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THE QUANTITATIVE ANALYSIS OF POLYCHLORINATED BIPHENYLS AND THEIR METABOLITES BY GAS CHROMATOGRAPHY AND ION-MOLECULE REACTION DETECTION IN A TANDEM MASS SPECTROMETER

presented by

Ronald Franklin Lopshire

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Christie G Enke
Major professor

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By

Ronald Franklin Lopshire

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ABSTRACT

THE QUANTITATIVE ANALYSIS OF POLYCHLORINATED BIPHENYLS AND THEIR METABOLITES BY GAS CHROMATOGRAPHY AND ION-MOLECULE REACTION DETECTION IN A TANDEM MASS SPECTROMETER

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An analytical method for the quantitative analysis of polychlorinated biphenyls (PCBs) using gas chromatography and mass spectrometry was developed. The method uses the exchange reaction of oxygen for chlorine with the molecular anions of PCBs in an MS/MS experiment with a triple stage quadrupole mass spectrometer. The instrumental parameters explored for instrumental optimization for this reaction include: type and pressure of moderating gas for the production of molecular anions; type and pressure of collision (reactant) gas; and collision energy and translational kinetic energy correction factor. Instrument scan modes are optimized for application of the method to sediment samples containing PCBs. The method focusses on analysis of coplanar PCB congeners that have been shown to exhibit dioxin-like toxicity. The specificity of the method is explored with respect to congeners that co-elute with the coplanar congeners in a GC experiment. The method is expanded to identify other chlorinated aromatic compounds in sediment samples containing significant amounts of PCBs.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

The detection and quantitation of polychlorinated biphenyls (PCBs) in a wide range of samples and matrices poses a unique and challenging analytical problem. The purpose of the present work has been to use tandem mass spectrometry (MS/MS) to develop a unique but robust and routine method for the detection and quantitation of individual PCB congeners in PCB-contaminated samples. In addition, the methods developed have been extended to include the detection of PCB metabolites in samples with a significant PCB contribution.

As a preface to the discussion of the analytical methodology involved with this work, a brief discussion of the history of PCBs as an environmental and clinical problem is included. This discussion also contains the terminology associated with the chemistry and analysis of PCBs.

The current work involves the development of an MS/MS method for the detection and quantitation of individual PCB congeners. It is, therefore, desirable to include a brief synopsis of the limitations of the analytical methods currently used for

routine PCB analyses. These methods include gas chromatography, electron capture detection and mass spectrometry, and have generally been used not for congener-specific analyses, but for the determination of total PCBs or for the determination of a specific commercial mixture, such as an Aroclor.

The method of analysis for individual PCB congeners presented in this work involves the detection of products of an ion-molecule reaction carried out in the collision chamber of a triple-quadrupole mass spectrometer. The molecular anions of PCB congeners are allowed to react with molecular oxygen to produce an exchange of an oxygen atom for chlorine, and the products of the reactions are monitored. The parameters involved in these reactions include the kinetic energy and collision pressure.

The exchange reaction of oxygen for chlorine with the molecular anions of PCB congeners is monitored in conjunction with chromatographic separation as a method of analysis for individual PCB congeners. The development of the method includes the optimization of several instrumental parameters involved in the chromatography and mass spectrometry. The precision and accuracy of the method are also compared to methods commonly used. The precision obtained is less than ten percent which is comparable for most congeners to MS techniques and of an order acceptable for methods such as GC/ECD. The accuracy of the method was determined by comparison with published results of analysis of Aroclor standards. Even though the composition of Aroclor mixtures varies widely, the results were of

the same order of magnitude for the congeners compared.

While the development of the MS/MS method for PCB analysis has merit on its own, the method is applied to the analysis of real samples. Specific applications include using the method for the analysis of PCB-contaminated soil and sediment samples. The method is applied to the analysis of samples used in the study of microbial action on individual PCB congeners.

An analytical method was also developed using the exchange reaction of oxygen for chlorine to include the analysis of PCB metabolites and other chlorinated aromatics in samples with significant amounts of PCBs. Specific metabolites include polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

A summary of the limitations and general observations of the method of analysis presented is offered. The primary advantage of the method is due to the high selectivity provided by the MS/MS technique. Other methods such as GC/ECD and GC/MS do not provide for the quantitation of lower mass congeners in the presence of higher mass congeners that chromatographically co-elute. The observations presented include a discussion of the critical parameters involved in the development of the method, the application of the method to specific samples and possible interferences observed with the method. The primary interference with the method occurs in some cases when congeners co-eluting with those of interest

exhibit rearrangement in the ion source to produce precursor ions of the same chemical composition. Future considerations offered include the further exploration of all possible interferences with the method.

Chapter 2

Introduction to Polychlorinated Biphenyls

Synthesis and Use. The synthesis of polychlorinated biphenyls (PCBs) was first reported in 1881 by Schmidt and Schultz *(1)*, but industrial production of the unnatural complex mixtures did not begin until 1929. Due to their unique physical and chemical properties, PCBs were soon in widespread use as nonflammable lubricants, plasticizers, flame retardants, insulators, pesticide extenders, dust suppressors, vapor suppressors and high-pressure sealants. These applications found use in transformers, capacitors, pesticides, printing inks, paints, de-dusting agents and several other products. PCBs were manufactured and marketed under several trade names including Aroclor, Clophen, Phenoclor, Fenclor, Kaneclor and Phyralene.

Commercial production of PCBs is accomplished by chlorination of biphenyl with chlorine gas. By controlling reaction conditions and using catalysts such as ferric chloride, mixtures of PCB congeners are produced with the desired overall chlorine content *(2)*. In theory, 209 chlorinated biphenyl isomers are possible, but in practice only a little over one hundred are probable. The properties of these

mixtures, as expected, are dependent on the degree of chlorination. Boiling points range from 278 C for the 21% chlorinated product to over 420 C for the 60% chlorinated product. These substances can be distilled at atmospheric pressure without decomposition and are soluble in hydrocarbons but only very slightly soluble in aqueous media. In addition, PCBs are also produced during incineration of chlorinated aromatics and as by-products in several commercial chemical processes.

Monsanto began production of Aroclors in 1930, and before ceasing production in 1977, became the world's largest producer of PCBs. It has been estimated that total U. S. production of PCBs through 1976 was 1.3 billion pounds, 93% of which was produced by Monsanto. Worldwide commercial production through 1980 is estimated to be 2.4 billion pounds. Annual production of PCBs as by-products is estimated to be 100,000 pounds *(3)*.

Environmental Ramifications. A unique aspect of the PCB problem is that unlike other environmental contaminants such as DDT, the commercial applications of PCBs did not generally include distribution in the environment *(4)*. And yet today PCBs are ubiquitous contaminants and have been found all over the globe. Three significant events occurred which led to worldwide concern. In 1966, the Swedish scientist Jensen drew attention to the fact that PCBs were found in fish and birds *(5)*. In 1968, an accident in western Japan poisoned 1300 people. The so-called "Yusho" poisoning occurred when a heat exchanger leaked PCBs into a batch of rice oil *(6)*. This accident was the precursor of several similar but

smaller events in this country. Then in 1975, Renate Kimbrough of the Centers for Disease Control found that high dosage feedings of Aroclor 1260 (a commercial mixture of highly chlorinated PCBs) caused liver cancer in rats *(7)*. These events spurred a tremendous amount of PCB research which led to their eventual regulation. Since these events, however, studies have shown that the clinical ramifications of the Yusho poisoning were actually due to the polychlorinated dibenzofuran (PCDF) content of the PCB-containing oil.

Surprisingly, or not, as early as 1936, occupational exposure was reported to cause toxic effects and limits were set. But it was not until the Yusho incident that the U.S. FDA conducted surveys to determine the extent and levels of PCB contamination which might find ways into the food chain. In the early 1970s, it was determined that 67% of food packaging and 19% of the food therein contained PCBs —— 75% of infant food cereals were found to be contaminated *(8)*. In 1973, temporary tolerances of PCB levels were established for several classes of food. Under the Toxic Substance Control Act of 1976, the EPA was granted authority to enforce their continued use. As of November 1, 1979, PCBs could no longer be used in new systems, and all equipment containing PCBs was to be phased out by July 1, 1984 *(9)*.

Biological Ramifications. As a biological hazard, chronic exposure tends to be more serious than acute exposure. The toxicity of individual congeners decreases with increasing chlorine substitution at the ortho position *(10),(11),(12)*. The

toxicity of PCB congeners is measured as dioxin-like toxicity, and only those congeners with no or one ortho chlorine and at least tetrachloro- substitution are particularly toxic. PCBs are soluble in human adipose tissue, leach into most biological fluids, particularly blood and milk, and tend to bio-accumulate. Symptoms of exposure include chloracne, fatigue, nausea, vomiting, mild jaundice, abdominal pain, loss of hair, loss of libido, numbness of extremities, headaches and darkening of the skin. Long range effects include neural disturbances such as memory loss, impaired circulation, impaired digestion, bone and joint deformities, morphological changes in adult teeth and poor teeth development in children. Of particular importance is the fact that many symptoms take years to manifest themselves after exposure.

Since the 1975 Kimbrough study showed that high levels of Aroclor 1260 caused liver cancer in rats, several other investigations have supported these findings. It does appear, however, that only highly chlorinated mixtures produce these effects, whereas mixtures such as Aroclor 1242 do not. Three significant conclusions have resulted from these studies. One, the PCB-treated rats, including those with liver tumors, lived significantly longer than the untreated rats. Two, the PCB-treated rats had significantly fewer cancers of all types. And three, the liver tumors, although officially classified as cancerous at that time, did not metastasize to other organs.

Several studies have been made on humans, specifically General Electric Company workers in New York State, exposed to PCBs and PCB-containing substrates. These

epidemiological studies included those done by the National Institute for Occupational Safety and Health (NIOSH) and David Wegman of the University of Lowell. In all cases, no association of PCB exposure and human cancer was found. Dr. Kimbrough, now with the Environmental Protection Agency, has reviewed these studies and has stated "No conclusive evidence thus far reported shows that occupational exposure to PCBs causes increased incidence of cancer." *(7)*

Analytical Significance. From an analytical standpoint, the analysis of PCBs has undergone several changes. In the U.S., most analyses in the past have classified PCBs as total PCBs or as individual or mixtures of Aroclors since Monsanto had been the major producer. Even so, procedures including extraction, clean-up, separation, identification and quantitation have been difficult at best. Now with the interest in individual PCB congeners, the analytical problem is formidable indeed. The future trend will no doubt be in the development of effective and efficient methods for the identification and quantitation of PCB congeners in a wide variety of environmental and biological matrices.

Nomenclature and Composition. Polychlorinated biphenyl isomers (congeners) are named with the numbers of the chlorine substitution (2-6 and 2´-6´) about the biphenyl structure with the two carbons at the linkage being number 1 and 1´——for example, 3,3´,4,4´-tetrachlorobiphenyl *(Figure 2.1)*. Since free rotation is possible about the biphenyl linkage, the 2-position is identical to the 6-position for a mono-substituted ring. Numbering is always done to achieve the lowest possible numbers

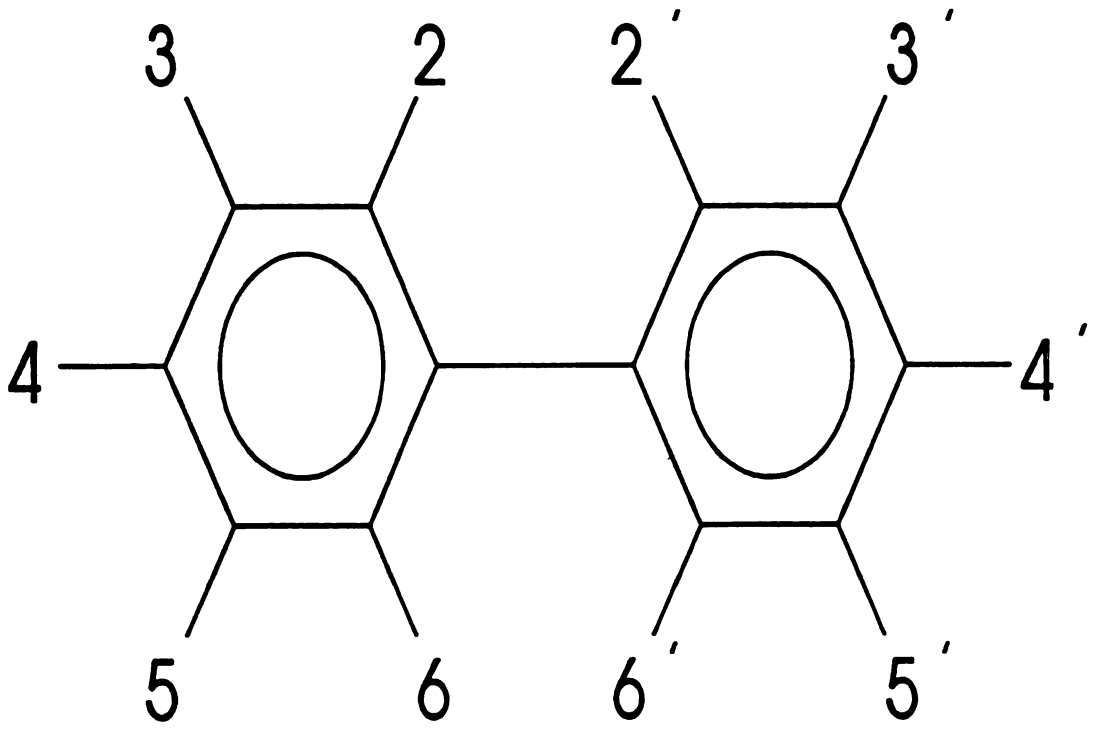


Figure 2.1. The biphenyl structure indicating numbering convention.

with the primed numbers reported second. In addition, each congener has been assigned a number from 1 to 209 *(13)*. This numbering convention has been adopted by IUPAC such that IUPAC #77 is a valid designation for the above-mentioned tetrachlorobiphenyl. The logic for the numbering of all 209 congeners was originally followed for all but two of the 209 congeners *(14)*. This has since been corrected for classification by IUPAC. Nonetheless, early work in the literature often refers to IUPAC #200 as #201 and #201 as #200.

All PCB isomers with the same number of chlorines are classified as belonging to the same homologous series *(Table 2.1)*. This is not strictly a correct use of the term homologous, since a homologous series generally refers to a class of compounds differing only by some structural unit such as $-CH_2$ in $CH_3(CH_2)_nCH_3$ for a series of alkanes. Thus, each PCB isomer (or congener) belongs to one of eleven (including biphenyl itself) homologous series. A homolog, therefore, of a pentachlorobiphenyl is any other pentachlorobiphenyl.

The vast majority of PCBs found in the environment in the United States are from commercial mixtures produced by Monsanto and marketed under the trade name Aroclor. These mixtures were designated as Aroclor *12pp* where the *12* is indicative of the two phenyl rings (12 carbons) and the *pp* is indicative of the percent chlorine by weight in the total mixture such as Aroclor 1242 being 42% by weight chlorine *(Table 2.2) (15)*. Aroclor 1016 is composed of a mixture of PCB congeners with 16% by weight chlorine, but it is a distillation product of another Aroclor and not

Table 2.1. The number of possible congeners for each homolog.

Composition of PCB Homologs			
Empirical Formula	Molecular Weight	Percent Chlorine	Number of Congeners
$C_{12}H_{10}$	154.1	0	1
$C_{12}H_9Cl$	188.0	19	3
$C_{12}H_8Cl_2$	222.0	32	12
$C_{12}H_7Cl_3$	256.0	41	24
$C_{12}H_6Cl_4$	289.9	49	42
$C_{12}H_5Cl_5$	323.9	54	46
$C_{12}H_4Cl_6$	357.8	59	42
$C_{12}H_3Cl_7$	391.8	63	24
$C_{12}H_2Cl_8$	425.8	66	12
$C_{12}HCl_9$	459.7	69	3
$C_{12}Cl_{10}$	493.7	71	1

Table 2.2. Average Aroclor composition by homolog (percent by weight).

Aroclor Composition by Percent Weight							
Homolog (Chlorines)	Aroclor						
	1221	1232	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
10							

from chlorination of biphenyl. Thus, the *10* in Aroclor 1016 is not indicative of ten carbons.

A major focus of the current work is with the toxicity of sediment samples containing PCBs. The twelve (12) PCB congeners that have been classified as exhibiting dioxin-like toxicity *(14)* are listed in Table 2.3. All of these congeners contain either one or no chlorine ortho to the biphenyl linkage. This allows for these congeners to form a conformation where the two aromatic rings are coplanar. Also, all of these congeners contain at least four (4) chlorines.

Table 2.3. Twelve PCB congeners that have been shown to exhibit dioxin-like toxicity.

IUPAC NUMBER	ORTHO CHLORINES	CONGENER STRUCTURE
81	0	345,4-CB
77	0	34,34-CB
123	1	345,24-CB
118	1	245,34-CB
114	1	2345,4-CB
105	1	234,34-CB
126	0	345,34-CB
167	1	245,345-CB
156	1	2345,34-CB
157	1	234,345-CB
169	0	345,345-CB
189	1	2345,345-CB

Chapter 3

Analytical Methodology for Polychlorinated Biphenyls

Introduction. Most of the currently employed methods of analysis for PCBs (and other chlorinated aromatics) involve extensive sample preparation followed by chromatography with one of a wide variety of detectors. The amount and type of sample clean-up is generally dependent on the specificity of the detection scheme used and on the need to protect the cleanliness of the chromatographic apparatus. The various types of detectors used offer a wide range of detection limits and dynamic working ranges, and no one method can be justified for all applications.

This chapter offers a brief literature review of many of the more commonly used methods of analysis for PCBs. PCB analyses may be categorized as one of three types —— total PCBs, Aroclor composition or individual PCB congeners. With the current emphasis on classifying PCB samples with respect to toxicity, the need for congener specific analytical methods is becoming more important. It is this need to which this research is focussed. As such, this discussion of the analytical methodology currently in use for PCBs places emphasis on the inadequacies of these methods with respect to congener-specific analysis. In addition, the specificity of

each method is examined for the required sample preparation.

Sample Preparation. The extent of the preparation of PCB-containing samples is defined by the specificity of the method(s) being used for analysis and by the type of sample. Typically, samples are subjected to extraction with non-polar solvents and extensive clean-up involving separation with adsorbent and/or conventional chromatographic columns. Often, further contaminant removal is done with chemical degradation and precipitation.

Extraction. The extraction of PCBs from solid samples such as soils, sediments and sludges is usually done with a non-polar solvent such as hexane. The most commonly employed extraction method involves the use of a Soxhlet apparatus with continual extraction and distillation for several hours up to overnight. For sludge samples, due to their high organic content, several extraction procedures have been evaluated *(16)* with respect to sample recovery. For Soxhlet extraction, the sludge sample is often mixed with sodium sulfate and extracted with a mixture of dichloromethane and hexane. The efficiency of this method varies greatly with respect to the particle size of the sample due to solvent-sample contact. The best recoveries (>80%) have been reported using centrifuging of an emulsified mixture of the sample with acetone/dichloromethane/hexane *(3)*. In all cases, repetition of the extraction process improves recovery.

With sediment and soil samples, the lower organic content generally allows for

simplified extraction and clean-up procedures. A most important step involves pulverization and thorough mixing of the sample. Soxhlet extraction works exceptionally well for these samples using a variety of solvent mixtures including acetone, dichloromethane and hexane *(3,17-20)*. In almost all cases, recoveries are improved if the sample is first wetted with an agent such as water, acetone or acetonitrile or mixtures of same. Paper samples are similarly extracted, but with KOH saponification as adopted by the Association of Official Analytical Chemists (AOAC) *(21),(22)*.

In recent years, the use of supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE) have been applied to analysis and sample clean-up for many environmental methods *(23),(24)*. The main advantage of SFE for sample clean-up is that the method is very amenable to automation and typically does not require the use of large volumes of organic solvents. Much of the early work with SFE for PCB samples was devoted to those samples containing relatively high concentrations of total PCBs *(25-30)*. More recently, results have been published for the use of SFE for low nanogram per gram levels of PCBs in sediment samples *(31-33)*. An important consideration with SFE is the choice of trapping systems. These include liquid, cryogenic and solid-phase systems. Much of the advantage of using SFE for PCB samples is often lost with liquid or cryogenic systems, however, since extensive clean-up is still required. With solid-phase trapping, extraction, clean-up and concentration for PCB samples may be performed simultaneously *(31)*. An SFE method for PCB-containing sediment samples has also

been developed that includes sulfur removal using elemental copper *(34)*. This allows for the elimination of an additional clean-up step in sample preparation, and also eliminates the need for using elemental mercury which has other environmental ramifications.

