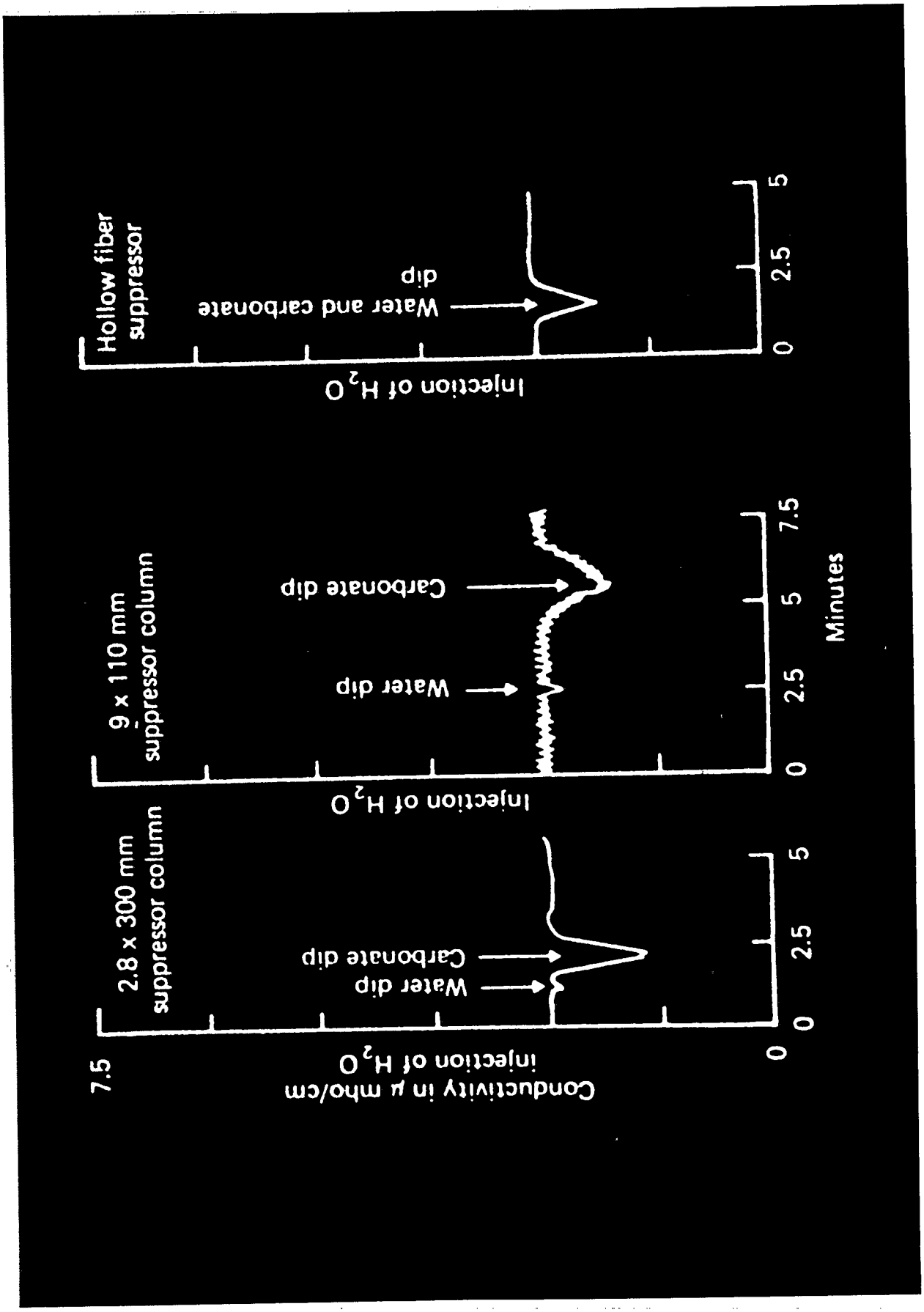


Concentrations

mg/l (ppm)	
F^-	3
Cl^-	4
NO_2^-	10
HPO_4^{2-}	25
Br^-	10
NO_3^-	30
SO_4^{2-}	25

Variation of Retention Time with Analyte Concentration

Fluoride		Nitrate		Sulfate	
ppm	min	ppm	min	ppm	min
100	1.24	100	5.22	500	7.94
0.5	1.26	0.5	7.95	1	11.60