With aqueous samples, classical liquid-liquid extraction with a non-polar solvent (hexane, dichloromethane) is commonly employed *(35)*. For many samples, prior to extraction, adjusting the pH of the samples offers no advantages. For paper mill effluents, extraction followed by KOH saponification provided for best recoveries *(36),(37)*. Other extraction techniques for aqueous samples include continuous liquid-liquid extraction (CLE) *(38),(39)*, sorbent column extraction *(40-43)* and purge-and-trap techniques *(44)*. In addition, the sorbent column extraction technique is commonly employed in the field as a sampling technique.

In the interest of completeness, extraction techniques for air and biological samples are also presented. Air samples are usually acquired with a sorbent material such as polyurethane foam, Florisil or XAD-2 *(3)*. The sorbent matrix is then extracted with a non-polar solvent system. Blood samples are typically extracted with liquid-liquid partitioning according to the protocols of the U.S. EPA *(45)* or the U.S. Centers for Disease Control *(46),(47)*. Adipose tissue and food substances may be extracted with petroleum ether and then the PCBs extracted from the petroleum ether with acetonitrile *(48)*. This method may also be used for fish samples.

Milk, both bovine and human, is similar in fat content to adipose tissue and may be extracted similarly as outlined by AOAC *(49)*. Other biological samples routinely extracted for analysis for PCBs include eggs *(50)*, bird tissue *(51)*, earthworms *(52)* and plant tissue *(53)*.

The determination of PCB content in oils has been of analytical significance because the major commercial use of PCBs was as transformer and related oils. Also, the Yusho incident (see Chapter 2) prompted interest in oils used in animal and human food sources. For most oil samples, simple dilution with hexane or other non-polar solvent systems is the most common technique as outlined by EPA methods *(54)*.

Regardless of the sample under consideration or the analytical methods of detection being employed, extraction or dilution with or into a non-polar solvent system is necessary. Even with the MS/MS method involved in this research, placing a raw sample directly into the source could conceivably interfere with maintaining a constant and reliable flux of thermal electrons in the ECNI plasma.

Clean-up. After extraction or dilution of the sample, the amount and extent of sample clean-up is dependent on the specificity of the analytical method, the detection scheme employed and on the type of analysis —— total PCBs, Aroclor composition or identification and quantitation of individual congeners. These clean-up steps often include back-partitioning with solvents, column elution and chemical

treatment. Of course, the extent of this clean-up is also dependent on the matrix effects inherent in the type of sample being analyzed.

Back-partitioning of many samples is employed as a clean-up and extraction procedure. The most common procedure is with acetonitrile partitioning. In this classic procedure *(45)*, the PCBs are extracted from the non-polar solvent system with acetonitrile. After dilution with water, the PCBs are partitioned back into the non-polar solvent by aqueous dilution of the acetonitrile extract. This method allows for the removal of many of the polar components from the extracted sample. Other partitioning techniques have also been employed *(55)*.

Perhaps the most common clean-up technique is the use of adsorbent column chromatography. Adsorbents include Florisil *(22),(45),(56)*, silica gel *(57-60)*, alumina *(61)* and carbon *(62),(63)*. Carbon column techniques are also used to separate PCB congeners with no ortho chlorines from those with ortho chlorines *(64)*. Other column clean-up techniques include HPLC *(65)*, thin layer chromatography (TLC) *(3)* and gel permeation chromatography (GPC) *(66)*.

Chemical methods of clean-up include degradation and sulfur removal. Commonly used chemical methods include acid degradation *(65,67-70)*, chromic trioxide oxidation *(71)* and saponification *(72)*. Sulfur removal from sample extracts is typically done by shaking with elemental mercury *(73)*, treatment with

tetrabutylammonium sulfite *(74)* or treatment with cyanide *(75)*.

For GC/ECD analysis of PCBs, most samples are subjected to extraction (or dilution), back-partitioning, adsorbent column elution and sulfur removal. All of this clean-up is required due to the lack of specificity of the detector. For GC/EI-MS methods, most of these procedures are still followed in an effort to keep the chromatographic apparatus clean. For direct insertion EI-MS methods for some targeted compounds, some of these steps may be reduced or eliminated. This still requires much method development for each sample matrix encountered, though since high background ion current may be detrimental. For other modes of MS ionization, background levels are again dependent on the sample matrix. For direct insertion MS/MS methods, simple extraction (or dilution) may be the only requirement as a screening method. For the current work, however, all samples used were subjected to the same clean-up procedures for purposes of comparison to the methods used by collaborators.

Non-chromatographic Methods. Many non-chromatographic techniques have been used for the analysis of PCBs. Generally, though, none of these techniques possess the specificity and/or sensitivity to be of use quantitatively for trace PCB determinations. Nuclear magnetic resonance (NMR) has been used to characterize the chemistry of PCB congeners *(76-78)*, but has not been used for the quantitative analysis of real samples. Infrared (IR) *(79-81)* and Fourier transform infrared (FTIR) *(82)* spectrometry have been employed as screening techniques for total

PCB content. FTIR has also been used in the field for determining the PCB content of transformer oils *(83)*. While some of these techniques have been used as chromatographic detectors (FTIR), most are used for confirmation of other techniques.

Chromatographic Methods. The majority of chromatographic methods for the analysis of PCBs has been with gas chromatography. Other techniques, though, have included high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and hyphenated methods such as GC/GC. These methods may be used for sample clean-up and/or determination.

Gas Chromatography. Both packed column (glass or stainless steel) and wall-coated open tubular (WCOT - typically fused silica) gas chromatographic with a wide variety of detectors have been employed for the trace analysis of PCBs. Packed column GC has been the most widely used technique, and several reviews have appeared in the literature *(84-89)*. One work investigated 13 different GC stationary phases for observed theoretical plates and overall PCB resolution *(90)*. It was calculated that of the 21,945 possible pairs of PCB congeners, 465 would be indistinguishable using the best column available. It would take multiple analyses using at least five columns to resolve all possible pairs.

Increasingly, high resolution (WCOT) GC has become the method of choice for PCB analysis due to the extremely high number of theoretical plates (up to 100,000)

and hence, resolution available *(76,77,90-93)*. For congener specific analysis, high resolution GC is required. The majority of methods have used methyl silicone, 95% methyl/5% phenyl silicone or 94% methyl/5% phenyl/1% vinyl silicone as stationary phases. A comparison of packed column GC to capillary column GC has been presented in the literature *(94)*.

The most common detectors used with GC for PCB analysis are ECD and MS. Mass spectrometric detection is discussed later in this chapter. The electron capture detector (ECD) is selective for analytes such as halogenated compounds and is generally the most sensitive of all GC detectors for PCBs *(95)*. Other halogenated compounds will interfere in a PCB analysis, as will some esters, nitrogen-containing compounds and sulfonated compounds. With the use of an ECD, extensive sample clean-up is necessary to eliminate many of these interferences. Even so, for some samples an ECD is not suitable for PCB detection *(96)*. Mullin has determined the relative responses to an ECD of all 209 PCB congeners *(77)*.

Other GC detectors used for PCB analysis include the Hall electrolytic conductivity detector (HECD), the flame ionization detector (FID), the photoionization detector (PID) and the thermal conductivity detector (TCD). While the HECD is selective for chlorinated and brominated compounds, the other detectors are generally regarded as universal detectors, and as such, are not as selective (or sensitive) as an ECD. The literature contains many reviews of the relative merits of the various GC

detection methods. Pellizzari has reviewed the GC detectors used for PCB analysis *(95)*.

High Performance Liquid Chromatography. High performance liquid chromatography (HPLC) may be normal phase or reverse phase. Normal phase HPLC consists of using a non-polar solvent system such as hexane and a polar stationary phase such as alumina or silica. With reverse phase HPLC, a polar solvent system (water, methanol, acetonitrile, etc.) is used with a non-polar stationary phase such as chemically bonded C_{18} - silica gel. Reverse phase HPLC is more commonly used for PCBs. Commonly used HPLC detectors in increasing order of selectivity are ultraviolet, fluorescence and electrochemical. HPLC is primarily used for PCB determination when combined as part of the clean-up procedure *(97),(98)*. The major limitation of HPLC as a routine method for PCBs is that none of the commonly employed HPLC detectors are as sensitive or selective as those used with GC.

Hyphenated Methods. Several investigators have used GC/GC or "heart cut" methods where the effluent of one column is redirected onto a second column using a different stationary phase *(14),(99),(100)*. With this method, Schulz, et. al., were able to resolve all detectable congeners of several commercial mixtures of PCBs. Since this technique is not commercially available, though, this could not be termed a routine method.

Other Chromatographic Techniques. Other chromatographic techniques used for PCB analysis include thin layer chromatography (TLC) *(101),(102)*. TLC is most commonly used as a method for analyzing food samples for PCBs and as a qualitative confirmation technique for other methods. Perchlorination techniques are actually modified GC methods where all congeners are exhaustively perchlorinated in-line and the resultant decachlorobiphenyl content detected *(103)*. The major problem with this technique is that biphenyl itself is detected, and generally high blanks result. A technique just the opposite of perchlorination is termed carbon skeleton chromatography where all congeners are dechlorinated in line and the resultant biphenyl content is detected *(104-106)*. The same problems with perchlorination techniques are inherent in this method.

Mass Spectrometric Detection. The use of mass spectrometry for PCB analysis has generally been limited to gas chromatographic detection. The modes of MS usually employed are electron impact (EI) and electron capture negative ionization (ECNI). EI methods provide structural information for purposes of identification, but ECNI methods are generally two to three orders of magnitude more sensitive. Positive chemical ionization (PCI) may also be used with some improvement in selectivity over EI, but is generally employed only in situations where ECNI is unavailable. As tandem techniques (MS/MS) become more routine, methods are developed for PCB analysis by MS/MS due to enhancements in selectivity. MS/MS methods may be applied to any of the ionization modes, and these methods are categorized as one of two types —— collisionally-induced (or activated) dissociation (CID or CAD) or

ion-molecule reactions. Any of these methods may be used as gas chromatographic detection schemes or used with direct sample introduction such as with a solids probe. Additionally, with the advent of ionization modes such as electrospray (ESI) and atmospheric pressure chemical ionization (APCI), HPLC/MS methods may become more routine.

The choice of mass spectrometer is usually made with regard to analytical needs and to cost. A simple GC/EIMS system may cost as little as $50,000 USD, and a high resolution double-focussing sector instrument with many modes of sample introduction and ionization may cost close to $1,000,000 USD. GC/MS systems with chemical ionization and both positive and negative ion polarity modes are readily available but at higher costs than that of EIMS systems. If available, high resolution mass spectrometry can provide much more information than low resolution instruments particularly with regard to possible interferences. For example, high resolution MS would be needed to distinguish between the hexachlorobiphenyl ion $C_{12}Cl_6H_4$, m/z 357.84443, and the pentachlorodibenzo dioxin ion $C_{12}O_2{}^{35}Cl_3{}^{37}Cl_2H_3$, m/z 357.851662. A low resolution MS instrument is typically tuned such that at m/z 358, the resolution (< 5% valley) calculated as $m/\Delta m$ where m and Δm are the m/z values of two adjacent peaks in the mass spectrum is 358. In order to resolve the two aforementioned ions then, the resolving power of the instrument must be at least 49,480. Using this method of calculating resolution, with a quadrupole instrument, the peak shape remains constant across the mass range, but the resolution increases with m/z. With a high resolution sector instrument, the resolution is fixed by slit

widths and focussing and remains relatively constant across the mass range, but the peak widths broaden with increasing m/z.

Electron Impact Ionization. GC/EIMS has become a routine method for the analysis of PCBs *(78,83,107-109)*. While analytical response varies from instrument to instrument, the reproducibility from day to day is actually often better than that of an ECD. Using full scan modes of operation, EIMS spectra provide much more information than other modes of detection but at limits of quantitation (LOQ) up to four orders of magnitude higher than that of an ECD. As such, this technique is often used for confirmation for ECD methods. Using single ion monitoring (SIM) or multiple ion detection (MID) modes, much information is sacrificed but at an improvement in LOQ of up to two orders of magnitude. Many other limited scan range techniques have been proposed *(91),(110),(111)*.

Differences in response are generally observed between sector, time-of-flight (TOF) and quadrupole mass spectrometers. Since there are many libraries of EI spectra available, it is important that data are generated under standard conditions. Most EI data are acquired using electron energies of 70 eV. Also, instrument tunes are generally adjusted according to the spectral characteristics of a calibration and tuning compound such as decafluorotriphenylphosphine (DFTPP). An alternative to this approach, is for the analyst to generate his or her own library of MS data with standards for comparison with sample data.

Positive Chemical Ionization. By introducing a moderating gas such as methane into ion source of a mass spectrometer, a plasma is generated that allows for the protonation of analyte molecules (see Chapter 4). These processes, positive chemical ionization (PCI), are much "softer" in that little fragmentation is observed. For many analytes, this is a complementary technique in that PCI provides molecular weight information and EI provides structural information. For PCBs, however, this is not much of an advantage since the molecular ion is of significant abundance for all congeners. Also, PCI is not as reproducible as EI. Nonetheless, several researchers have used the PCI technique for the screening and/or determination of PCBs *(107,112-118)*.

Electron Capture Negative Ionization. Using the same plasma of methane ions as in PCI, a good source of thermalized electrons is produced. Under these conditions, good electron-capturing analytes such as PCBs may be analyzed by electron capture negative ionization (ECNI - see Chapter 4) *(62,119-125)*. Basically, the same ionization processes occur in ECNI as in ECD modes of detection. Also, as with PCI techniques, instrumental parameters are more critical in maintaining reproducible results. Operated in a SIM or MID mode (m/z 35, m/z 37 and/or the molecular anion), ECNI analysis of PCBs approaches the detection limits of an ECD *(126)*. And, as with an ECD, responses of the 209 PCB congeners varies over several orders of magnitude. Since this method offers an enhancement in selectivity over that of an ECD, some of the sample clean-up may be eliminated *(127)*.

Tandem Mass Spectrometry. By selecting a specific ion (cation or anion) produced in the source, reacting this ion in a collision cell and mass selecting the resultant product or products, an MS/MS spectrum is produced. This technique is necessarily one of the most selective analytical techniques available *(128),(129)*. In addition, the reaction spectra allow one to regain the structural information lost when more sensitive modes of ionization such as chemical ionization are used *(124),(130)*. The majority of early MS/MS work done with PCBs has involved collisionally induced dissociation (CID) *(131)*. Even with the enhanced selectivity, CID MS/MS is not very useful for distinguishing positional isomers.

Neither the ECD nor the MS detectors are totally specific for halogenated species; the ECD responds to many electron-capturing species besides chlorine, and MS responds to virtually all hydrocarbons often present in real samples. MS/MS, on the other hand, generally provides extremely high chemical selectivity. A GC/MS/MS method could easily be restricted to chlorinated biphenyls; at worst these interferences can be identified and compensated for *(132)*. It has been shown with GC/MS/MS that the analyte fragments can be identified with little interference *(133)*. This technique as implemented, though, was not used for quantitation. Attempts have been made to use direct-probe MS/MS for PCB analysis, but again quantitation was difficult and yielded erroneously high results *(134)*.

None of the MS techniques have been very helpful in resolving positional isomers of molecules *(135),(136)*. MS/MS techniques, however, hold some

promise for alleviating this problem *(137)*. For example, PCB isomers have been distinguished by GC-MS/MS using oxygen-enhanced negative chemical ionization (ONCI/MS/MS) by Guevremont and co-workers *(138)*. In this work, congeners of the same molecular weight and similar retention times were shown to produce significantly different precursor-to-product fragmentation patterns resulting in the relative abundances of each being distinguishable for the co-elutants. Several rules for the identification of congeners were then postulated based on the stability of precursor ions $[M\text{-}Cl+O]^-$ with respect to chlorine position (and/or hydrogen position) about the biphenyl structure. The development and application of such rules would allow the possibility for the identification of PCB congeners and their relative concentrations even if two isomers co-elute. Further development of this relative intensities method using other modes of ionization and other MS/MS techniques has yet to be explored.

In most cases, positional isomers do not yield unique precursor-product ion combinations but rather different intensities of the same combinations. At a given collision energy, then, it may be possible to distinguish one positional isomer from another based on the ratio of two product ions. The same difference in product ion intensities, however, may also be induced by varying instrumental parameters such as ion source voltages and temperature. Thus, the use of such ratios is rarely reliable and reproducible as an analytical method. In these cases, isomer-specific ion molecule reactions with MS/MS shows some promise with regard to the characterization of positional isomers even without GC separation. Some reactions

have been shown to be highly selective with regard to positional substitution. Preliminary results obtained from reactions of the brominated analogs (PBBs) with nitrogen, ammonia and deuterated water clearly distinguish the isomers with respect to bromine position *(139)*. For example, resultant spectra for 2,2'-dibromobiphenyl and 4,4'-dibromobiphenyl using the reaction of the negative molecular ions with D_2O are clearly distinguishable. This allows the possibility for developing rules (and hence software) for these reactions based on chlorine position on the biphenyl structure.

Data Reduction. Many methods have been used to process the MS data produced in the analysis of PCBs. Most of these, however, have been used to characterize samples as to total PCB content or Aroclor content. For the sake of completeness, a few of these are presented.

One of the most prominent quantitation techniques for PCBs was originated by Webb and M^cCall for packed column GC/ECD methods *(140)*. In this technique, samples are quantitated as total PCB content or as the content of specific Aroclors for the case of mixtures of Aroclors. All PCB peaks are identified by retention times relative to that of *p,p'*-DDE, and a flow chart *(Figure 3.1)* was developed for quantitating samples with mixed Aroclor patterns.

Most GC peaks that contain more than one component are not precisely co-eluting but rather "overlapping" with maximum concentrations at slightly different times. In

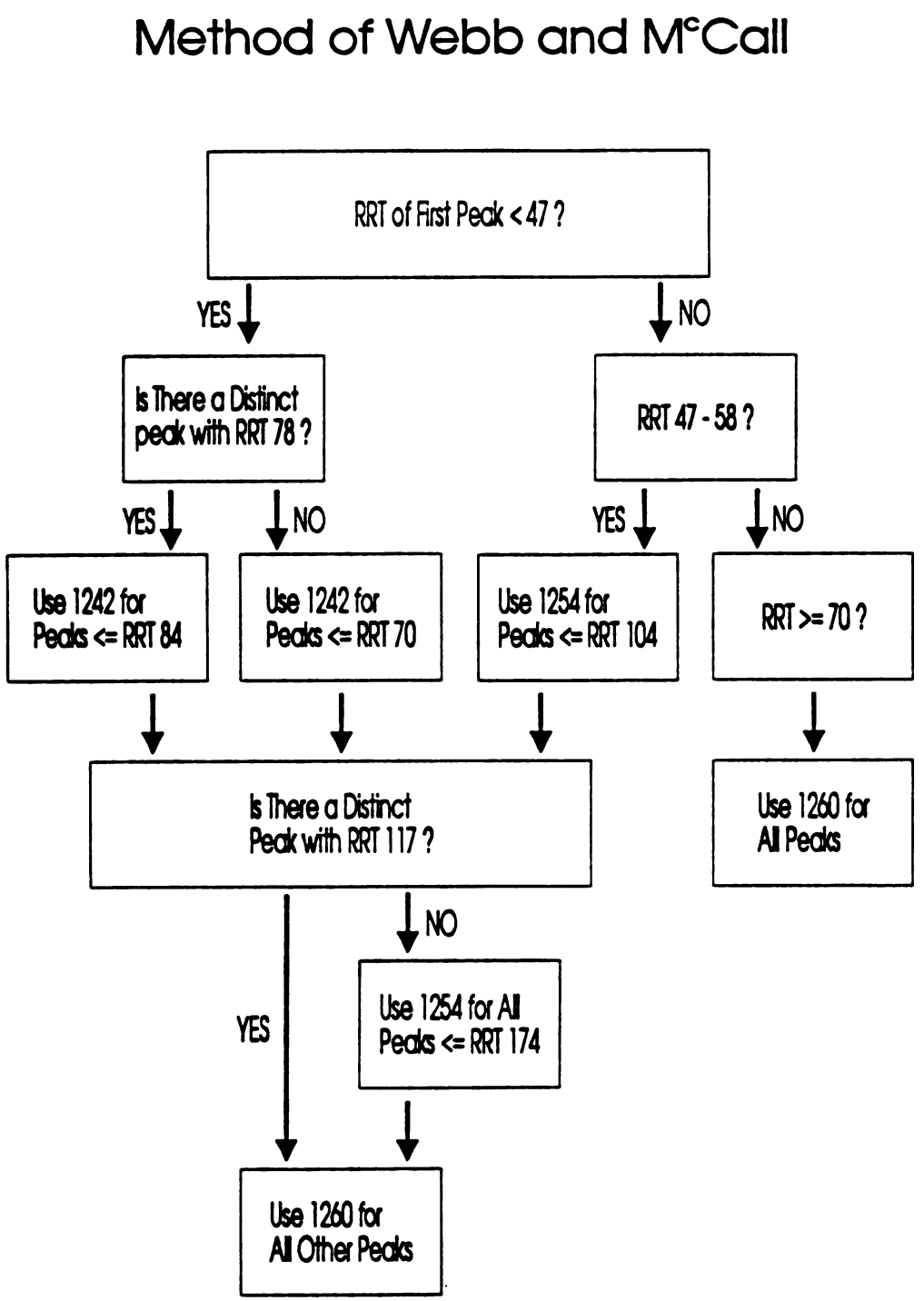


Figure 3.1. A flow chart (method of Webb and McCall) for the quantitation of samples with mixed Aroclor patterns.

these cases it is possible to produce clean MS spectra for each component effectively improving peak recognition over the observed GC resolution by an order of magnitude. Most MS spectra obtained in the course of a GC/MS run are a superposition of several individual component spectra. By examining each spectrum it is possible to identify the individual component spectra by testing the intensity of each peak for synchronicity with the others. In this way, components can be identified which do not even have sufficient concentration to appear on the total ion current mass chromatogram. The identification of structural isomers which have similar spectral masses at different relative intensities is sometimes possible by comparing ratios of the intensities of fragment ions. This method for extracting the necessary information from the GC/MS database has been shown to be feasible and effective and has only recently been implemented in modern data systems.

Biller and Biemann generated reconstructed mass spectra and mass-resolved gas chromatograms from total ion current (TIC) mass chromatograms *(141)*. By plotting m/z versus relative intensity for each scan number for only those ions which exhibit their maximum intensities in that scan, a clean spectrum is produced which is free of interference from the co-elutant. Also, by then regenerating the mass chromatogram from the reconstructed mass spectra, much higher resolution is achieved than from a TIC plot since the leading and tailing edges have been discarded. The reconstructed mass spectra for samples spiked with known compounds have been subjected to automated identification with library data-bases with excellent results. Also, Blaisdell and Sweeley have developed the algorithms

used in clean-up methods for MS data fields *(142)*, and Gates and workers have developed a computer system for quantitative GC/MS *(143)*. It would be of use to develop the software compatible with existing data systems to analyze the data from standard GC/MS experiments for synchronicity of MS spectra. This would include an improvement over current methodology with new smoothing and correlation techniques. The result would be the production of a modified file of mass spectra versus chromatographic time in which the mass spectra are essentially free of background noise and random mass peaks and in which the chromatographic time resolution is improved by approximately ten-fold.

Several workers have reported using regression techniques for both GC/ECD *(144)* and GC/MS *(145)* analyses of Aroclors and their mixtures. Results of such techniques have ranged from fair to good. By identifying up to 40 GC peaks from several representative Aroclors, a matrix is generated for each sample, peak by peak, as linear combinations of known Aroclors. In addition to this multiple regression analysis (MRA), other more sophisticated classification schemes have been reported *(146),(147)*.

Summary. In this chapter, many of the commonly employed methods of analysis for PCBs have been presented. All of these methods have limitations with respect to cost and ease of implementation. In addition, as alluded to earlier, most have moderate to severe limitations with respect to congener specific analysis. It was toward these limitations that the research presented in this work was directed.

Chapter 4

Exchange of Oxygen for Chlorine in Molecular Anions of Chlorinated Aromatics in a Tandem Mass Spectrometer

The products of the reaction of PCB molecular anions with molecular oxygen were monitored as a detection scheme for chromatographically-separated PCB congeners in an MS/MS experiment. In order to develop this method, the effects of several instrumental parameters on this reaction were studied. These parameters included the choice of reagents and pressures for both the ion source, and the collision cell and the energy of the reaction as controlled by the collision cell offset voltage. Also studied were the effects of translational kinetic energy correction, electron energy, and source and manifold temperatures on ionization and product formation.

Experimental. The Aroclor standards were provided by John Quensen, and all other individual congeners and standards were provided by George Frame. All standards were prepared by dilution with Organic Residue Analysis grade hexane (J.T. Baker).

Gas Chromatography. All gas chromatographic methods were performed with a Varian 3400 gas chromatograph equipped with a 30 meter x 0.250 mm column of

0.25 mm DB-1 phase (methyl silicone, J & W Scientific). Helium was used as the GC carrier gas. The Varian 3400 was equipped with an injector body that accommodates both split and splitless modes of injection. All experiments were performed using the splitless mode of injection.

Mass Spectrometry. Early MS and MS/MS experiments were done with a Finnigan TSQ-70B mass spectrometer operated in a negative ion mode. Later, the TSQ instrument was upgraded to a TSQ-700. The pertinent changes included the installation of an octapole as a collision cell (the TSQ-70 instruments use a quadrupole) and replacing the PDP-11/73 data system with a DEC Station. Ammonia, methane, argon or deuterated ammonia were used as chemical ionization reagent gases.

Generation of the M^- Ion. As a prelude to a discussion of negative ion formation in MS, a discussion of positive chemical ionization is presented. It is important to note that those instrumental parameters such as ion source pressure and electron energy must be set differently for all three modes of classical ionization——electron impact, positive chemical ionization and negative ion production under high pressure ionization conditions.

Positive Chemical Ionization. Ions formed by true chemical ionization are those ions that result from secondary and tertiary ionization processes. Generally, for positive chemical ionization (PCI), the analyte molecules react with an abundance of reagent

gas ions produced by electron impact ionization of the reagent gas *(148)*. When methane is used as the reagent gas, the primary reagent gas ions produced are:

$$CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2e^- \qquad m/z\ 16$$

$$CH_4^{+\cdot} \rightarrow CH_2^{+\cdot} + H_2 \qquad m/z\ 14$$

$$CH_4^{+\cdot} \rightarrow CH_3^{+} + H^{\cdot} \qquad m/z\ 15$$

$$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^{+} + {}^{\cdot}CH_3 \qquad m/z\ 17$$

$$CH_3^{+\cdot} + CH_4 \rightarrow C_2H_5^{+} + H_2 \qquad m/z\ 29$$

The major analyte ions produced are then:

$$M + CH_5^+ \rightarrow [M+H]^+ + CH_4 \qquad [M+1]^+$$

$$M + CH_5^+ \rightarrow [M+CH_5]^+ \qquad [M+17]^+$$

$$M + C_2H_5^+ \rightarrow [M+C_2H_5]^+ \qquad [M+29]^+$$

While there may be the formation of free protons with these processes, their abundance is probably limited due to the high abundance of species with significant proton affinities. As such, the formation of protonated analytes from association with free protons is generally ignored as insignificant compared to other processes. Fragmentation observed with PCI generally includes elimination of a functional group X together with a hydrogen atom from the protonated molecule. This process typically involves transfer of a proton from a reagent ion to a specific site (such as a heteroatom) on the sample molecule *(149),(150)*. This process may be summarized as:

$$AX + RH^+ \rightarrow R + AXH^+ + HX^+$$

where AX is the sample molecule, X is the functional group or heteroatom and RH^+ is the reagent ion. Other fragmentation reactions observed with PCI include hydride abstraction *(150-157)* and electrophilic attack of a carbon/carbon single bond by a proton with the resultant cleavage of the sigma bond *(154),(158)*.

Optimum PCI conditions generally exist when the reagent gas pressure in the CI ion volume is approximately 1 Torr. This roughly corresponds to 5-10 Torr on the ion source Convectron gauge or 5-10 x 10^{-3} mTorr on the ion gauge. The optimum pressure for analyte ion production is ***not*** the pressure that results in the highest abundance of reagent gas ions. At higher reagent gas pressures, reagent gas ions become more abundant, but the abundance of analyte ions ***decreases*** as their formation and the ability to be extracted from the ion source is suppressed.

While EI tuning compounds (such as PFTBA - FC43) are used to tune the instrument for positive chemical ionization, the tuning compound ions are ***not*** the result of chemical ionization. Although the higher mass PFTBA ions are generally more abundant with PCI, the same ions are observed with PCI as with EI. If one wishes to optimize the CI reagent gas pressure by monitoring the abundance of an analyte ion that undergoes PCI, the analyte must be introduced in some steady state fashion such as via a micro valve in plumbing connected to the solids probe.

Positive chemical ionization is generally a *softer* ionization technique than EI in that much less fragmentation is observed. The choice of reagent gas is analyte dependent. Those analytes that have proton affinities less than that of the reagent gas will not become protonated, but may form positive adduct ions. For analytes having a proton affinity greater than that of the reagent gas, the analyte becomes protonated, and quite often, the base peak represents the protonated molecule $[M+H]^+$. As such, PCI is a complementary technique to EI. Molecular weight information is provided by PCI, and structural information by EI.

Electron-Capture Negative Ionization. Negative ions are produced in all modes of ionization including EI. Generally, though, molecular anions are produced in a significant abundance only in the presence of thermal electrons, which can be provided by a significant amount of a moderating or reagent gas. While positive ions observed under CI conditions are usually adducts of the neutral analyte molecule with protons or protonated species, almost all negative ions observed with high

moderating gas pressures are the result of the capture of a thermalized electron or electron capture negative ionization (ECNI). There are, however, five prominent types of negative ion production *(149),(159),(160)*:

$M + e^- (< 1\ eV) \rightarrow M^-$ Resonance Electron Capture

$MX + e^- (< 15\ eV) \rightarrow M^- + X\bullet$ Dissociative Electron Capture

$MX + e^- (> 10\ eV) \rightarrow M^- + X^+ + e^-$ Ion-pair Formation

$M + X^- \rightarrow MX^-$ Negative Ion-molecule Reaction

$M + X^- \rightarrow M^- + X$ Charge-exchange Reaction

Notice that only the negative ion-molecule reaction is analogous to PCI, and thus, could properly be called NCI. The vast majority of negative ion processes observed are a result of one of the first two processes which produce M^- or $[M-H]^-$. Nonetheless, all negative ion processes are quite often categorized (incorrectly) as negative chemical ionization or NCI. Notice, also, that the electrons involved in these processes are not those that result directly from the higher energy filament emission. The thermalized electrons that are involved in electron-capturing processes are 1) those electrons released during the ionization of the moderating gas and 2) those electrons emitted from the filament that lose most of their energy

during collisions with molecules in the relatively high pressure of the ion source. In addition, unlike in PCI, since the ions observed during tuning *are* the result of the same ionization processes by which analyte ions are produced, these ions may quite often be used for the optimization of all instrumental parameters. The ions observed (M^- and $[M-X]^-$) with both analyte and tuning compounds are the result of resonance or dissociative electron capture, and as such, optimizing instrumental parameters such as moderating gas pressure with the tuning compound should also allow for optimum conditions for analyte anion production.

Electron capture negative ionization is generally one of the *softest* ionization techniques available in mass spectrometry. The molecular anion is almost always represented by the base peak, and very little fragmentation is generally observed. As such, ECNI is usually the most sensitive technique available for those analytes amenable to these processes. Generally, any species that can be detected by electron capture detection (ECD) is amenable to ECNI-MS. These include halogenated compounds, sulfonated compounds, many nitrogen containing compounds (particularly those with basic nitrogens such as amines), many oxygen containing compounds, and some aromatic and polyaromatic compounds. While this work is primarily concerned with chlorinated aromatic compounds, other electron-capturing compounds must not be overlooked as possible interferences.

A major difference between ECNI and other ionization techniques, is that these low-energy processes are much more susceptible to variations in source conditions. Small

voltages or charging phenomena on source elements can drastically compromise the instrument tune. Also, it is quite possible to saturate the ionization capacity of the source (depletion of thermalized electrons) without saturating the analyzer or detector. With ECNI, very little fragmentation is observed in the primary mass spectra of PCB congeners *(161),(162)*. Generally, disregarding chloride ions, the most abundant ion produced is in the molecular ion region of the spectrum. This ion may be the $[M]^-$ or $[M-H]^-$ ion or the $[M+2]^-$ or $[M+4]^-$ ions in the case of the more chlorinated species due to the presence of ^{37}Cl isotopes (taking M as the PCB with only ^{35}Cl). Also observed in the primary mass spectrum of a PCB congener is the isotopic cluster at -19 u (^{35}Cl-^{16}O or 35-16) due to the oxygen-chlorine exchange reaction products formed in the source from the molecular anions. These ions are usually ten to thirty percent as abundant as the molecular ions. The molecular ions can also lose a chlorine to form the isotopic cluster at -35 u, but this region is generally only one to twenty percent as abundant as the molecular ion region. This does not imply that the loss of chlorine from a molecular ion is not favorable, only that the charge may stay with the chlorine as opposed to the biphenyl structure. Rarely are any other ions observed with relative abundances more than a few tenths of a percent. Thus, only ions from these three regions are logical choices as precursors in an MS/MS experiment. This is shown in Figure 4.1, which is the ECNI mass spectrum of a tetrachlorobiphenyl.

Negative ion formation, particularly in the case of chlorinated aromatic compounds, is generally due to the capture of a thermal electron produced during the ionization

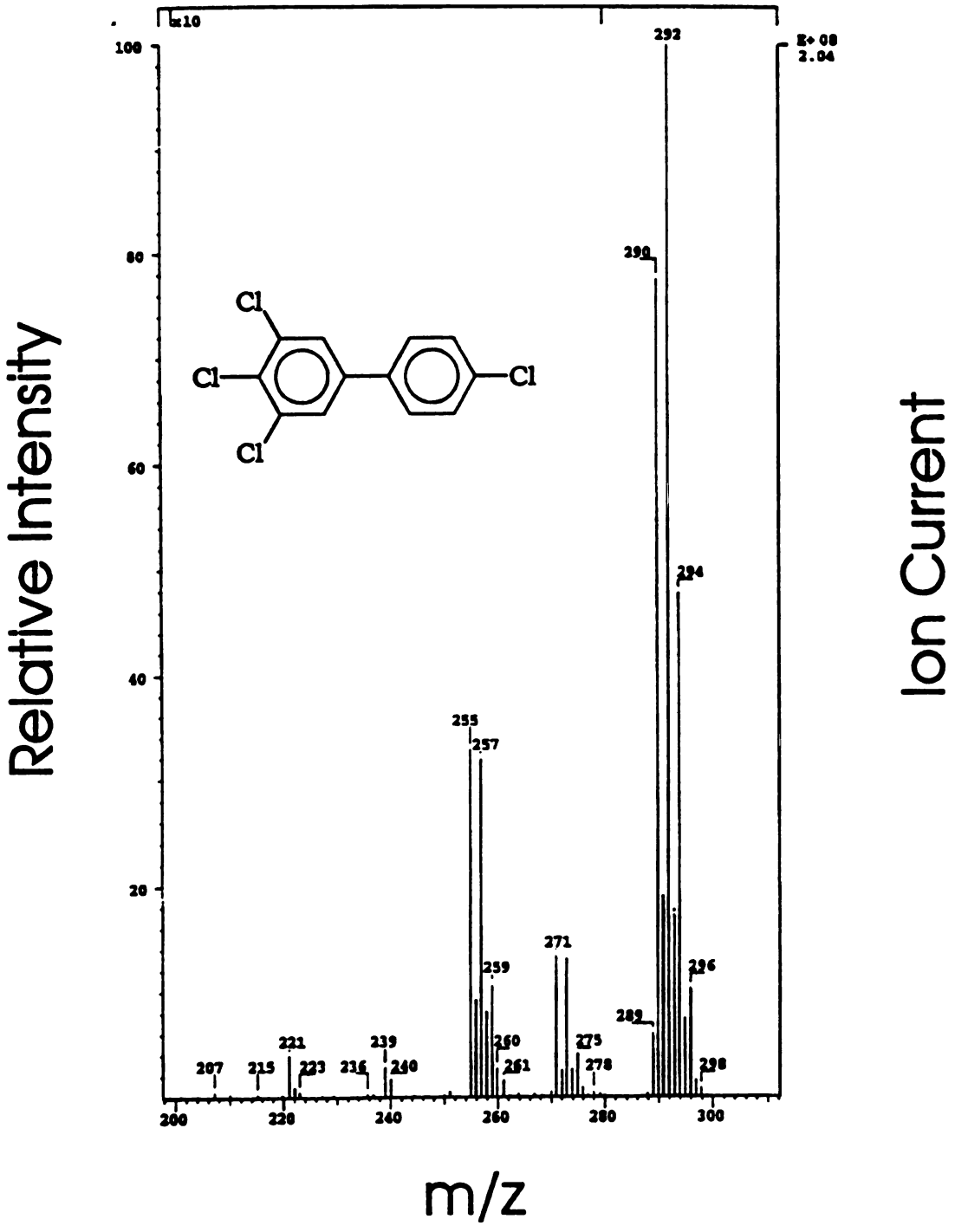


Figure 4.1. The ECNI mass spectrum of a tetrachlorobiphenyl. The three regions of interest are the molecular ion region, the oxygen-for-chlorine exchange reaction region and the loss of chlorine from the molecular ion region.

of the moderating gas in the source and may proceed by one of the two electron-capturing processes. For this work, the two ionization products of interest are the $[M]^-$ and $[M-H]^-$ ions. Whether one or the other or both are produced is a function of the electron affinities of the species involved. The major difference in these two species other than mass is that $[M]^{-\cdot}$ is a radical anion and $[M-H]^-$ is not. It has been previously reported that for PCBs, those species with five or more chlorines generally yield $[M]^{-\cdot}$ ions as a result of resonance electron capture and those with four or fewer chlorines yield $[M-H]^-$ ions as a result of dissociative electron capture *(163),(164)*. In the present work, resonance electron capture has also been observed with a few trichlorobiphenyls and all the tetrachlorobiphenyls, producing a significant amount of the $[M]^{-\cdot}$ ion. In Figures 4.2 through 4.5 are shown the mass spectra of selected tetra- through heptachlorobiphenyls. As is shown, the predominant ions are molecular anions $[M]^{-\cdot}$ with nominal masses of m/z 290, m/z 324, m/z 358 and m/z 392. The anion $[M-H]^-$ is significant only with the tetrachlorobiphenyl. For each of these, though, the ratio $[M]^{-\cdot}/[M-H]^-$ could be estimated from the excess abundance of $[M+1]^-$ over 13.1%.