Conductivity in μ mho/cm
injection of H_2O
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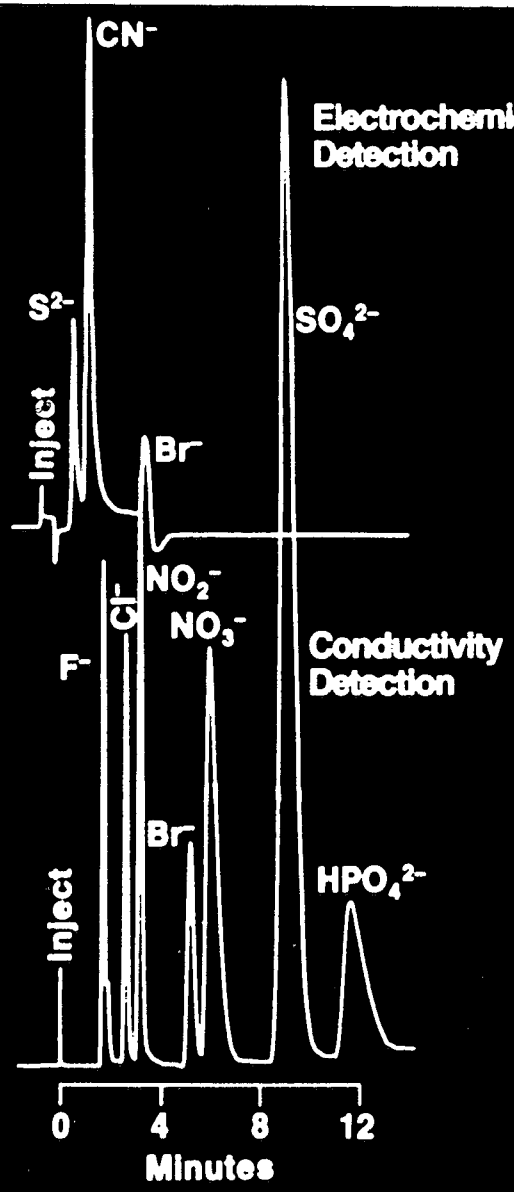
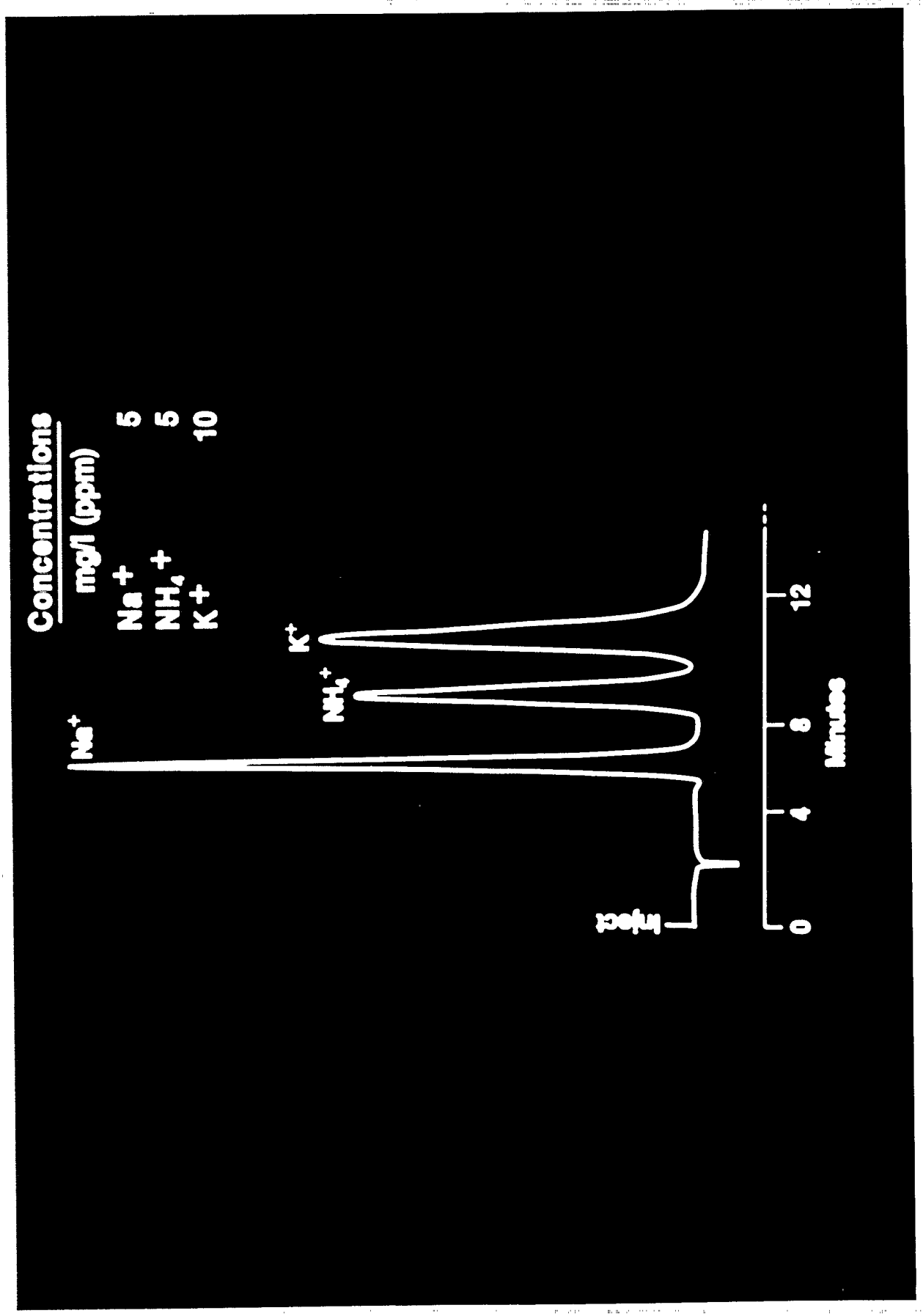
0
2.8 x 300 mm
suppressor column
Water dip
Carbonate dip
0
2.5
5
Injection of H_2O
9 x 110 mm
suppressor column
Water dip
Carbonate dip
0
2.5
5
7.5
Minutes
Injection of H_2O
Hollow fiber
suppressor
Water and carbonate
dip
0
2.5
5