Ion Source Optimization for ECNI. The major parameters to be considered for generating negative ions are moderating gas pressure, type of moderating gas and filament electron energy. Since the primary ionization processes involve the interaction of analyte molecules with thermalized electrons, the generation of a large abundance of thermal electrons is desired. This usually requires that the electron energy be lower than that used for EI or PCI and the moderating gas pressure to be

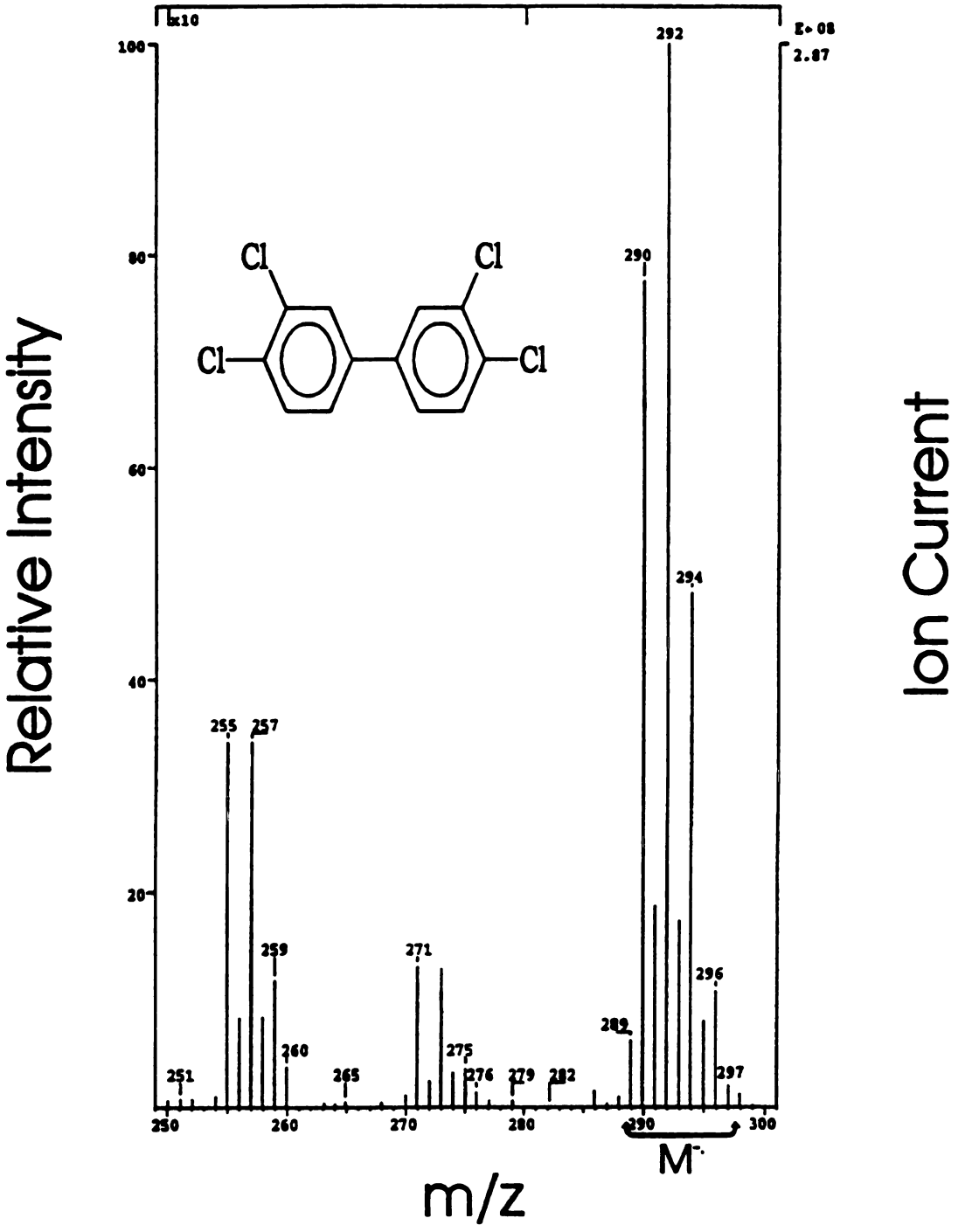


Figure 4.2. The ECNI mass spectrum of a tetrachlorobiphenyl indicating the $[M]^{-\cdot}$ radical ions.

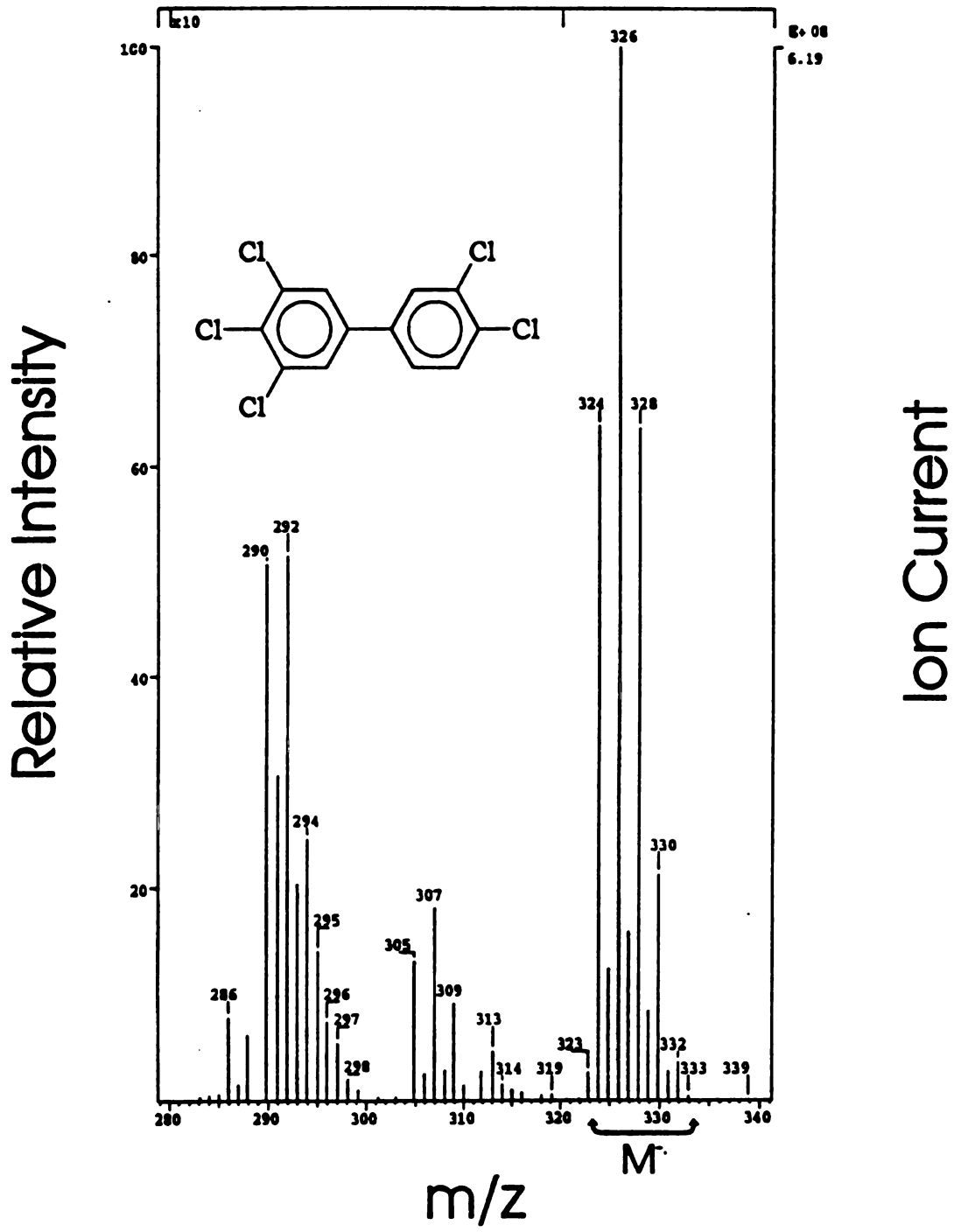


Figure 4.3. The ECNI mass spectrum of a pentachlorobiphenyl indicating the $[M]^{-\cdot}$ radical ions

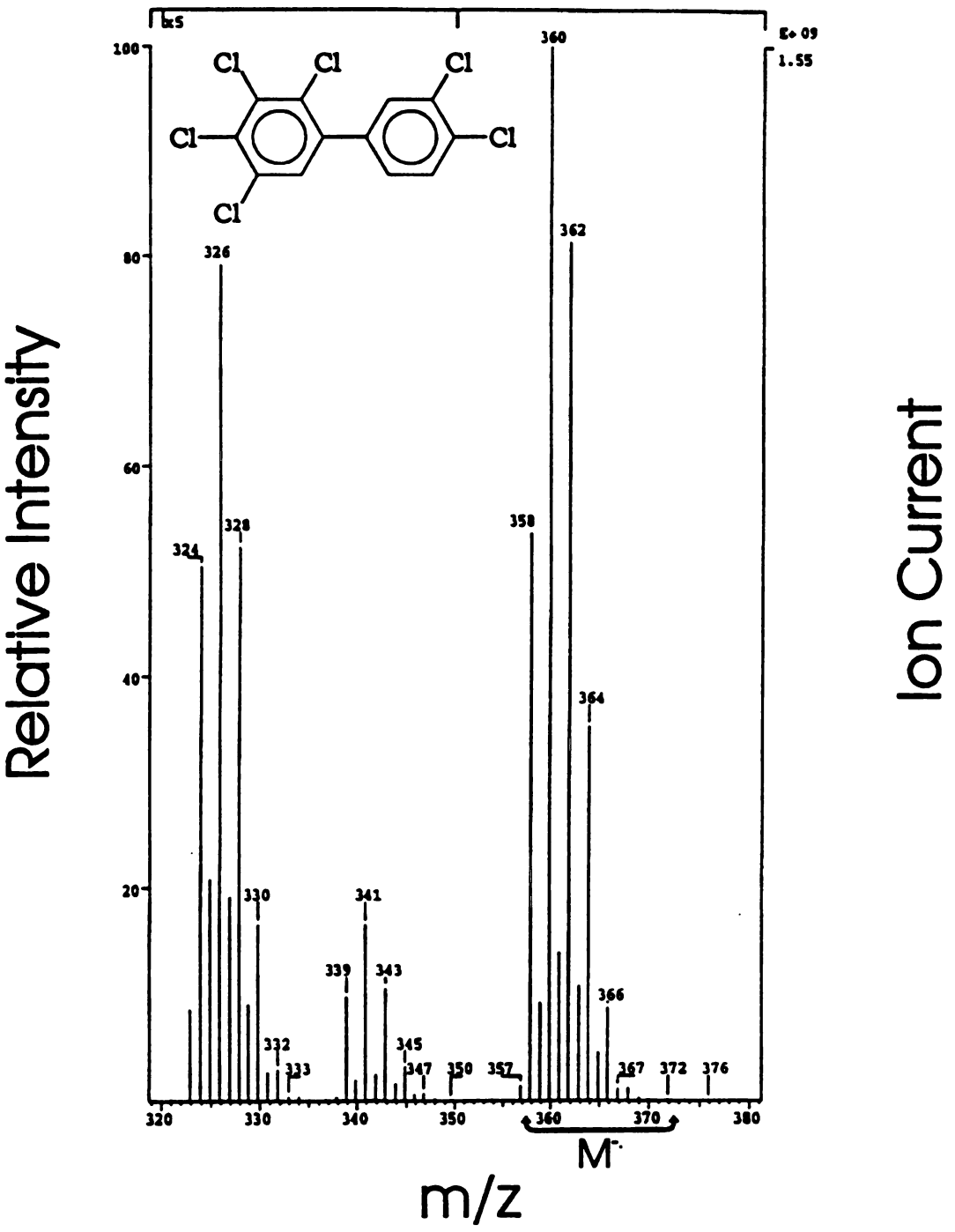


Figure 4.4. The ECNI mass spectrum of a hexachlorobiphenyl indicating the $[M]^{-\cdot}$ radical ions.

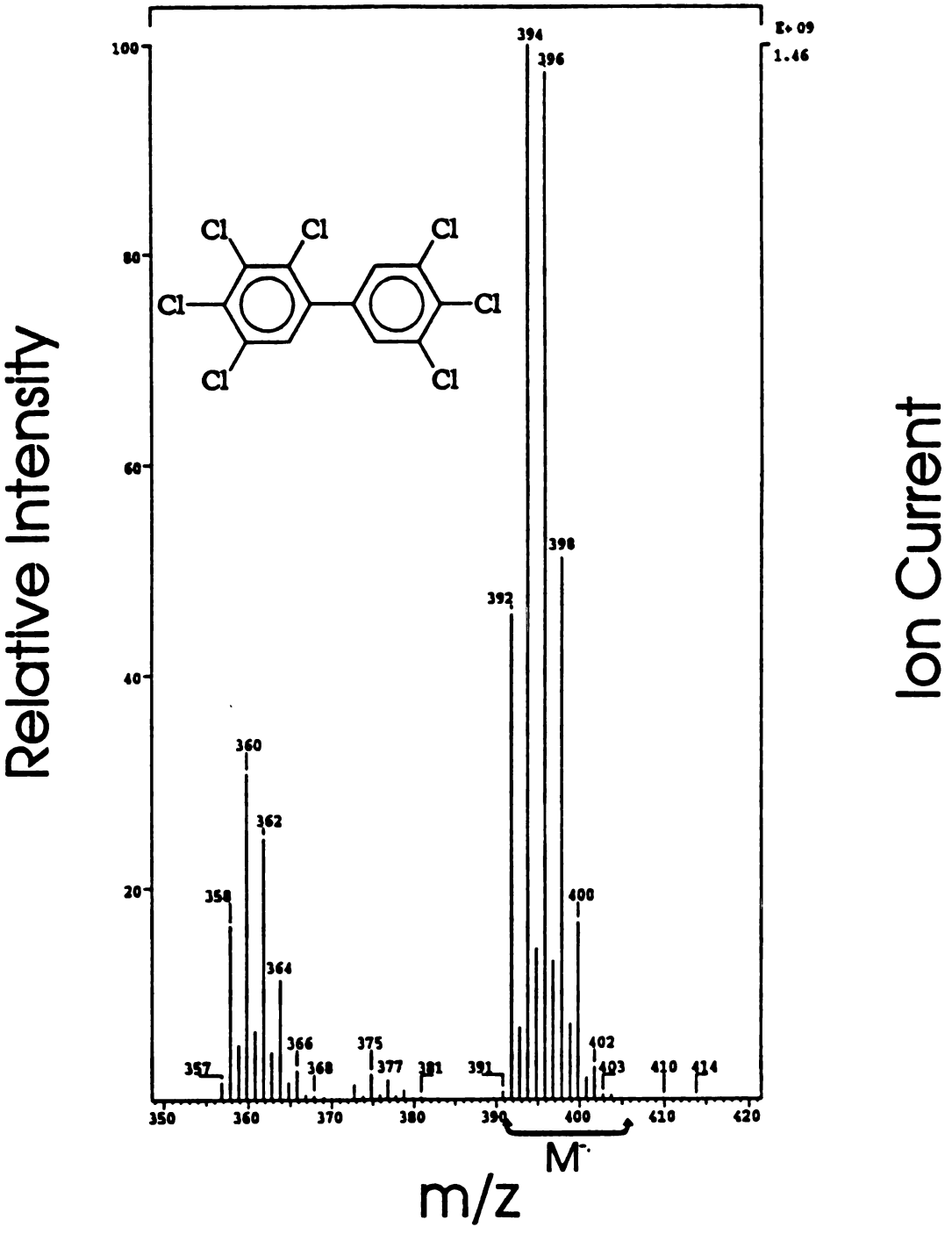


Figure 4.5. The ECNI mass spectrum of a heptachlorobiphenyl indicating the $[M]^{-\cdot}$ radical ions.

somewhat higher than that used for PCI.

The use of highly basic reagent gases can sometimes result in the production of higher abundances of thermalized electrons. While many CI/MS techniques employ methane as a moderating gas, it was observed that ammonia offers advantages for negative ion formation. This difference was not studied in a quantitative fashion, but the abundance of negative ions of PCBs produced in the source and extracted into the precursor analyzer appeared to be up to two to three times more abundant when using ammonia rather than methane as the moderating gas. In addition, maximum $[M]^{-\cdot}$ ion formation was observed when the ammonia pressure was approximately ten percent higher than the pressure normally used with methane. Not only did this choice result in somewhat greater negative ion formation in the source, but greater MS/MS product formation was observed with the use of ammonia as opposed to methane. This could be due to either somewhat reduced fragment ion formation with ammonia or to a greater abundance of thermal electrons. These phenomena were observed early in the development of this method, and no attempt at quantifying these observations was made. The optimum pressure of ammonia was determined to be approximately 9500 mTorr as measured by the ion source Convectron gauge by monitoring the formation of radical anions in an MS experiment with a steady state of Aroclor 1242 being leaked into the source *(Figure 4.6)*.

On a TSQ-700 there are two voltages associated with the filament. The voltage

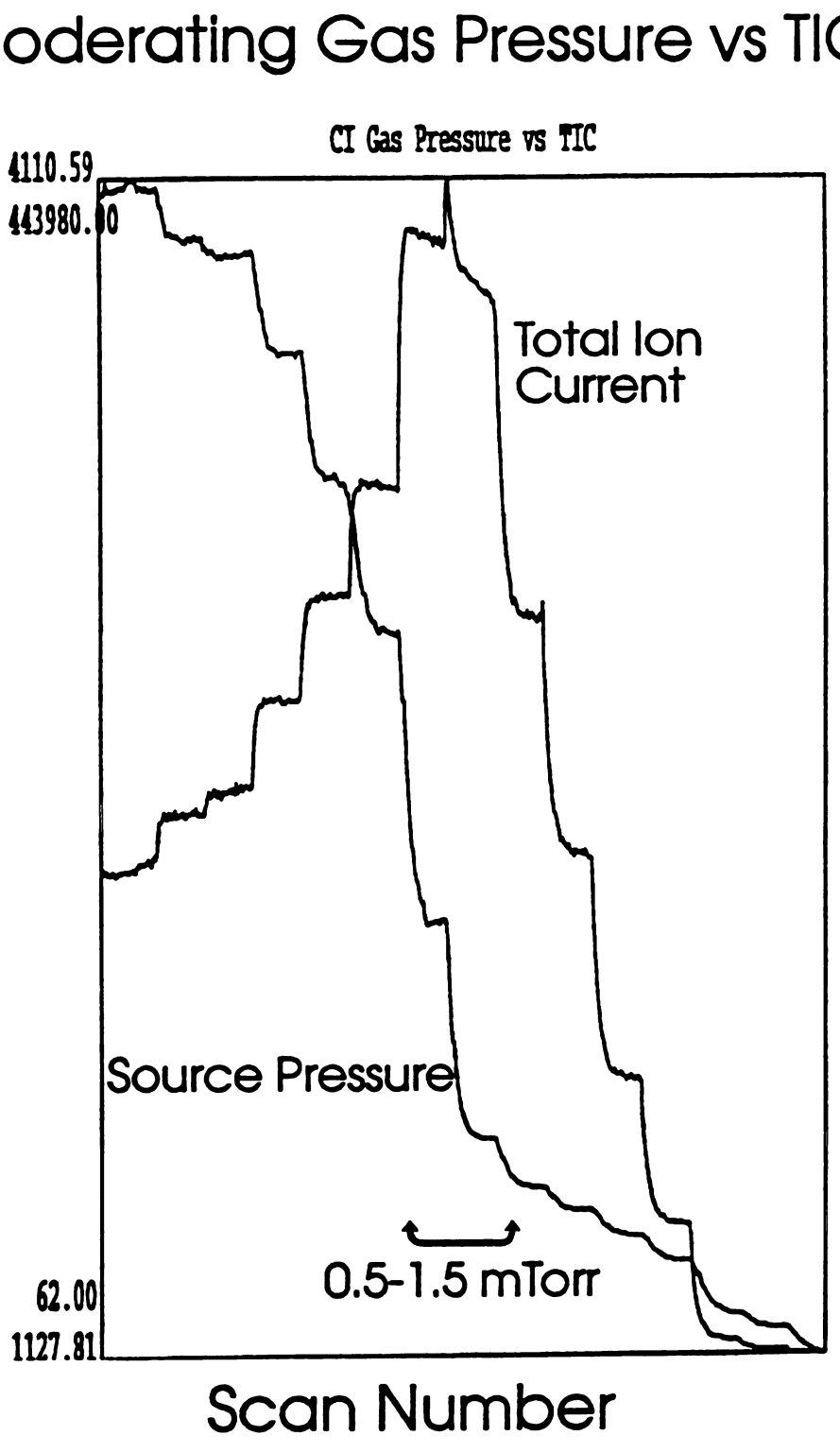


Figure 4.6. The ion current of the molecular anion of a pentachlorobiphenyl as the pressure of the CI moderating gas is varied from high to low.

across the filament is set such that resistive heating of the filament occurs with electrons being emitted. In order to form a stable environment for ionization, the electron emission is set (0-1000 μA). The voltage across the filament is varied to maintain that emission as source conditions change with analyte introduction (such as chromatographic elution). In addition to the voltage applied across the filament, the entire filament is biased (negatively) with respect to ground. Since the ion source (ion volume) is at ground potential, this filament bias is the energy of the electrons being emitted. With EI experiments, the filament electron energy is usually set to 70-eV. This is due to the ion production with respect to fragmentation of most MS ion sources being relatively stable at this value. Also, most EI analyses are performed for the purposes of searching generated EI spectra against a database (library) which was also generated with 70 eV electrons. With PCI, most analyses are performed with higher electron energies (100-200 eV) again for reasons of stability and for purposes of enhancing the production of $[M+H]^+$ molecular ions.

For the current work in ECNI modes of ionization, the filament emission was maintained at 300 μ-amperes. Higher emission currents generally provide for more molecular anions being produced, but at a compromise in filament lifetime. At an emission of 300 μ-amperes, the production of $M^{-\bullet}$ optimized at an electron energy of approximately 50 eV *(Figure 4.7)*. The energy was varied from 35-200 eV and the ion current monitored. Lower energies were not studied since the instrument automatically lowers the filament emission at low energies. This is a feature that prevents the user from destroying the filament by "boiling off" significant amounts

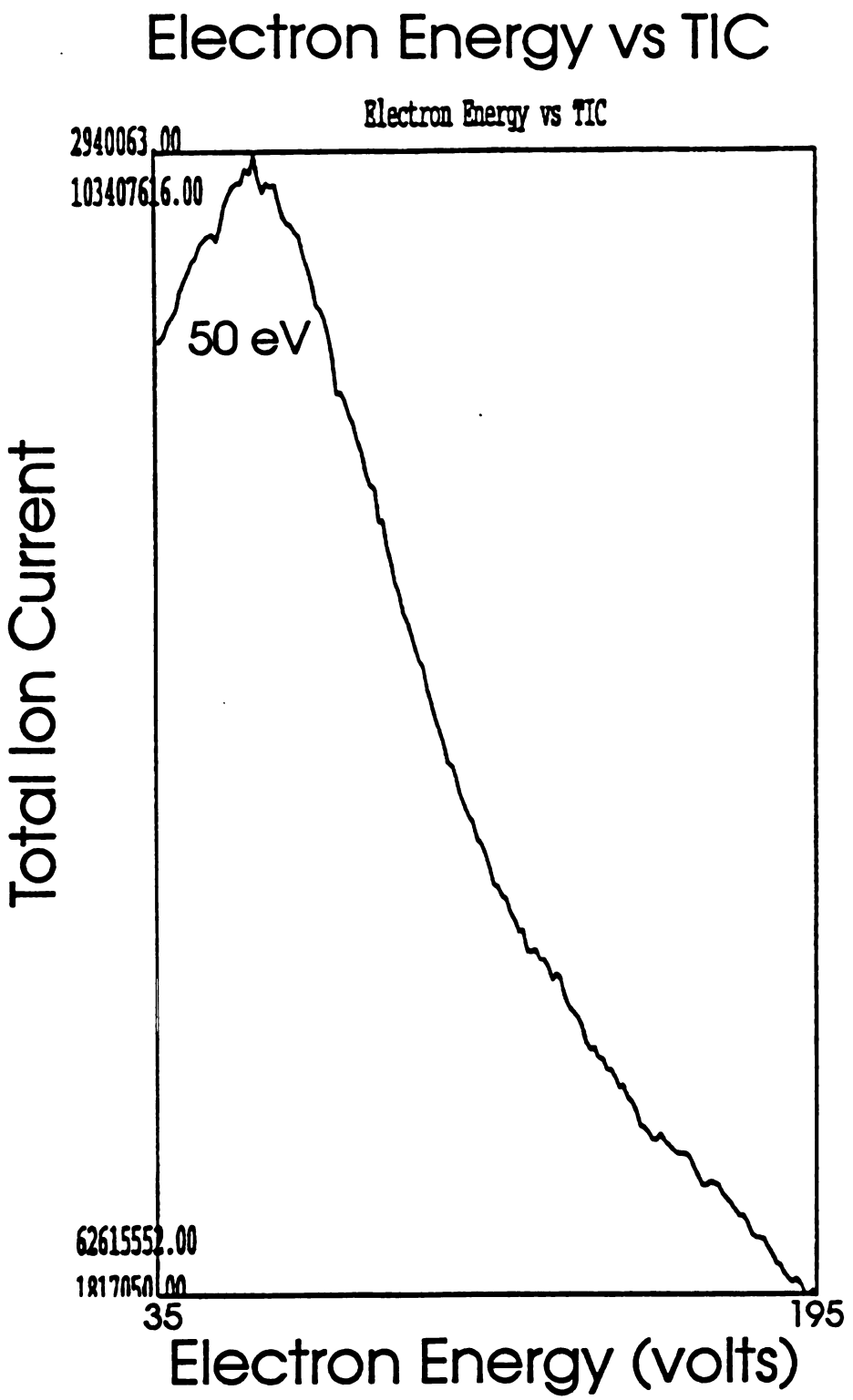


Figure 4.7. A plot of electron energy versus ion current of the molecular anion of a pentachlorobiphenyl.

of electrons, but not removing them from the region with an adequate electron energy.

The temperature of the ion source has been shown to have an effect on the production of molecular anions *(165)*. For the present work, MS chromatographic analyses were conducted at 100 C, 150 C (TSQ-700 default) and 250 C (See Chapter 5). At the low temperature, a more significant amount of background was observed than at the higher temperatures. At the high temperature, more fragmentation in the source was observed than with the lower temperatures. As such, all experiments were conducted with the source at 150 C and the manifold (analyzer) at 70 C. In an effort to eliminate chemical background, the system was generally "baked out" during the evening with a source temperature of 250 C and a manifold temperature of 150 C, and then returned to 150 C and 70 C, respectively, prior to analysis.

Oxygen-Chlorine Exchange. By forming the precursor ion $[M]^-$ under electron-capture negative ionization conditions and selecting it in the first quadrupole of a triple-stage quadrupole mass spectrometer and introducing oxygen into the second quadrupole (collision cell), the exchange reaction of oxygen for chlorine may be observed. The same reaction is not observed with the $[M-H]^-$ species. This phenomenon may be attributed to the fact that the former is an odd-electron moiety and the latter even-electron.

Odd- vs. Even-Electron Anions. It has been observed that only radical anions of PCBs will undergo the oxygen-chlorine exchange reaction. Other precursor ions such as the $[M-H]^-$ ions will not undergo oxygen exchange. These ions are even-electron aromatic species for which the oxygen-chlorine exchange reaction is not energetically favorable. The radical anions, however, are odd-electron and non-aromatic, and as such, are amenable to reactions that produce more stable products. The m/z 290 ion, which is the ^{35}Cl molecular anion of tetrachlorobiphenyls, is also present in the primary mass spectrum of many pentachloro- congeners as a result of the loss of chlorine from the ^{13}C molecular anion. The ion formed by loss of chlorine in the source has an even number of electrons. The oxygen-chlorine exchange reaction, however, is specific for the odd-electron molecular anion. Therefore, the MS/MS detection of m/z 271 ions formed from m/z 290 will be completely free of interference from the even-electron m/z 290 ions formed by the loss of chlorine from pentachlorobiphenyls in the source. This also explains the fact that the oxygen-for-chlorine exchange reaction in the source is only observed in the mass spectrum in the neutral loss of 19 u from the molecular ion and its isotopes.

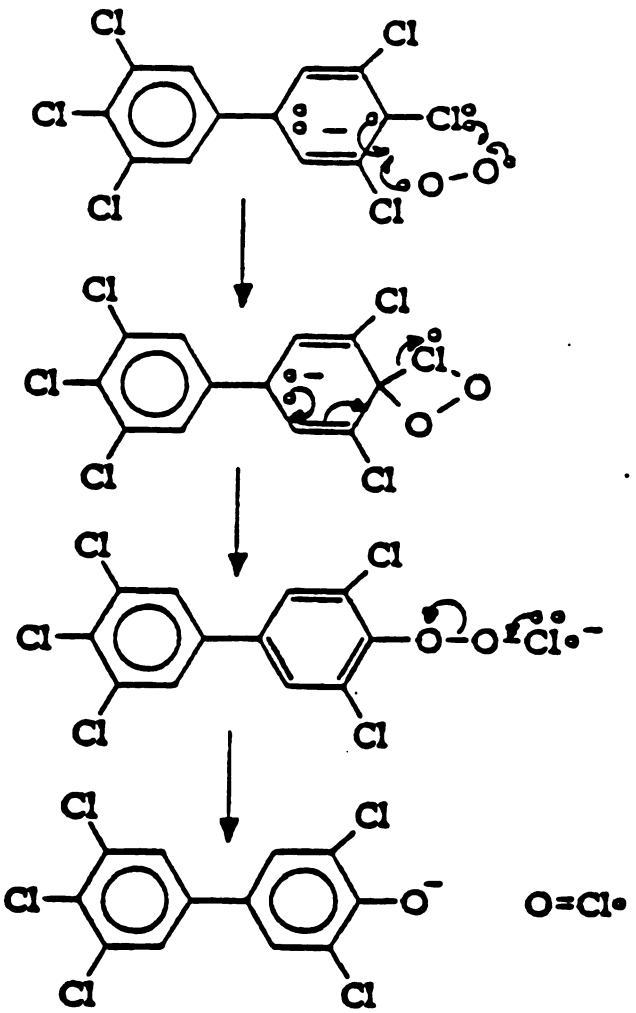
Several researchers have investigated the reaction of molecular oxygen in an MS source with chlorinated hydrocarbon radicals in both MS *(166)* and MS/MS *(138)* experiments. Indeed, it has long been known that oxygen will quench radical-initiated chain reaction mechanisms *(167)*. Recently, the use of this reaction in an MS/MS experiment has been explored as a detection scheme for tetrachlorodibenzo-p-dioxins and tetrachloro-dibenzofurans *(168),(169)*.

It has been proposed that the oxygen atom(s) in these species are instrumental in the reactions of their precursor ions with molecular oxygen. While this may be true, it is obviously not a prerequisite for reaction because the same reaction is observed with PCBs, and this reaction has been reported for other chlorinated aromatics *(170)*.

Reaction Scheme. The reaction of a hexachlorobiphenyl molecular anion with molecular oxygen is shown in Scheme I. The molecular ion shown is only an example, and presumably, due to resonance, any of the carbon atoms of the biphenyl skeleton could exhibit the higher radical electron density. In addition, in the case of asymmetric congeners, the charge may exist on either of the two rings or on both in resonance equilibrium. Perchlorinated aromatic compounds, such as decachlorobiphenyl and octachloronaphthalene, exhibit responses to this reaction that are considerably less than that of the congeners with lower degrees of chlorination. This would indicate that, while hydrogen atoms attached to the ring are not necessary for this reaction to occur, those positions of chlorination which are ortho to hydrogens may be more favorable due to steric considerations.

While the exact mechanism of this reaction is not certain, the product ion is most likely that represented in Scheme I. This product, unlike the precursor anion, is a highly stable moiety with total aromatic character. In addition, the charge residing on the oxygen atom is the only configuration possible in which each atom of the product is surrounded by a full complement of eight electrons. The stability of this

SCHEME I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product may also explain the fact that the exchange reaction products do not undergo further exchange of chlorine for oxygen.

MS/MS Reaction Parameters. When acquiring data in an MS/MS mode (product, precursor or neutral loss), the voltages applied to all of the analyzer elements *after* the collision cell need to be modified from those set during tuning to compensate for the collision energies of the MS/MS reactions. On a TSQ-700 this is accomplished automatically using a voltage programming algorithm that takes into account the original tune voltages, the energy of the collisions and the masses and energies of the product ions. The elements affected are Lens Stack 3 or the Einsel lens assembly between the collision cell (Q2) and the product analyzer (Q3) —— **L31, L32 and L33** —— and the product analyzer offset (**DOFF**). These analyzer elements are referred to as *link parameters* since their values are linked to the mass currently being filtered. As such, link parameters are used to tune the instrument.

Voltage Programming. The goal of voltage programming is to set the voltages on the analyzer elements such that the Translational Kinetic Energy of the product ions (TKE_d) is the same as that experienced by isobaric ions in the Q3MS mode of scanning. As an example, the Daughter (product) OFFset (DOFF) parameter for an MS/MS reaction will be explored. For the moment, Lens Stack 3 will be ignored. Specifically, the reaction of m/z 500 (positive) at 100 eV to produce a product at m/z 250 will be used. Also, it is assumed that the Q3MS tune resulted in **DOFF** = -5.2 volts at m/z 250.

As a first approximation, it is assumed that no loss in *total* energy of the products occurred as a result of the dissociation reaction. This assumption will be dealt with later. This means that the product ion would have a TKE_d = 50 eV —— half the mass, half the energy. Since the product ion must have only 5.2 eV in Q3, it must experience a decelerating potential (DP) of 44.8 volts. Now, since COFF = -100 V, DOFF must be set at -55.2 V; i.e.,

(1) $$DP = DOFF_{MS} - TKE_d$$ and

(2) $$DOFF_{MS/MS} = COFF + DP$$ or

(3) $$DOFF_{MS/MS} = DOFF_{MS} + COFF - TKE_d .$$

Now, since

(4) $$TKE_d = COFF\ (M_d/M_p) ,$$ where

M_d is the mass of the product and M_p is the mass of the precursor, substituting for TKE_d leaves

(5) $$DOFF_{MS/MS} = DOFF_{MS} + COFF - COFF(M_d/M_p)$$ or

(6) $$DOFF_{MS/MS} = DOFF_{MS} + COFF(1-M_d/M_p) .$$

Now, substituting the values for the above experiment into Equation (6) does indeed result in **DOFF** = -55.2 V. This equation would work well, however, only for those ions not losing energy in the collision process.

Translational Kinetic Energy Correction. Since most reactions result in some energy loss, TKE_d will be some fraction of Equation (4). This is accounted for by incorporating into the equation a translational kinetic energy correction factor, **MSMSC**, or

$$(7) \qquad TKE_d = COFF\ (M_d/M_p)\ (MSMSC/100) \qquad \text{where}$$

MSMSC is expressed in percent and is a number between 0 and 100. This allows the instrument to account for an MS/MS product ion losing all, none or part of its theoretical energy. Now, substituting into Equations (5) and (6) leaves

$$(8) \qquad DOFF_{MS/MS} = DOFF_{MS} + COFF - COFF(M_d/M_p)(MSMSC/100) \quad \text{or}$$

$$(9) \qquad DOFF_{MS/MS} = DOFF_{MS} + COFF[1-(M_d/M_p)(MSMSC/100)]\ .$$

As an example, the two extreme cases are considered. For the case of metastable decomposition (i.e., no collision gas), no energy is lost during the reaction, and **MSMSC** should be set at 100. This would be identical to the above example of m/z

500 producing m/z 250 at 100 eV —— $DOFF_{MS/MS}$ would be set at -55.2 V. For the other extreme, all translational kinetic energy is lost during CID (i.e., TKE_d =0). This is common for many reactions at high CID gas pressures. In this case, Equation (9) reduces to

$$(10) \qquad DOFF_{MS/MS} = DOFF_{MS} + COFF \qquad (MSMSC = 0).$$

Thus, the product ion must be *accelerated* —— DP is negative for positive ions. For the ion at m/z 250 with COFF = -100 and TKE_d = 0, $DOFF_{MS/MS}$ should be -105.2V.

In the above examples, only the DOFF parameter was considered. These are shown graphically in Figures 4.8 and 4.9. As originally stated, the voltages of *all* of the analyzer elements *after* the collision cell (L31, L32, L33, and DOFF) must be programmed to reflect the energies of the collisions and products. The more generalized form of Equation (9), therefore, is

$$(11)\ LTAB_{MS/MS} = LTAB_{MS} + COFF[1-(M_d/M_p)(MSMSC/100)] \qquad \text{where}$$

$LTAB_{MS/MS}$ and $LTAB_{MS}$ are L31, L32, L33, and DOFF. Thus, the voltages of these elements in MS/MS modes of acquisition are *calculated* from Equation (11) and not derived from an MS/MS link table. MSMSC becomes more significant for experiments conducted at low pressures where product ions do not lose all theoretical energy. From Equation (11), it is also apparent that the use of MSMSC

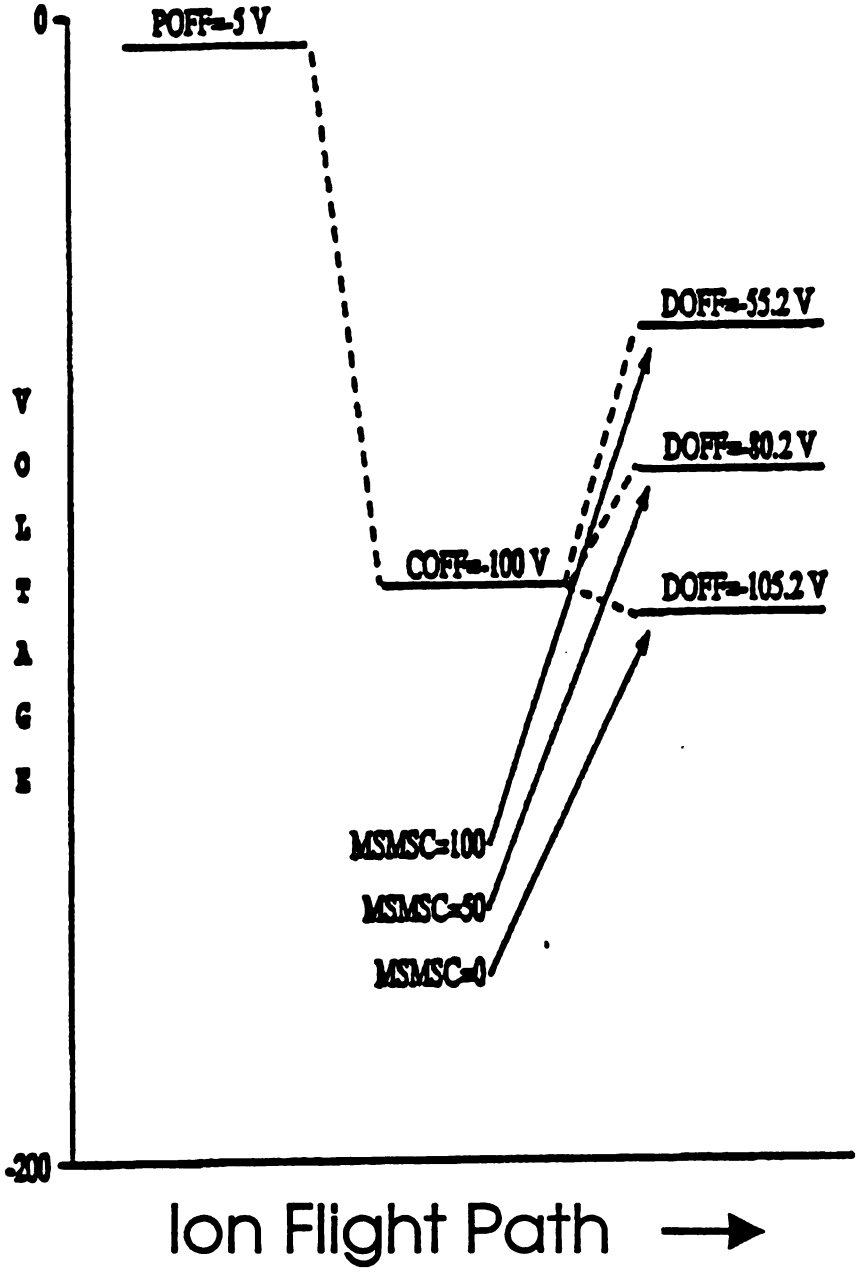

Figure 4.8. The offset voltage settings for Q1, Q2, and Q3 for three settings of MSMSC.

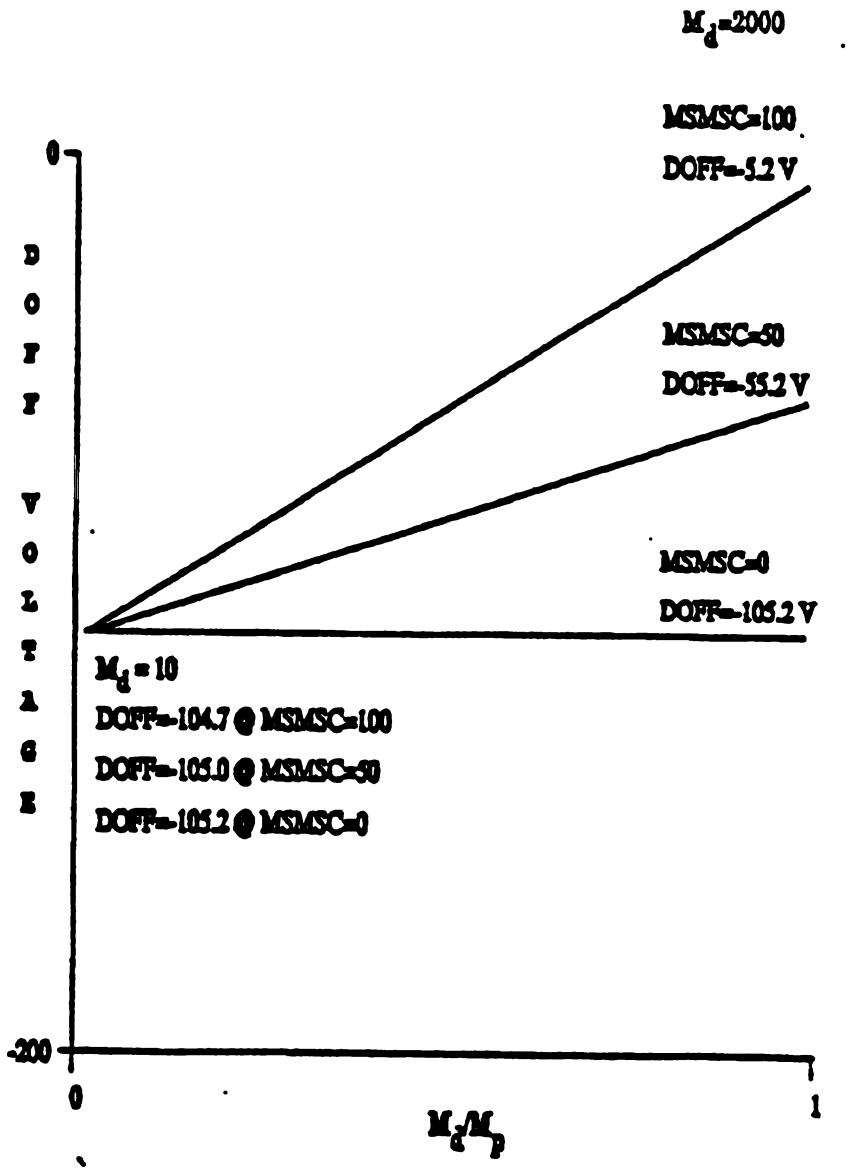

Figure 4.9. The plots of the DOFF voltage as a function of the ratio of the daughter mass to the parent mass.