Concentrations

mg/l (ppm)	
S^{2-}	0.5
CN^-	0.5
F^-	3
Cl^-	4
NO_2^-	10
Br^-	10
NO_3^-	10
SO_4^{2-}	50
HPO_4^{2-}	50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mg/l (ppm)
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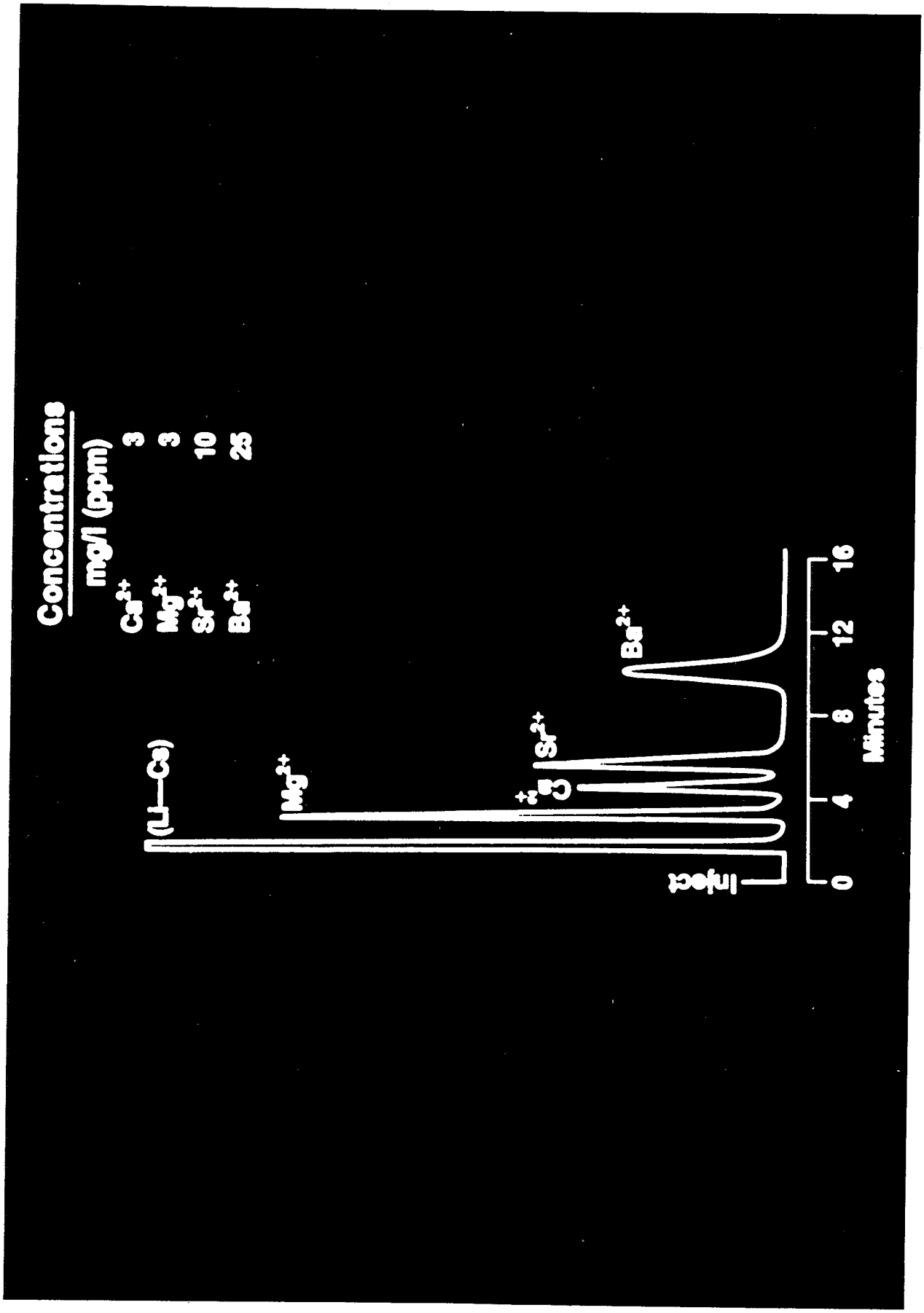
NH4+ 5
K+ 10
Na+
NH4+
K+
Inject
0
4
8
12
Minutes


Concentrations
mg/l (ppm)
Ca2+ 3
Mg2+ 3
Sr2+ 10
Ba2+ 25
Ca2+
Mg2+
Ca2+
Sr2+
Ba2+
Inject
0
4
8
12
16
Minutes

MR. TELLIARD: Our last speaker this morning is Suzanne with Environment, Canada. She's going to talk about some HPLC with electroconductivity detection for phenols.

SUZANNE LESAGE, PH.D.

WASTEWATER TECHNOLOGY CENTRE
ENVIRONMENTAL CANADA
ENVIRONMENTAL PROTECTION SERVICE

DIRECT ANALYSIS OF PHENOLS BY HPLC

DR. LESAGE: Thank you for inviting me here. The little ship break this morning reminded me much of the situation that we have. Our offices are located on Lake Ontario between Toronto and Niagara Falls, right on the lake and beside a lift bridge, very similar to the one that is here. Whenever my guests stop listening to me, I'll turn around and inevitably there's a ship going by. Fortunately, we've got winter when we manage to work because then there are no ships going through.

Before I start, I will acknowledge the people I work with. The data that I will present today was mostly produced by Mrs. Sharon Hay-Pole and Mrs. Sandra Abbott. Mr. Ken Conn is the supervisor of the lab and is the person who allowed me to do this kind of work, and Mr. Fowlie is our quality control chemist. Maybe I should say the data

didn't quite pass his criteria, but I told him, "It's organic analysis, you have to be understanding."

I work for Environment Canada, the Canadian Federal Department of the Environment, more specifically for the Environmental Protection Service in the Wastewater Technology Center. We are a small research facility, mostly staffed with engineers, and our role is to study industrial discharge problems and try to solve them by developing appropriate control technology.

Our laboratory works in support of those research programs, and also acts as a national center for GCMS analysis of industrial wastes. We've done all kinds of wastes--tanneries, textiles, wood preservation, steel mills, coal conversion wastewater, resin manufacturing, pulp and paper. We're only one lab, so we do everything.