(greater than 0%) becomes more critical for experiments conducted at high energies and with products from small neutral losses.

By introducing Aroclor 1242 into the source in a steady state, the kinetic energy correction factor (MSMSC) was varied from 0 to 100% and the ion current from the reaction of m/z 326 with molecular oxygen to produce m/z 307 monitored *(Figure 4.10)*. As was expected with these low collision pressure, low energy experiments, MSMSC was shown to have minimal effect. This was observed early in this work but not tabulated until later. As such, all experiments were conducted with MSMSC set to the default value of 70%.

Collision Reagents and Pressures. The maximum product formation from a given abundance of precursor ion is dependent upon both collision pressure and collision energy. Optimization of product ion production for this ion-molecule reaction occurs when the pressure of oxygen in the collision cell is such that the collision cross-section allows for a high probability of interaction with a limited amount of scattering and fragmentation. Thus, higher pressures of oxygen require higher collision offset energies for maximum product formation. As the amount of oxygen is increased, however, the energy required to maintain optimum conditions becomes so great that either the likelihood of reaction is diminished or the products cannot be focussed into the entrance of the product analyzer. This relationship is shown in Figure 4.11 where the reaction of a pentachlorobiphenyl was monitored as the collision gas pressure was varied from 10 mTorr to non-existent. The optimum

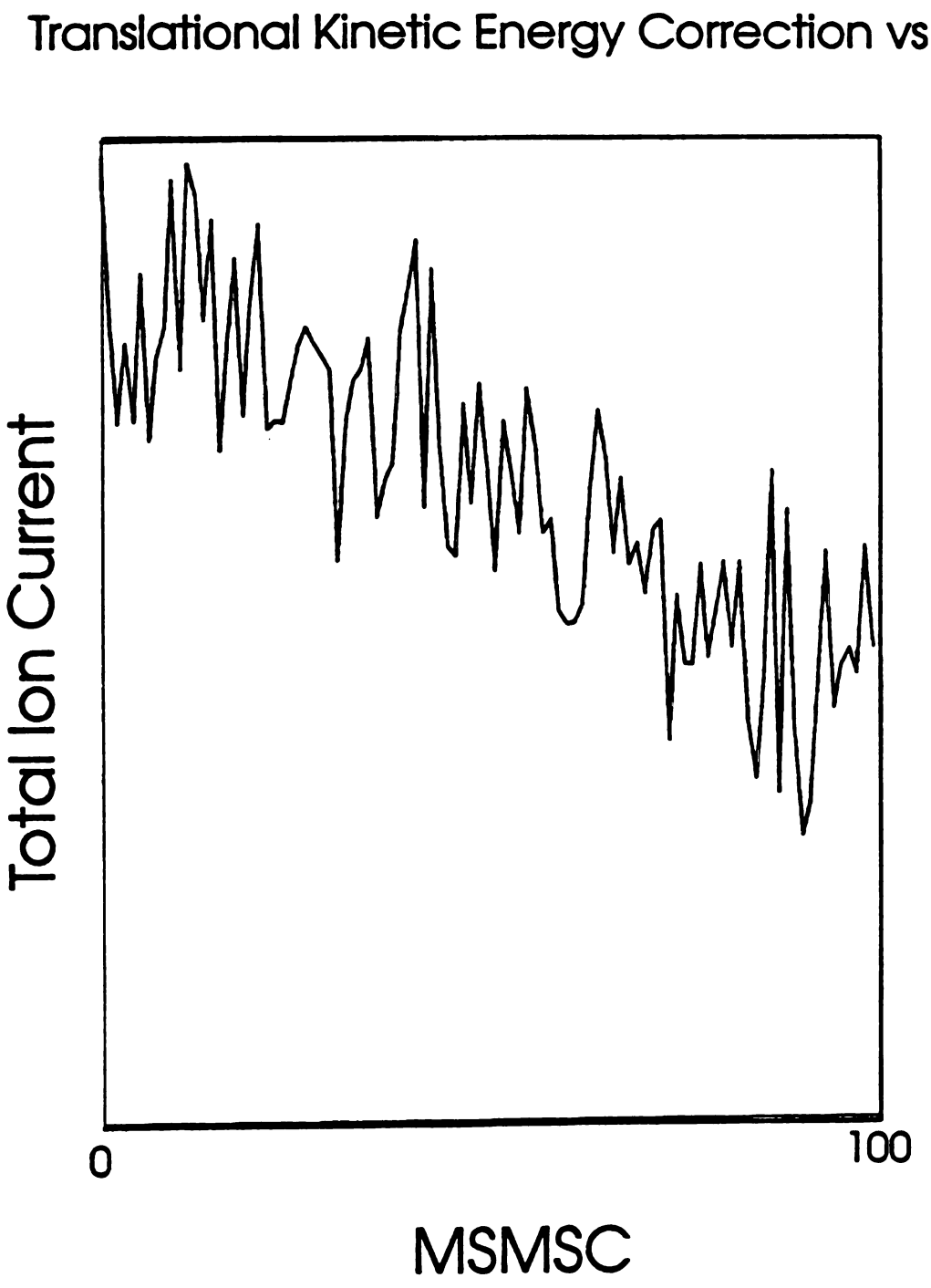

Figure 4.10. The ion current of the product of the oxygen-for-chlorine exchange reaction as a function of the translational kinetic energy correction factor (MSMSC).

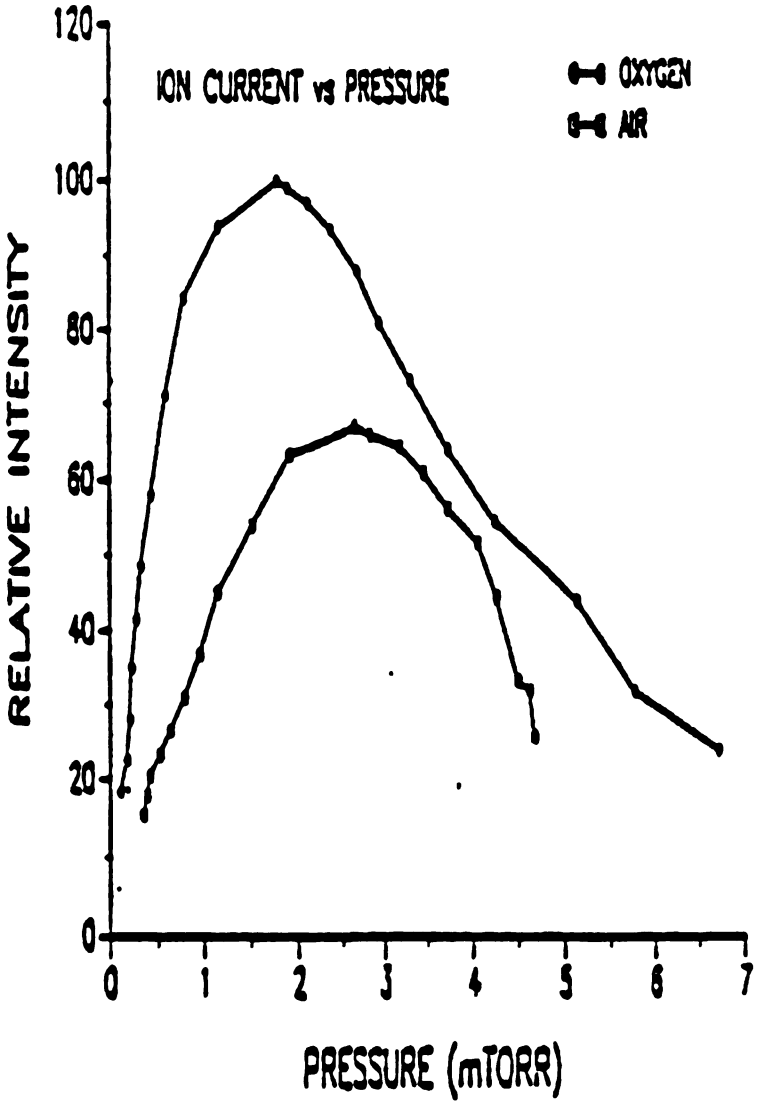

Figure 4.11. Ion current of the product of the oxygen-for-chlorine exchange reaction as a function of the pressure of the collision gas.

reaction parameters were determined to be about 1 mTorr of oxygen in the collision cell with 1.5-eV (laboratory) precursor ions. This corresponded to collisions of about 1.0 eV in energy *(See below)*.

Early MS/MS experiments with PCBs were done by monitoring the oxygen-chlorine exchange reaction products formed by reaction with ambient air. While this proved to be a viable alternative to pure oxygen, a reduction in sensitivity was observed. Dilution of the oxygen with unreactive gases results in optimization at higher pressures where the rates of scattering and fragmentation are also increased *(Figure 4.11)*.

Collision Energy. With the collision gas (oxygen) pressure set to approximately 1 mTorr, the voltage offset of Q2 was varied from -2 volts to 8 volts and the ion current of oxygen-for-chlorine exchange reaction products of tetra-, penta- and hexachlorobiphenyls monitored *(Figures 4.12-4.14)*. Although the optimum energy of an ion-molecule reaction in an MS/MS experiment is dependent on all parameters such as collision pressure and type of collision gas, the energy (Q2 offset) is clearly the most critical.

The optimum energy for an MS/MS reaction with a triple-stage quadrupole instrument is determined and set by varying the voltage offset of the collision cell. This offset is the energy of the MS/MS reaction in the laboratory frame of reference. In order to determine the true energy of the reaction(s), the precursor ion

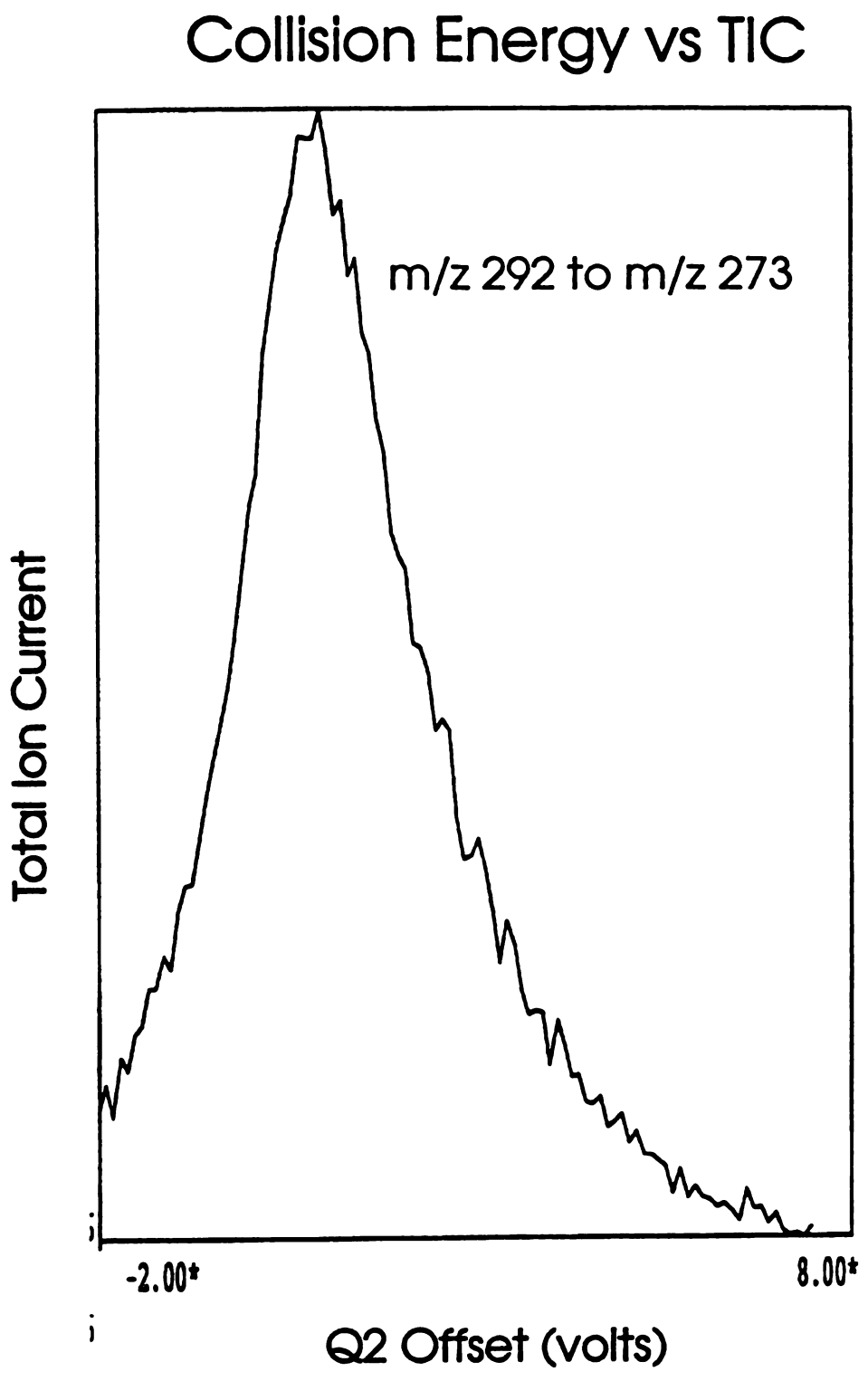

Figure 4.12. The ion current of the oxygen-for-chlorine exchange reaction product of a tetrachlorobiphenyl as a function of collision energy.

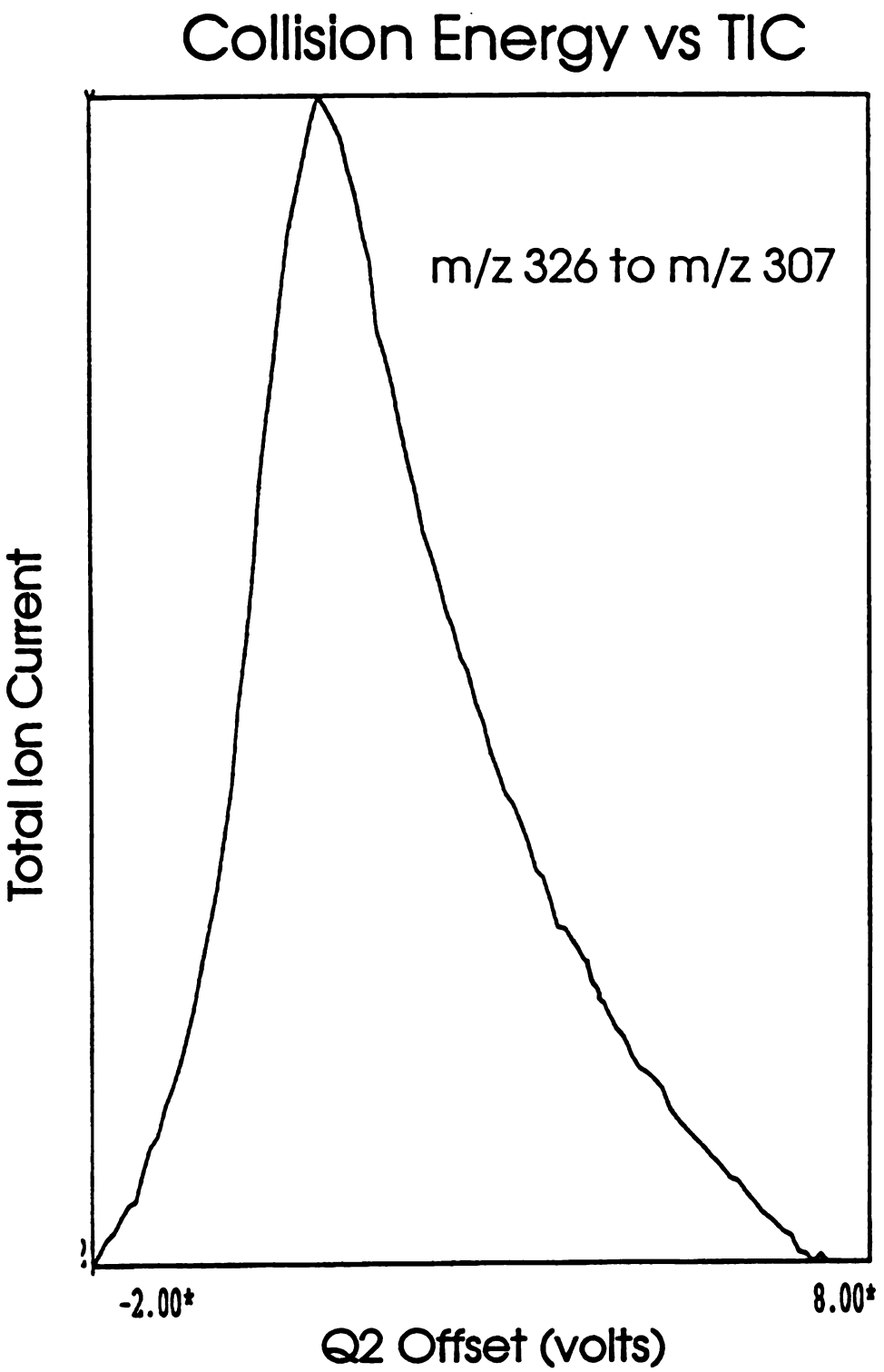

Figure 4.13. The ion current of the oxygen-for-chlorine exchange reaction product of a pentachlorobiphenyl as a function of collision energy.

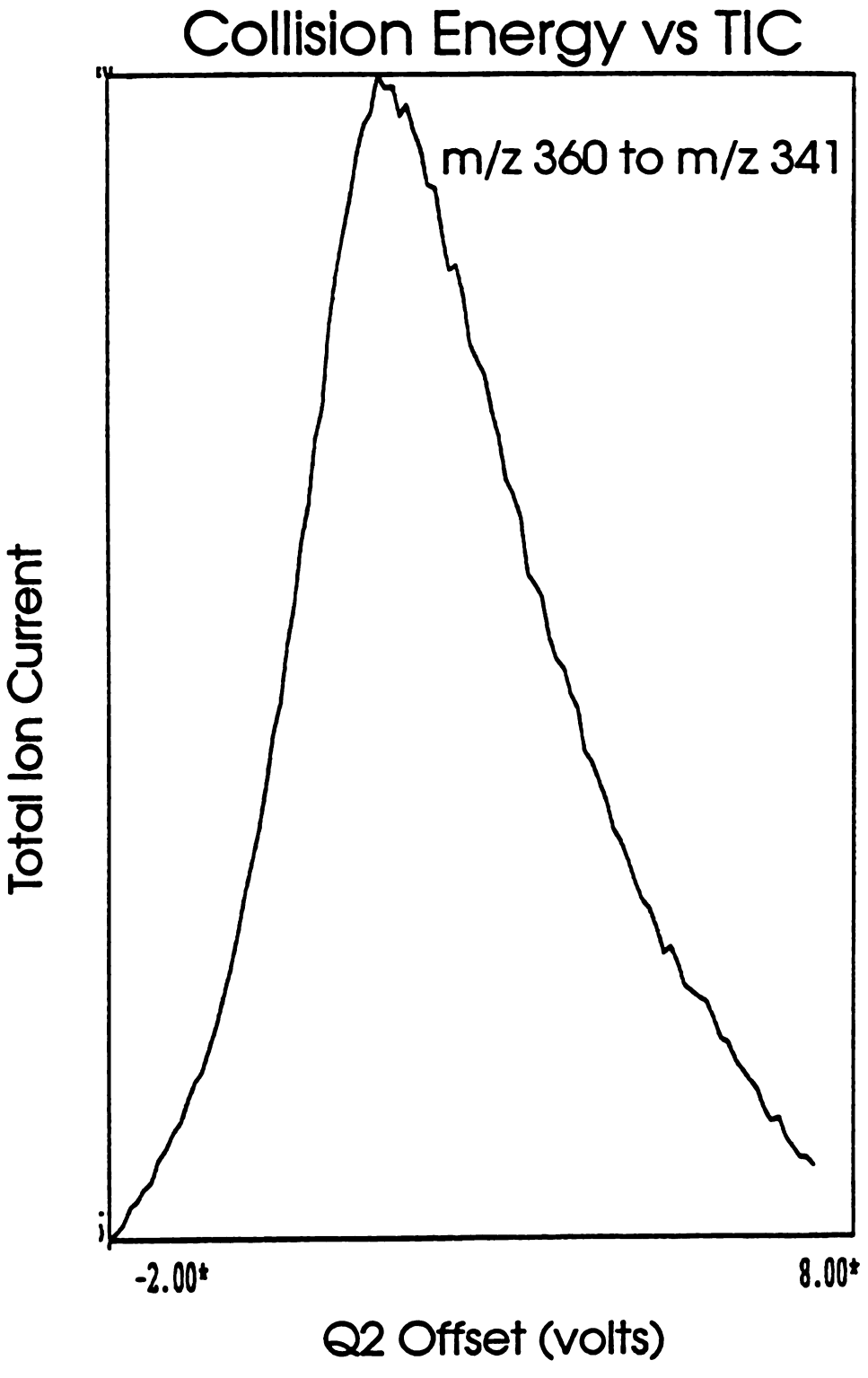

Figure 4.14. The ion current of the oxygen-for-chlorine exchange reaction product of a hexachlorobiphenyl as a function of collision energy.

transmission is monitored as a function of the Q2 voltage offset with no collision gas being introduced *(Figure 4.15)*. The difference of the optimum Q2 voltage offset for product ion formation and the inflection point of the energy plot for the precursor ion transmission is the true optimum energy for the given reaction. As shown, the inflection point occurred at approximately 0.5 volts and the optimum offset at approximately 1.5 volts. Figure 4.15 shows the energy range of the ions that are extracted from Q1. All ions to the left of the offset used get in with the excess energy given by their displacement to the left.