Whenever possible, we use the EPA methods. To us it only makes good sense; why reinvent the wheel? Well, of course, like everybody else, we get the urge once in a while to try, and our attempts at reinventing the wheel for phenol analysis is what I will be describing now.

This program was started because we needed

to turn out a lot of phenol results in coal conversion wastewater. We have a GCMS, which we prefer to use where we can. Unfortunately, we only have one, and when you have several pilot plants running continuously, we have to be able to provide data relatively quickly, and the engineers with whom we work are more used to the BOD, TOCs type of turnaround time and they don't have the patience to wait for six months for their samples to be analyzed by GCMS. Of course, their needs are to run the pilot plants, thus they require results much faster.

The situation we had here, was we were looking at the effluent from a pilot plant, consisting of a biologically active fluidized bed treating coal conversion wastewater. As an aside, the coal conversion wastewater used actually was imported from the H-coal Pilot Plant in Catlettsburg, Kentucky. We don't seem to have enough Canadian problems, so we import some.

We looked at the alternate method 604, the GC method for phenols, so we could turn out results a little bit faster than with GCMS. Unfortunately, coal conversion wastewater contains a lot of other chemicals. Typically it's full of heterocyclic nitrogenous compounds--aniline, quinoline, carbazole

and the like. So we got a tremendously complex chromatogram. The only way really we could handle it was GCMS. The other problem we had with this as with all GCMS analysis, is that typically with this kind of matrix, our recoveries from phenol ran from very poor to simply pathetic. That means 30 down to about 5 percent, if we found any. We knew from the conventional phenol, 4-amino-antipyrene method, that there was a substantial amount of phenols in there, the only problem is that the GCMS just couldn't find it.

SLIDE 1

So we turned ourselves to HPLC as a possible substitute. The method we tried is in essence very simple. We used a five micron ultrasphere ODS column that can be purchased anywhere, and the mobile phase is acetonitrile and water with acetic acid and phosphoric acid as modifiers at 1.5 mL/min. We used a BAS amperometric detector running at 1.2 mV, and 20 mA current. That is really middle range for that particular detector, but we find that although the label says it goes down to .1, the actual life situation is different. We also have to use a UV detector to follow the response of nitrophenols.

For PCP analysis, the percentage of acetonitrile has to be increased to 60 percent, otherwise it takes something like an hour and a half to elute PCP with detection that would be very poor. We find that usually it's a lot more practical to do PCPs one day and the rest of them the other day. In coal conversion, PCP was of no concern whatsoever. We were actually looking more at phenol, methylated phenols and dihydric phenols, and when we do PCP, it's usually for wood preservation plants where tetra and pentachlorophenol are most important. It would be possible to do all of priority pollutant phenols by solvent programming, but the electrochemical detector does not like any kind of programming whatsoever. The response of the detector depends on the mobile phase, on its electroconductivity. When you increase the percent of acetonitrile, the baseline drifts down and the detector does not recover fast enough to enable you to do a lot of analysis in a day, so it's easier to change mobile phase over night.

SLIDES 2&3

This is a typical standard chromatogram. There's not really much to see looking at this standard chromatogram, other than everything is

nicely separated. There is response to dinitrophenol, and 2- and 4-nitrophenol on the electrochemical detector. The amount injected on column here is representative of 100 to 500 micrograms per liter solution. That represents 2 to 10 ng. injected in a 20 microliter loop. We also followed the analysis with the U.V. and visually checked the presence of nitros from both detectors. You can see that for 4-nitrophenol one actually is better off using the electrochemical detector rather than the U.V. detector. For the 2,4 nitrophenol and 4,6 dinitrocresol, the response is not sufficient in the electrochemical detector. But as I'll show you a little bit later, we have done some work and it is possible to enhance the response by addition of specific buffers. Unfortunately, it doesn't really quite work.

SLIDE 4

These are the retention times of all the compounds of interest. As you can see here, we can complete analysis within 25 minutes. One thing I might not have stressed enough here is that we're doing direct analysis. We get a sample, filter it through to a .45 micron filter and inject. That is the end of sample preparation. This is not quite

the type of work that we're used to. In a normal organic lab, if you haven't done three extractions and five cleanups, you haven't done analysis. But it has the advantage of being able to do duplicates, triplicates, spikes and all.

SLIDE 5

We analyzed the standards in mobile phase and we did five replicate analyses. This is the typical kind of recovery we got. Now, looking at 97.8 percent recovery for phenol, and really, I don't know that anybody can honestly report more than 50 percent, when you're doing extractions, I got quite excited, especially with really rather low standard deviation, nothing above 10 percent. This is not the kind of numbers I'm used to in the lab.

The nitrophenols and dinitrophenols can be done either way. For the 2- and the 4-dinitrophenols, we usually get slightly better standard deviation using the electrochemical rather than the U.V. detector.

SLIDE 6

To prove that this really worked, we used the standards which are most generously provided by the EPA for quality control. These standards are provided for quality control under method 604. That's the GC method with or without derivatization. The

first column is the true value of the standard. These are the results obtained with Method 604 and provided with the standards. This is the kind of number that we got in our method. Again, where we got most excited was finding that with a true value of 75 microgram per liter of phenol, we were actually reporting 78 rather than 34. To us that is most crucial. The other thing is that this really isn't very much work. It's just basically get the sample, filter and inject.

For the other phenols, we think that the results either are equivalent or better than ones obtained with Method 604, with the exception of PCP. PCP at 70 micrograms per liter, that's below the detection limit. Now, I must stress that is for direct analysis. We could get better by extracting the sample and injecting the extract, which is obviously a very easy way to go. We had very little interest in actually doing that because when we have to do PCPs, we get samples that are high rather than low. We get levels that would be running around 50 or 60 milligrams per liter, so dilution is more of a problem than actual detection limits.

SLIDE 7

Now, in this next slide we decided to try a method in real life and to do so, we selected two typical, or maybe not very so typical wastewaters that we deal with. These are not nice wastewaters. They were very yellow, fairly high solids content. I don't have the exact TOC's, but it's fairly high. The other reason we chose those is that we had GCMS data to back up our analysis and we knew roughly how much phenol was in there to start with. For instance, the conversion wastewaters I'm talking about here, by GCMS we were reporting a figure of 6 micrograms per liter. My supervisor got a bit upset because the 4-AA method was reporting something to the order of 400 micrograms per liter in total phenols, and he said well, what are these other phenols? I said, I don't know. When we ran this, phenol itself accounted for over 100 micrograms of the total and there were a substantial number of methyl phenols and other analytes. So this method usually gives a better correlation with the total phenols.

These numbers here are not the measured values but actually the percentage of recovery. The water data is basically what was presented in the previous

slide. Everything is in fairly good standing and would pass except for some of the nitros. We all know, of course, problem with 2-nitrophenol.

I must say that we did not throw away any data whatsoever. So, what happens here when you see 193 +/-111 on triplicate, we found it in two samples and didn't find it in the other. At 150 micrograms per liter with 2-nitrophenol, that's basically at the detection limit, so it is not that surprising that there is such a variation.

SLIDE 8

We repeated the experiment at twice the level to see whether the method is linear. The percentage of recovery in water again is very similar to what we had obtained at the lower level and in the two samples, everything is more or less above 50 percent recovery except for the nitrophenols, and I think that's one of the nightmares of the analytical chemists.

OVERHEAD 1

Briefly I'll touch on the effect of adding buffers, one interesting fact we found here. At the top part here, that's the chromatogram that I showed you in the slides previously (Slide 2). That's our normal standardization chromatogram.

Here, below, is a chromatogram we obtained where we added .01M sodium acetate buffer to the solution. If you look at the 4-nitrophenol response here compared to the 4-nitrophenol here, you can see that there's a substantial difference. The same case with the 2-nitrophenol which is substantially enhanced by the addition of buffer.

You will ask probably why on earth didn't we use the buffer all the time. Well, the problem is that not only is the 2 and the 4-nitrophenol response enhanced, but so is the 2, 4-dinitrophenol and the 4, 6-dinitrocresol. Their response is enhanced and the retention time shifts. What we think is happening in this case is that the species we're actually measuring is not nitrophenol itself, but an ion-pair.

OVERHEAD 2

There are the typical detection limits that one gets, based on 20 microliters analyzed directly. It isn't great for dinitrophenol, but neither is the GC method. If there is a need for lower detection limits, I think one would have to go back to the extraction with all the problems it entails. It isn't as good as one would want, maybe, for some clean effluents, but is usually sufficient in

a real life situation for the monitoring of an industrial discharge.

SLIDE 9

Finally, here are what we believe are the advantages of the method. It has all of the advantages of direct analysis. There is no sample preparation. The method would be very easily automated because there is no need for an operator. It's low cost. We estimate that getting a single pump with the BAS detector and a column would cost you less than $10,000 U.S. For us, of course, it's about $20K Canadian.

It is a very rapid method and the reproducibility of standard deviation is usually better than one would obtain having to extract samples, especially very dirty samples. It's also fairly free of interferences. The coal conversion wastewater that we used, for instance, we really could not use anything else but GCMS and this provided us an alternative. So we believe this is ideally suited for routine monitoring of effluents when the samples are already characterized. Thank you.

MR. TELLIARD: Any questions?