Having performed the energy vs. ion current experiments on several occasions over many months, it was observed that the optimum value is dependent on ion source conditions. Specifically, as the ion source becomes more and more contaminated, the optimum Q2 voltage offset occurs at higher values. This is also true of the precursor ion transmission. Thus, even when the optimum reaction energy (laboratory) occurs at higher voltages, the true energy of the reaction(s) remains relatively constant at approximately 1 eV.

Collision RF Linkage. The default setting for the RF power on the collision cell (Q2) on a TSQ-700 is linked to the precursor analyzer (Q1). This peak-to-peak voltage will be set from 2-8 kV depending on the mass being filtered by Q1. This is a problem for MS/MS reactions produced at high collision pressures (> 1 mTorr), low energies (< 40 eV) and where the ratio of the product-to-precursor mass is low (< 0.5). For reactions under these conditions, product transmissions may be extremely

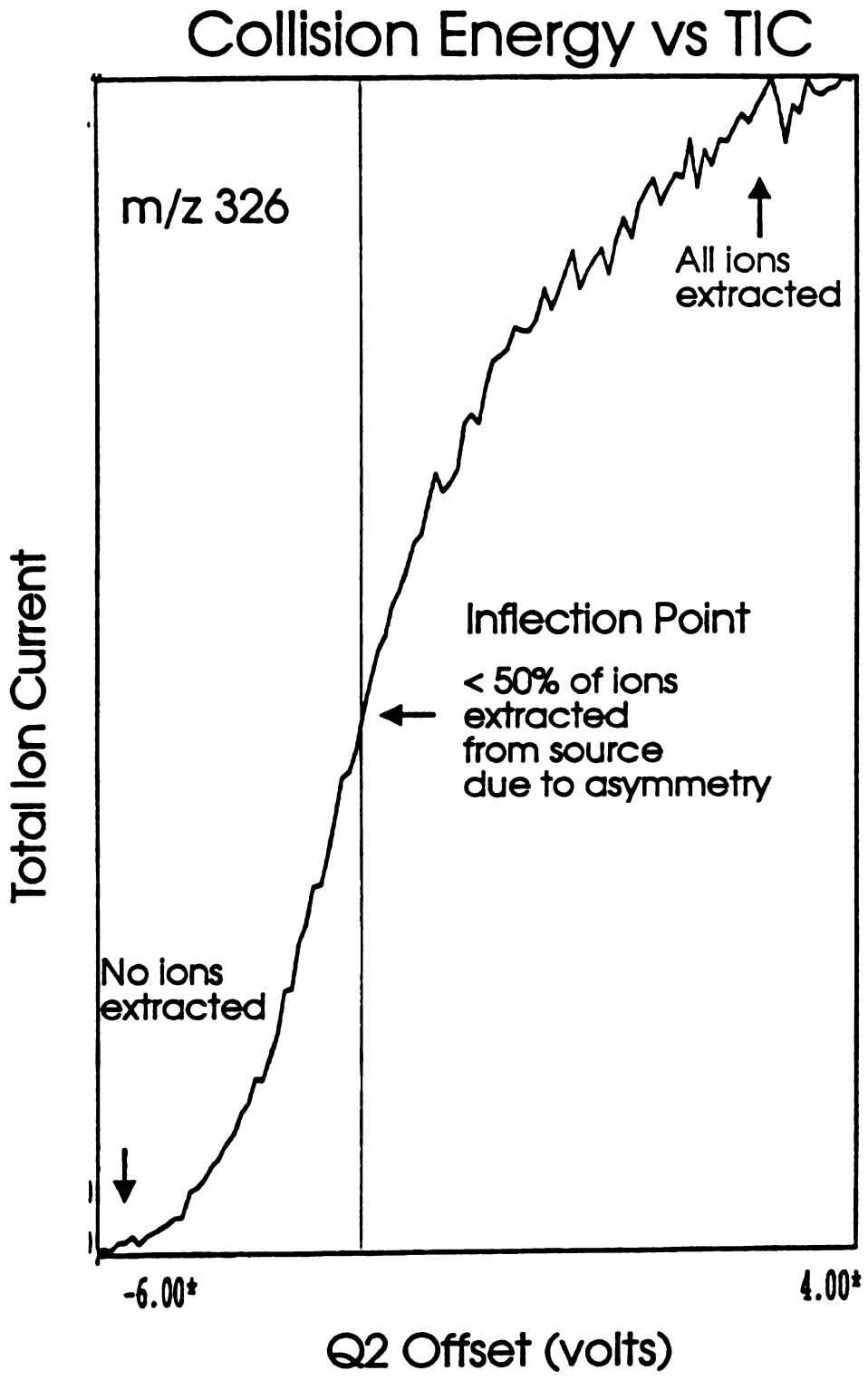

Figure 4.15. The transmission of the molecular anion of a pentachlorobiphenyl as a function of the collision energy (Q2 voltage offset) with no collision gas being introduced.

low or even non-existent due to the product ions being produced near the entrance of the collision cell. At the relatively high RF powers that are optimum for the precursor ion, quite often the lower mass product ion is unstable. The provision for changing the Q2 linkage to Q3 is available on the TSQ-700, and this capability offers a significant improvement in product transmission for many experiments.

For the present work, changing the linkage of the Q2 RF power to Q1 offered little or no improvement in product ion production. This was not surprising since the collision gas pressures of the reactions being studied were so low and the product ions were only 19 u less than that of the precursor ions. This phenomenon was observed on several occasions and was not tabulated. As such, all experiments were conducted under default conditions —— Q2 RF power linked to Q1.

Summary of Experimental Details. In conclusion, the following results were observed with the reactions of the molecular anions of PCBs:

- The odd-electron molecular anions $M^{-\bullet}$ of PCBs will undergo an exchange of chlorine for oxygen in an MS/MS experiment in a triple-stage quadrupole mass spectrometer. It will be shown later that the even-electron $[M-H]^-$ species will not.

- Under CI conditions, mono- and di-substituted PCBs produce negative anions only by dissociative EC ($[M-H]^-$). Some tri- and all tetra-substituted

PCBs produce a significant amount of $M^{-\bullet}$ through resonance EC. All penta- through deca-substituted PCBs exclusively produce molecular anions ($M^{-\bullet}$) through resonance EC.

- Molecular anion production maximizes with moderating gas pressures of approximately 9500 mTorr as measured by the TSQ-700 ion source gauge. This pressure is slightly higher than the optimum (8500 mTorr) observed with PCI.

- Using a filament emission of 300 μ-amperes, molecular anion production optimizes with an filament electron energy of 50 eV.

- Ammonia was observed to be a better CI moderating gas for negative ion production than methane.

- Product ion formation for the oxygen-for-chlorine exchange reaction optimized with a collision offset of approximately 1 eV. This represents the optimum compromise between transmission and fragmentation and/or scattering losses.

- Translational kinetic energy correction through Q3 is not a critical factor for these small neutral-loss, low-pressure reactions.

- **Collision cell (Q2) RF linkage is not a critical factor for these small neutral-loss experiments.**

- **The use of pure oxygen as a collision gas provides an enhancement of product ions over the use of ambient air.**

Chapter 5

Development of an Analytical Method for the Analysis of Polychlorinated Biphenyls using the Exchange Reaction of Oxygen for Chlorine with Molecular Anions of Chlorinated Aromatics in a Tandem Mass Spectrometer

Introduction. Using the exchange reaction of oxygen for chlorine with polychlorinated biphenyl anions, an analytical method for monitoring reaction products in a GC/MS/MS experiment was developed. The method development included optimization of chromatographic and mass spectrometric instrumental parameters such as GC temperature programming, the MS tune and MS scan functions for both full scan and selected reaction monitoring (SRM) modes of acquisition. For quantitation, the selection of an internal standard or standards was determined based on both GC retention time and relative instrumental response.

Experimental. The Aroclor standards were provided by John Quensen, and all other individual congeners and standards were provided by George Frame. All standards were prepared by dilution with Organic Residue Analysis grade hexane (J.T. Baker).

Gas Chromatography. All gas chromatographic methods were performed with a Varian 3400 gas chromatograph equipped with a 30 meter x 0.250 mm column of 0.25 mm DB-1 phase (methyl silicone, J & W Scientific). Helium was used as the GC carrier gas. The Varian 3400 was equipped with an injector body that accommodates both split and splitless modes of injection. All experiments were performed using the splitless mode of injection.

Mass Spectrometry. Early MS and MS/MS experiments were done with a Finnigan TSQ-70B mass spectrometer operated in a negative ion mode. Later, the TSQ instrument was upgraded to a TSQ-700. The pertinent changes included the installation of an octapole as a collision cell (the TSQ-70 instruments use a quadrupole) and replacing the PDP-11/73 data system with a DEC Station. Ammonia, methane, argon or deuterated ammonia were used as chemical ionization reagent gases. The CI reagent gas pressure was set to approximately 9500 mTorr as measured by the source Convectron gauge and was optimized daily using perfluorotributylamine. Pure oxygen or ambient air was introduced into the collision cell. The source was held at 150 C and the manifold at 70 C. The instrument utilizes a 20-kV dynode which was set at 10 kV. All reactions were generated using electron energies of 50 eV, collision offset energies of 1-2 eV (laboratory), and collision pressures from 0.5 mTorr to 1.5 mTorr.

Gas Chromatography. The three most common stationary phases used for the GC

analysis of chlorinated aromatics are methyl silicone (DB-1), 95% methyl/5% phenyl silicone (DB-5) and 94% methyl/5% phenyl/1% vinyl silicone (SE-54). The latter phase, SE-54, provides resolution of more congeners (188 of 209) than the other two. For many modes of detection, such as ECD and FID, chromatographic resolution provides almost all of the selectivity of the method. With MS detection, quantitation of higher mass congeners that co-elute with other lower mass congeners is possible due to differences in mass spectral ion abundances. The purpose of the current research was to develop an MS/MS method that provided increased selectivity such that ultimate chromatographic resolution was not necessary. All chromatographic experiments were therefore performed using DB-1 even though other phases provide better chromatographic resolution of PCB congeners.

The gas chromatograph was equipped with a 30 meter x 0.250 mm column of 0.25 mm DB-1 phase (methyl silicone, J & W Scientific). The GC was temperature programmed —— 40 C for two minutes, 40 C to 160 C at 20 C per minute, 160 C to 270 C at 5 C per minute and held at 270 C for seven minutes. Helium was used as the GC carrier gas with a column head pressure of 22.5 psi which corresponds to a linear flow of approximately 45 cm/second. Splitless injection was used with the split valve closed for the initial two minutes and open for thirty minutes. The injector was held at 270 C and the transfer line held at 275 C. The effluent end of the column was placed directly into the ion source. These conditions corresponded to those used by collaborators in other laboratories with other modes of detection (MS and ECD). Under these conditions, the PCB congeners of interest eluted from 10 to 25 minutes

after injection.

Generally, the split valve is held closed as long as possible in order to place more analyte on column. The trade-off is that with longer times, excessive tailing of the solvent peak may interfere with the elution of the analyte. Using negative ion MS, the solvents generally used (methanol or hexane) do not exhibit a response, and therefore, solvent tailing is not an issue. However, the ECNI plasma used for ionization is affected by the solvent front. When monitored, the solvent typically suppresses the background and is observed as a "negative" peak. As such, it is still important for this method to open the split valve and sweep the solvent out of the injector but with a minimum of analyte loss.

Mass Spectrometry. In addition to the instrumental parameters discussed in Chapter 4, collision energy, collision gas pressure and translational kinetic energy correction, all other instrumental parameters were studied for the purpose of improving precursor and product ion transmission and detection. These involved those instrumental parameters that control both MS and MS/MS tunes and those that control the scan functions of the instrument in both full scan and single or multiple ion detection modes of acquisition.

Instrumental Tune. The voltages (RF and DC) of each element of the analyzer of the TSQ instrument are controlled by link tables. Each link table is specific for a given analyzer element for the given scan mode (Q1, Q3 or MS/MS) and ion polarity and

may consist from one to several tune points across the scan range. As the instrument is scanned from low to high mass, the voltage of each analyzer element is varied according to its specific link table. Also, with the TSQ, the instrument must be calibrated prior to acquisition. This is accomplished by setting the Q1 or Q3 calibration link table with the exact masses of the tuning compound (perfluorotributylamine —— PFTBA or FC43). For the current work, at least two instrument tunes were generated and used. One was generated at unit (baseline) resolution for MS acquisitions and the other an "open-resolution" tune for MS/MS experiments.

The resolution of a quadrupole mass filter is determined from the DC-to-AC ratio of the voltage placed on the quadrupole assembly. Thus, there is a compromise between resolution and transmission. This ratio was set to achieve baseline resolution (<5% valley between a peak and its isotope). The DC offset of the filtering quadrupole was set to achieve good Gaussian peak shapes. The DC offsets of the filtering quadrupoles will affect the resolution, and as such, both parameters must be set iteratively. This also involves a compromise with sensitivity, but good peak shape is of paramount importance with respect to centroiding and tune and calibration stability. All other parameters, lenses and RF powers of the non-filtering quadrupole(s) and collision cell, were set for maximum transmission. In some cases, there was a slight compromise between sensitivity and stability (such as the middle lenses of the Einsel lens assemblies). In these cases, stability was always chosen. By using these criteria, instrumental tunes were generated that were stable with respect

to calibration, resolution and sensitivity over several days and even weeks.

Signal Optimization. When using the TSQ in an MS/MS mode, two mass filters are used in line. As such, almost 99% of all ions generated in the source are filtered out in order to achieve unit mass resolution in both (Q1 and Q3) mass filters. Thus, it is advantageous to enhance the number of ions striking the detector without sacrificing selectivity. In an MS/MS experiment, one mass filter, typically Q1, is used for a set mass —— the quadrupole is maintained at a specific DC-to-AC ratio. The resolution may be "opened up" to a significant degree, however, but such that still only ion current at one m/z value is transmitted *(Figure 5.1)*. PRES and DRES are the instrumental parameters linked to mass that are used to set the resolution of the instrument. Also, typically with a baseline-resolved MS tune, ions are slightly over-resolved in an MS/MS mode due to over-filtering. As such, in MS/MS the resolution may be opened up slightly thus improving sensitivity but still achieving baseline resolution.

For full scan MS/MS acquisitions, the resolution of the precursor quadrupole (Q1) was adjusted to approximately 20% valley and the product analyzer (Q3) from 1% to %5 valley. This offered a factor of two improvement in sensitivity as measured with the PFTBA tuning compound. For selected reaction monitoring experiments, the resolution of both filters was further adjusted such that three to five times the ion current was observed over that with the MS tune. In both cases, selectivity for ion current at each m/z value was preserved. Also, the quadrupole offsets were

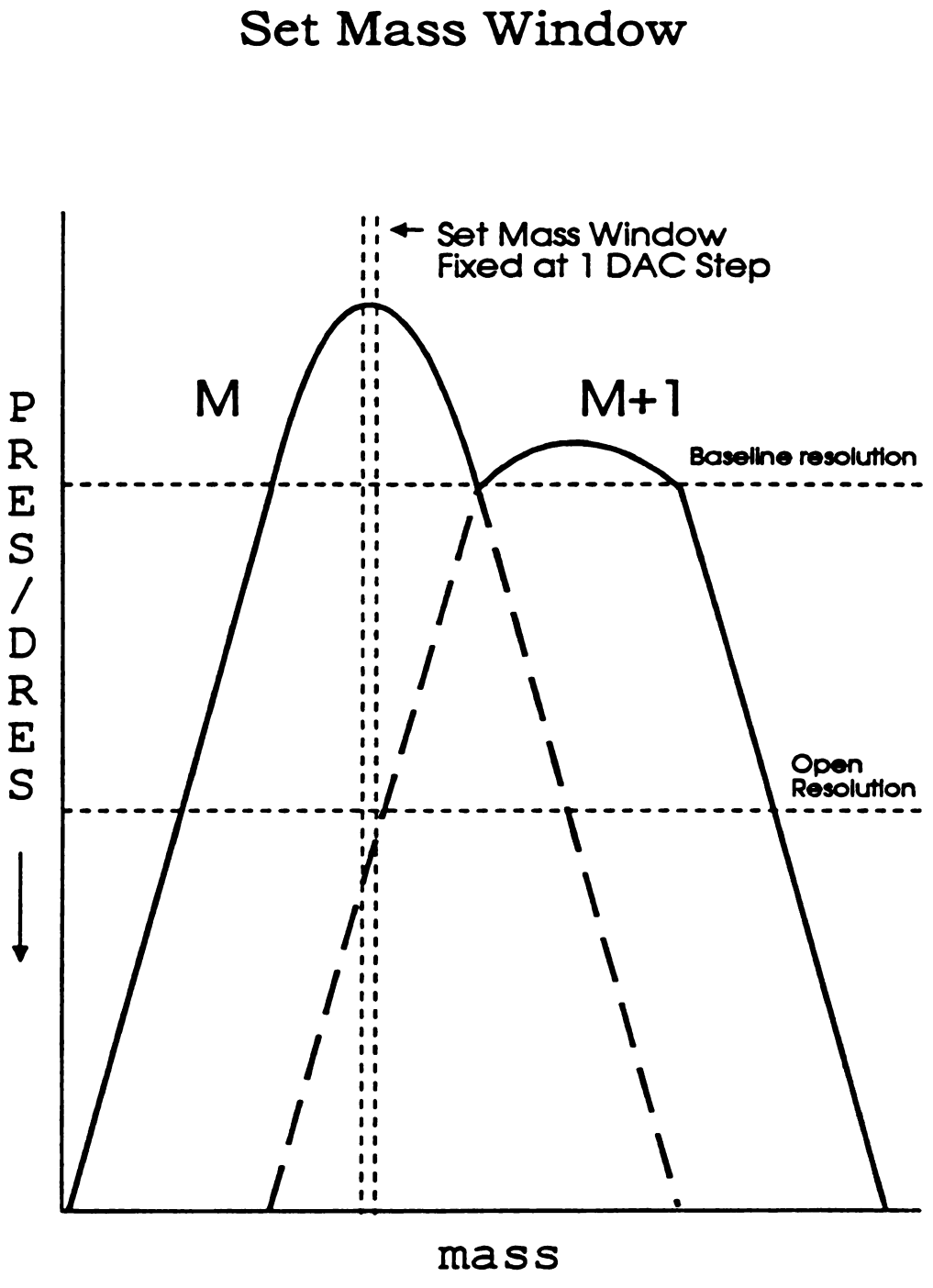

Figure 5.1. The relationship of an ion and its isotope indicating the significance of using the exact mass for a set mass in an MS/MS experiment and the effect on selectivity of under-resolving the instrument.

adjusted to maintain proper peak shapes. In all cases, after adjusting quadrupole resolution, the instrument was recalibrated.