ANALYTICAL CONDITIONS

COLUMN: 5 um ULTRASPHERE ODS

MOBILE PHASE: CH_3CN: WATER:ACETIC ACID:PHOSPHORIC ACID

40: 59.7 : 0.1 : 0.2

FLOW RATE: 1.5 ML/MIN

DETECTOR A: BAS AMPEROMETRIC

1.2 VOLT

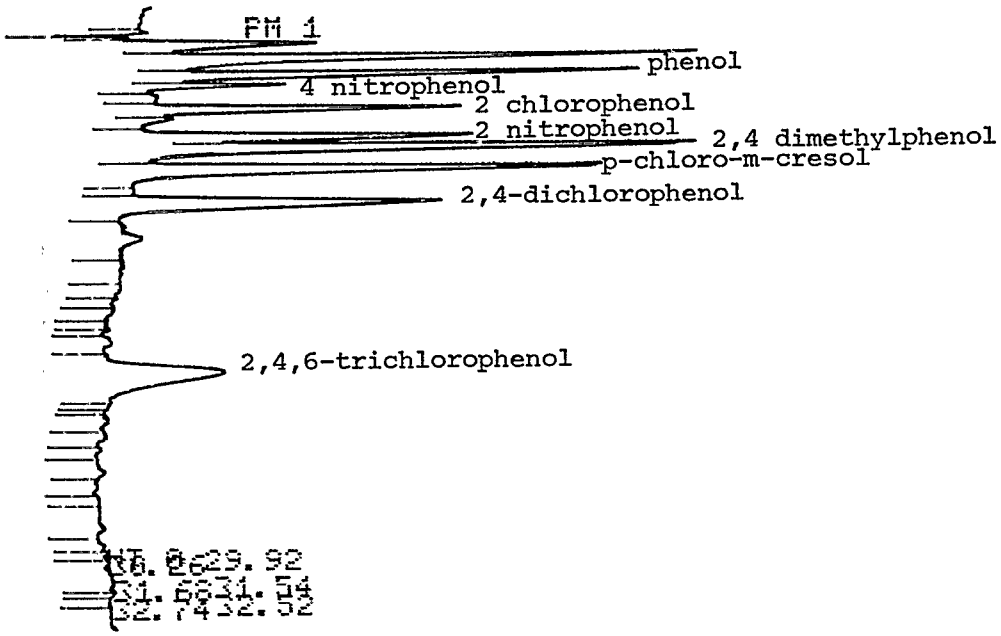
20 uA CURRENT

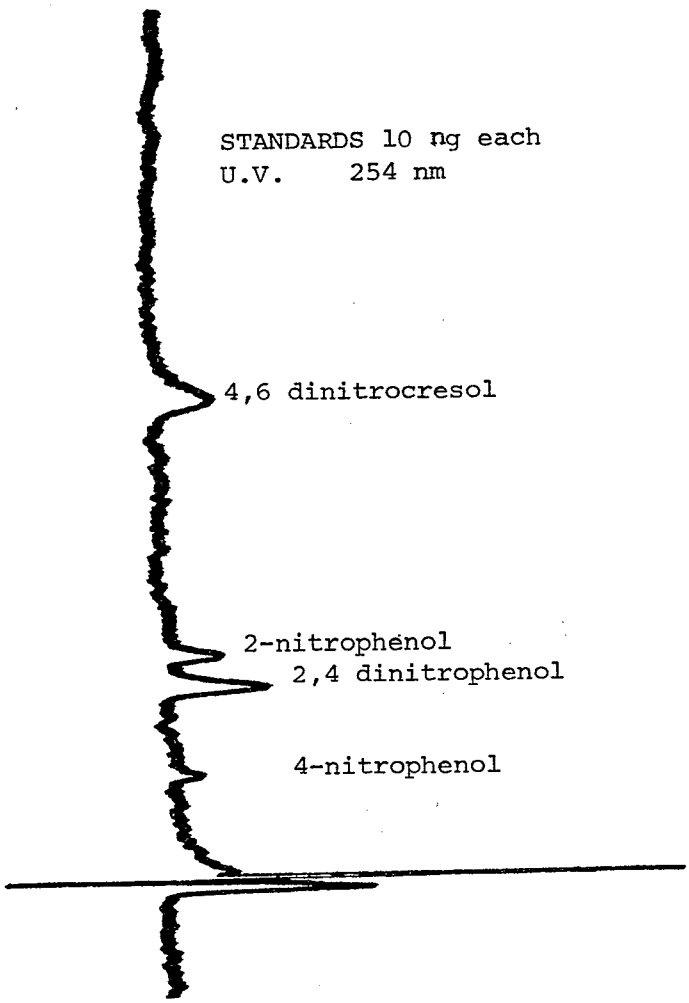
DETECTOR B: FIXED U.V. AT 254 nm

ATTENUATION: 0.005 A.U.

PCP: CHANGE TO 60% ACETONITRILE

STANDARDS by ELECTROCHEMICAL DETECTION
(2 to 10 ng per component (20 µl loop)




STANDARDS 10 ng each
U.V. 254 nm
4,6 dinitrocresol
2-nitrophenol
2,4 dinitrophenol
4-nitrophenol

COMPOUNDS OF INTEREST

	R_t
PHENOL	3.77
4-NITROPHENOL	4.46
2-CHLOROPHENOL	5.87
2,4-DINITROPHENOL	6.63
2-NITROPHENOL	7.54
2,4-DIMETHYLPHENOL	8.24
P-CHLORO-M-CRESOL	9.56
2,4-DICHLOROPHENOL	11.7
4,6-DINITROCRESOL	14.6
2,4,6-TRICHLOROPHENOL	22.2
PENTACHLOROPHENOL	13.3*

* USING 60% ACETONITRILE - 40% WATER WITH ACETIC ACID

ANALYSIS OF STANDARDS IN MOBILE PHASE ON 5 REPLICATES

COMPOUND	TRUE VALUE ug/L	MEAN $\bar{x}$	STD. DEV. n	REL. STD. DEV.
ECD				
PHENOL	100	97.8	2.6	2.7
4-NITROPHENOL	500	451	23.1	5.1
2-CHLOROPHENOL	100	95.5	5.0	5.2
2-NITROPHENOL	500	474	25.7	5.4
2,4-DIMETHYLPHENOL	250	238	9.2	3.9
P-CHLORO-M-CRESOL	250	244	13.9	5.7
2,4-DICHLOROPHENOL	250	246	6.3	2.6
2,4,6-TRICHLOROPHENOL	500	516	35.8	7.0
UV				
4-NITROPHENOL	500	427	31.9	7.5
2,4 DINITROPHENOL	500	534	28.2	5.3
2, NITROPHENOL	500	619	86.0	13.9
4,6 DINITROCRESOL	500	351	20.6	5.9

EPA STANDARD

PARAMETER	TRUE VALUE	METHOD 604 $\bar{x}$	METHOD 604 S	OUR METHOD $\bar{x}$	OUR METHOD S
PHENOL	75.0	34.1	11.1	78.1	0
2,4-DIMETHYLPHENOL	100	61.4	21.2	92.0	12
2-CHLOROPHENOL	75.0	60.3	13.6	73.8	6
4-CHLORO-3-METHYLPHENOL	125	106	19.0	125	16
2,4-DICHLOROPHENOL	100	79.6	17.0	99.4	42
2,4,6-TRICHLOROPHENOL	150	124	20.4	170	77
PENTACHLOROPHENOL	70.0	60.1	13.9		
2-NITROPHENOL	150	120	22.8	176	19
4-NITROPHENOL	160	73.0	31.2	118	43
2,4-DINITROPHENOL	250	205	67.8	221	16

RECOVERY OF SPIKED EPA STD

HIGH LEVEL

COMPOUND	TRUE VALUE ug/L	WATER % ± S.D.	SAMPLE A % ± S.D.	SAMPLE B % ± S.D.
ECD				
PHENOL	150	94±1.0	158±4.0	109±0.9
4-NITROPHENOL	320	84±1.7	40±44	124±17
2-CHLOROPHENOL	150	89±3.3	60±3.3	103±2.0
2-NITROPHENOL	300	107±12	62±0.5	71±1.2
2,4-DIMETHYLPHENOL	200	99±10	85±2.0	82±6.2
P-CHLORO-M-CRESOL	250	107±18	86±2.7	89±18
2,4-DICHLOROPHENOL	200	130±35	100±2.9	119±27
2,4,6-TRICHLOROPHENOL	300	144±17	69±7.5	110±20
UV				
2,4 DINITROPHENOL	500	86±36	93±3.5	92±0.7
4,6 DINITROCRESOL	NOT PRESENT IN STD.			

RECOVERY OF SPIKED EPA STD

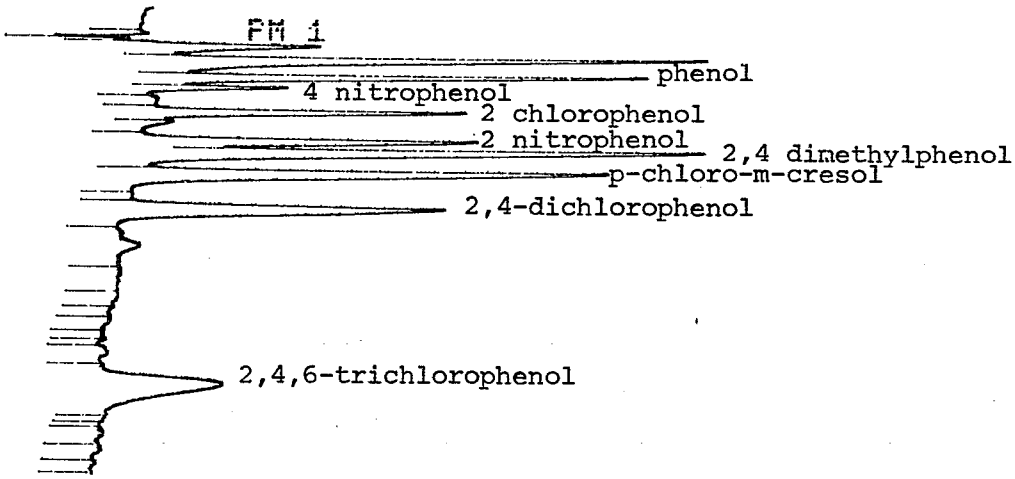
USED FOR METHOD 604

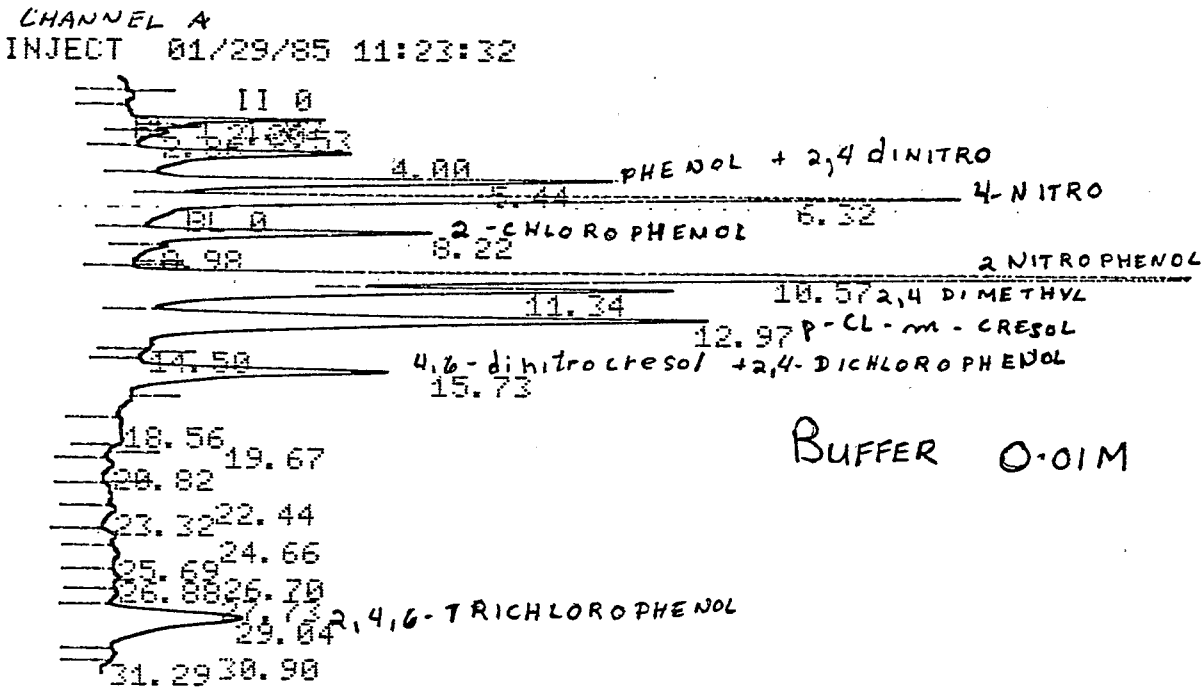
COMPOUND	TRUE VALUE ug/L	WATER % ± S.D.	SAMPLE A % ± S.D.	SAMPLE B % ± S.D.
ECD				
PHENOL	75	104±.3	155±14.6	120±3.4
4-NITROPHENOL	160	74±33	12±11	221±192
2-CHLOROPHENOL	75	86±9.1	39±34	84±13
2-NITROPHENOL	150	117±27	193±111	191±17
2,4-DIMETHYLPHENOL	100	92±16	92±6.5	86±2.2
P-CHLORO-M-CRESOL	125	82±13	86±2.6	91±2.9
2,4-DICHLOROPHENOL	100	86±45	110±4.5	101±4.9
2,4,6-TRICHLOROPHENOL	150	119±63	118±61	79±34
UV				
2,4 DINITROPHENOL	250	89±8.2	61±33	90±14
4,6 DINITROCRESOL	NOT PRESENT IN STD.			

SAMPLE A: COAL CONVERSION WASTEWATER

SAMPLE B: EFFLUENT FROM A WOOD PRESERVING PLANT

STANDARDS by ELECTROCHEMICAL DETECTION





12

DETECTION LIMIT

Based on 20 ul analyzed directly

	ug/L
Phenol	10
4 Nitrophenol	200
2 Chlorophenol	20
2 Nitrophenol	100
2,4 dimethylphenol	25
p-Cl-m-cresol	25
2,4-dichlorophenol	25
2,4,6 trichlorophenol	50
pentachlorophenol	150
U.V.	
4 Nitrophenol	300
2 Nitrophenol	300
4,6-dinitrophenol	300

ADVANTAGES OF THE METHOD

DIRECT ANALYSIS

- NO SAMPLE PREPARATION
- EASILY AUTOMATED
- LOW COST
- RAPID
- IMPROVED REPRODUCIBILITY

IDEALLY SUITED FOR ROUTINE MONITORING OF EFFLUENTS

QUESTION AND ANSWER SESSION

MR. STANKO: George Stanko, Shell Development. I have one question. Can you explain the difference in your procedure and Method 604 with respect to why the 604 method only has approximately 50 percent recovery of phenol itself and yours gets just about 100 percent recovery? Do you have an explanation or a reason?

DR. LESAGE: We do not extract. That's why. Method 604, I believe, is a methylene chloride extraction, and typically phenol doesn't seem to extract out of water. In this case, we just take a sample and inject it directly. So these recoveries are not really recoveries. The measurement is a direct measurement.

MR. STANKO: Then it's really nothing in the chromatography? In other words, there's nothing unique to your column or your chromatographic system, it is all in the extraction versus no extraction?

DR. LESAGE: I would tend to believe so, yet the chromatography on the GC column would obviously be better to get more theoretical plates than one gets with HPLC. The

detector is also more specific, so that there are fewer interference problems.

MR. McMAHON: Wayne McMahon, Martin Marietta. Did you do any comparison studies to compare the data from the 4-AAP method to your HPLC data to see how well there was correlation between the two methods?

DR. LESAGE: We always compared the data. One of the problems with comparison with the 4-AAP method is that of course, it is for total phenols, whichever ones are present, the color is the total. To be able to compare, it is necessary to know which species are present and what is their respective response for the 4-aminoantipyrine because as you know, that method is based on total phenols as phenol. The color response from other phenols is not equivalent. It would be something like 40 to 80 percent depending on the phenol. Anything that is substituted in the para position will not respond to the 4-aminoantipyrine. So when we correct for that and in samples that are not typically too bad, that is they don't have a lot of other interferences, the correlation is really quite good. Typically, we can usually account for about 80 percent of the total phenols.

There was a case where there was a lot of pentachlorophenol in the sample where the correlation fell through completely.

Now, when we try to do the same correlation with GCMS, there is usually absolutely no correlation, it never makes any sense. So in this case, it's a little bit better--quite a bit better, actually. At least the curves follow the same slopes.

MR. EDELMAN: Dave Edelman, Crown Zellerbach. When you do your percent recoveries on your real world effluent samples, how do you know you had positive interferences beforehand when you make your corrections for what's initially in there before you spike the samples?

DR. LESAGE: We did first an analysis of the sample as is, then spiked at the two levels. I just reported here the percent of recovery, not the amount that was found. There were some phenols that were not found at all and typically, phenol itself was running about 100 micrograms a liter as well as a few others that were present in there, and we corrected to percentage recovery.

MR. EDELMAN: Right. I understand that, but how do you know you don't have positive interferences initially that peak your chlorophenol before you spike it, as phenol, not something else, but one of the other peaks is...

DR. LESAGE: Yes, that's what I said earlier. A method like this has the same problem as Method 604. For instance, it's like any GC or HPLC method. You have to be able to correcterize your sample by an alternate method and check for those kind of possible interferences. We usually do not have very much problem with that and we have run very, very dirty samples. There is usually very few peaks in the electrochemical chromatogram.

One thing is that, basically because you're running a very specific buffer, something like aniline will not be in the same ionization state. It will be a positive ion in the solution, and be easily separated so you don't have a problem. It is more specific because you only elute in certain window. So it's not completely free of interferences, I'm not going to say that, but if

you have something that you have correcterized, you can fairly safely use it.

MR. TELLIARD: I'd like to thank Susan and Peggy and Jack for their presentations this morning. I'd also like to thank Dale Rushneck and Todd Fielding for working so much in getting this thing together, and, of course, Whitescarver Associates and County Court Reporters, Inc. for their work here.

This ends the eighth. We hope to see you for the ninth. Until we figure out where else we're going to have it, we might assume it's going to be here, unless somebody comes up with a real winner. It's rough to follow up on a battleship, but we'll try to figure out something. Thank you very much for coming; enjoyed having you here.

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