Full Scan Acquisition. In many MS/MS experiments, the products are the result of collisionally-induced dissociation (CID), and as such, the product analyzer is scanned from low mass to a mass near (above or below) the precursor mass. Since this mass range can be up to several hundred u, scan times must be at least several hundred milli-seconds in order to sample the mass spectral data adequately. Typically, in order to achieve precision at the 95% confidence level with respect to peak height and area, at least 10 samples per peak are required. The TSQ-700 has a limit of approximately 12,000 samples per second. With chromatographic peaks of 2 to 3 seconds, the compromise is therefore between mass spectral and chromatographic sampling. Since the current work involved monitoring only a single reaction product at a time, all quantitative work was done in selected reaction mode (SRM) at relatively short scan times, and the compromise in sampling was not an issue.

Full scan acquisition was used to generate MS precursor spectra but not for quantitative purposes. As such, mass spectral sampling was always maintained at a minimum of 10 samples per ion. This resulted in only a few samples per chromatographic peak which were averaged for qualitative purposes. These spectra were used to determine the exact mass of the molecular anions to be used as percursors for the MS/MS experiments. While the exact precursor masses could be

and were calculated, using the precursor masses as observed allowed for daily drifts in calibration. Also, full-scan MS data provided information on precursor ions that could interfere in the selectivity of the ion-molecule reactions being studied.

As previously stated, only odd-electron molecular anions will undergo the exchange of oxygen for chlorine in an MS/MS experiment. Since all of the tetra, penta, hexa and heptachlorobiphenyls produce this anion in the MS source, all of the coplanar congeners of interest for this work are amenable to this reaction. Most congeners exhibit ions in the source that are the result of the loss of chlorine (loss of 35u). These ions are not amenable to the exchange reaction, however. The specificity of this reaction for PCB congeners with a given amount of chlorine substitution is challenged only when the formation of two species occurs in the source. As mentioned previously, when a radical molecular anion loses a chlorine atom in the source, the resultant ion is an even-electron species that will not undergo chlorine exchange with oxygen. If this ion, however, gains or loses a hydrogen atom, whether concerted with the loss of the chlorine atom or not, before being extracted from the source, the product is once again an odd-electron species that will undergo oxygen-chlorine exchange for a net loss of 19 mass units. This is illustrated in Figures 5.2 through 5.5, where the primary mass spectra of tetra- through heptachlorobiphenyls show that there is a net loss of 35 mass units (u) from the molecular anion for the tetrachlorobiphenyl congener and a net loss of 34 u for the homologs of pentachloro- and greater substitution. This was found to be true for all twelve of the coplanar congeners and was not affected by varying instrumental

parameters such as temperature and CI moderating gas pressure. Due to these regions of the spectra not exhibiting typical isotopic patterns for polychlorinated species, there is apparently also some loss of 35 and 36 mass units.

While this reaction is specific for a given amount of chlorine substitution, it may not be totally specific for a given PCB isomer class. It was thought that the problem of a net loss of 34u in the source from a molecular anion might be overcome with the appropriate choice of moderating gas, since the source of the attached hydrogen atom might be due to the moderating gas. By choosing a gas that contains no hydrogen (such as argon), this interference might be eliminated. However, when either argon or deuterated ammonia was used as a moderating gas, the net loss of 34u was still observed. This demonstrated that the source of the H was not the moderating gas. The loss of HCl from a molecular anion would still be an interference, but would only be a problem if the combination of abundance and response to this reaction exceeded a few percent of that of a co-eluting congener of interest.

Even though all twelve coplanar congeners exhibit a net loss of 34u in the source, the specificity of this method is compromised only if a congener that co-elutes with one of these twelve also loses 34u in the source. When four of these co-elutants *(Table 5.1)* were mass analyzed, three exhibited losses of 35u and one showed a loss of 34u as shown for three of the four congeners in Figures 5.6-5.8. This is consistent with results reported by others in that some congeners lose 34u in the source and others

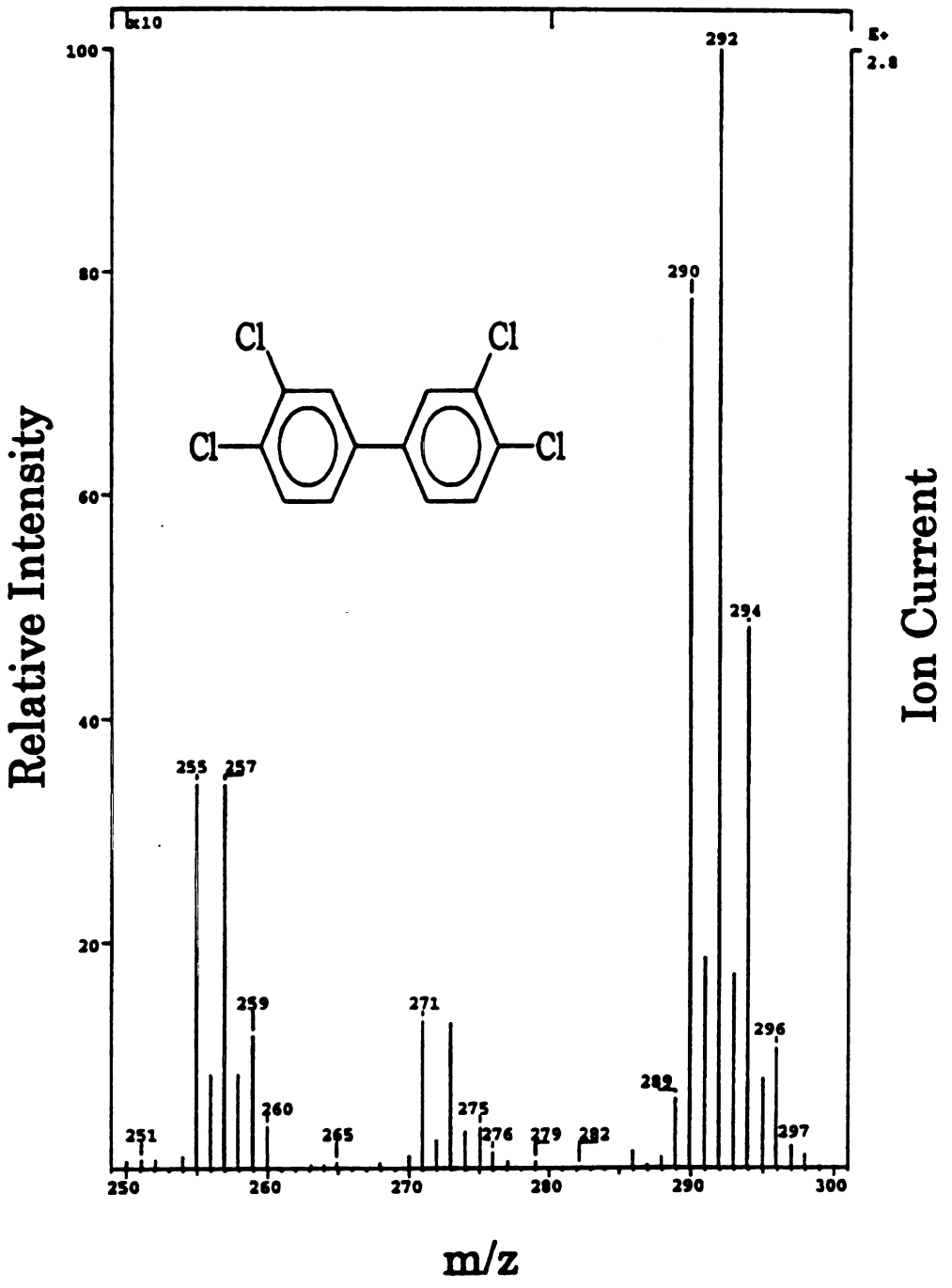

Figure 5.2. The ECNI mass spectrum of a tetrachlorobiphenyl indicating the loss of 35u in the ion source.

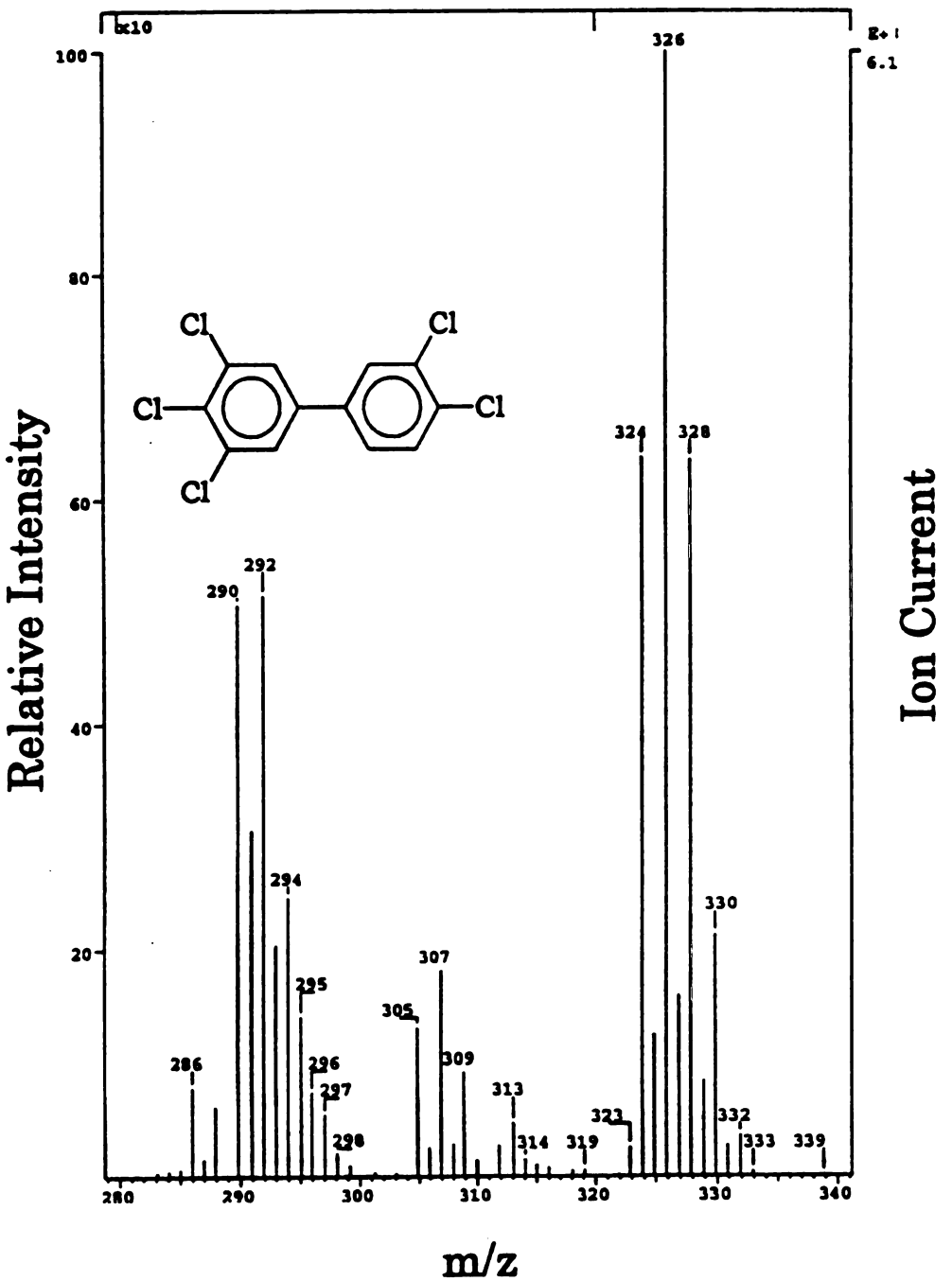

Figure 5.3. The ECNI mass spectrum of a pentachlorobiphenyl indicating the loss of 34u in the ion source.

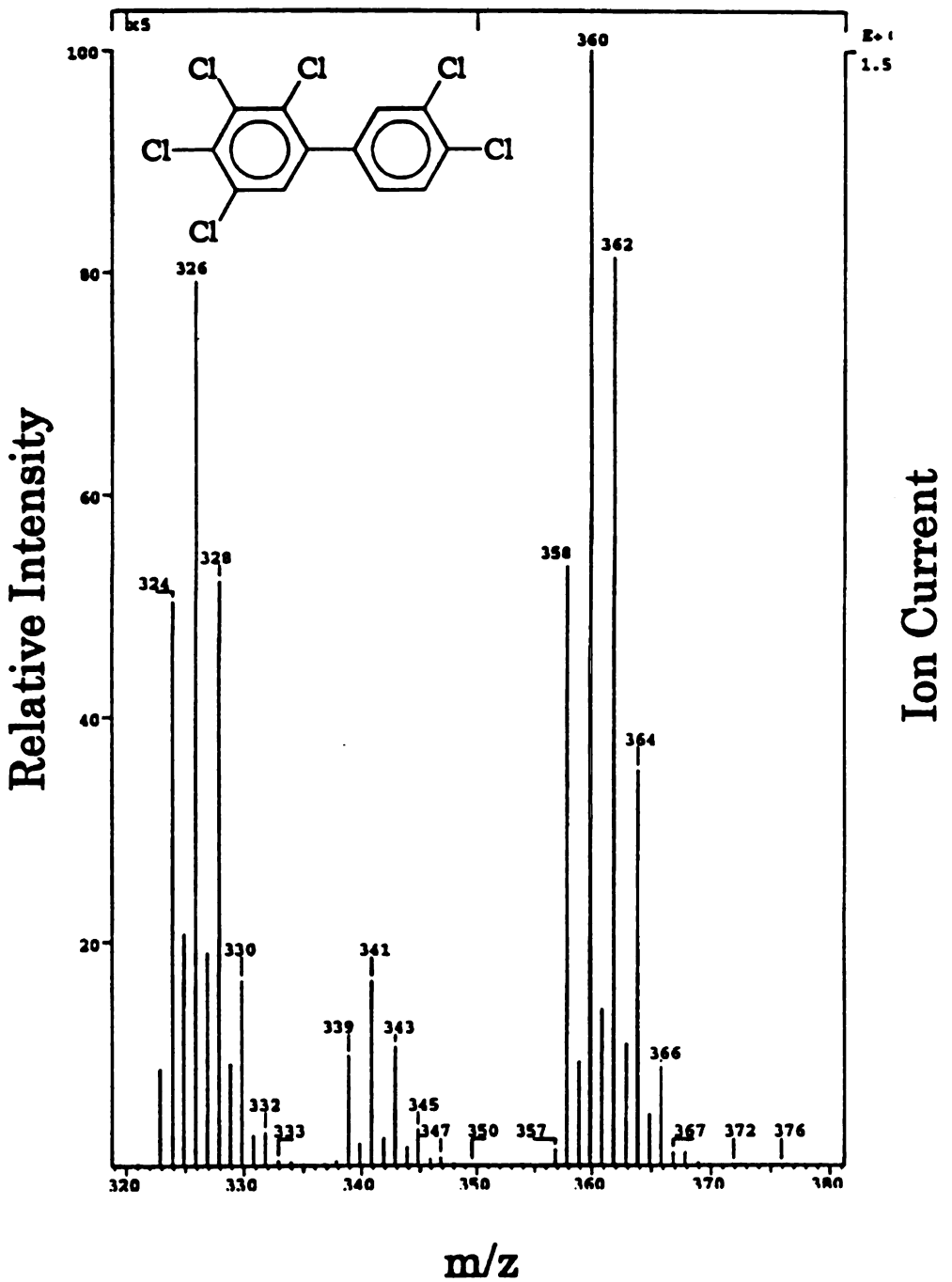

Figure 5.4. The ECNI mass spectrum of a hexachlorobiphenyl indicating the loss of 34u in the ion source.

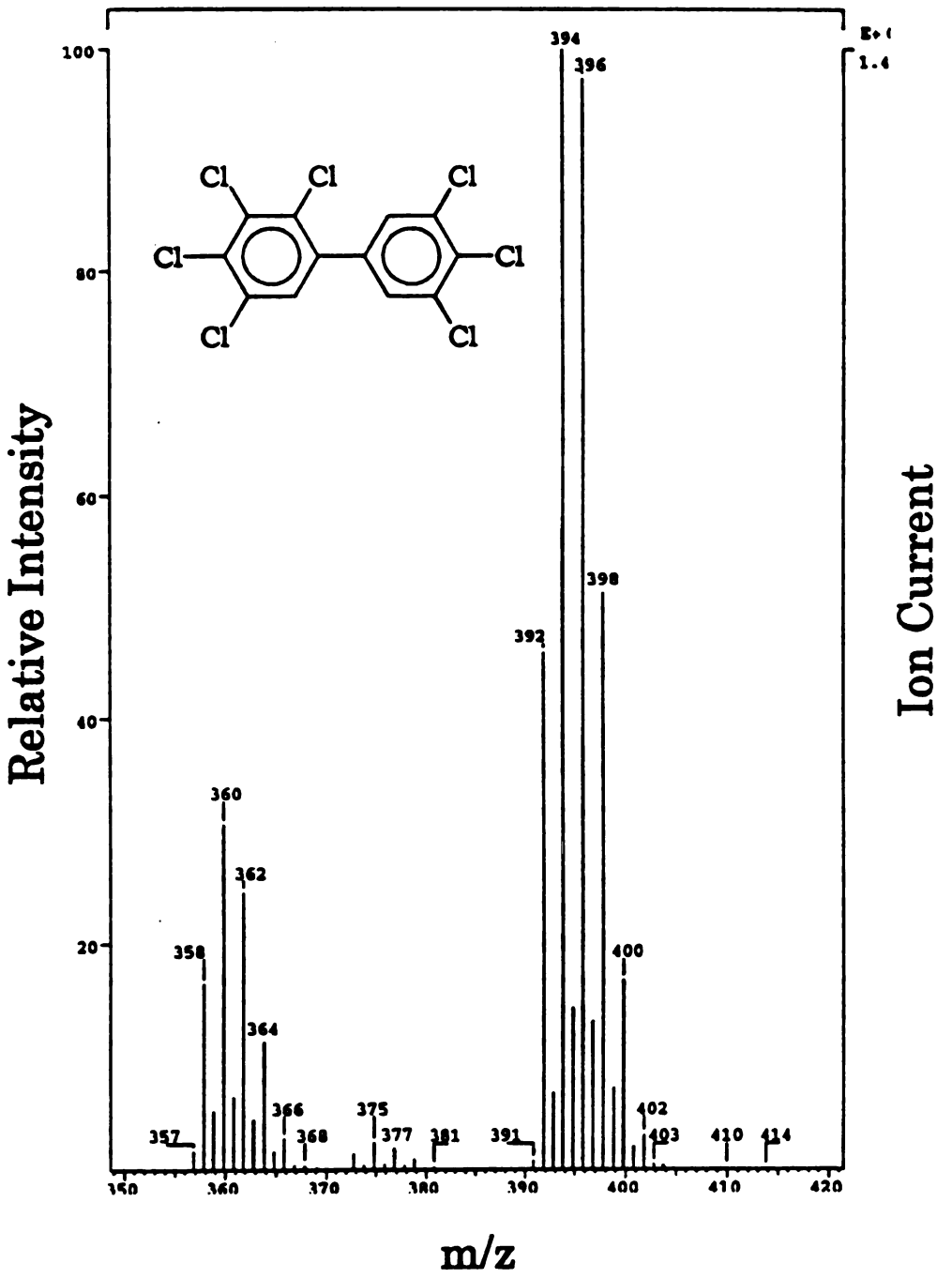

Figure 5.5. The ECNI mass spectrum of a heptachlorobiphenyl indicating the loss of 34u in the ion source.

Table 5.1. The structure of the twelve PCB congeners that have been shown to exhibit dioxin-like toxicity and those congeners that co-elute. Relative retention times (RRT) are with respect to octachloronaphthalene *(77)*.

IUPAC	TWELVE TOXIC CONGENERS	CO-ELUTANTS	RRT
81	345,4-CB	23456-CB 2356,4-CB 345,26-CB 2346,26-CB	.6149
77	34,34-CB	236,34-CB	.6295
123	345,24-CB	236,245-CB	.6658
118	245,34-CB		.6693
114	2345,4-CB	2346,23-CB 345,23-CB	.6828
105	234,34-CB	234,236-CB 245,245-CB	.7049
126	345,34-CB	2345,23-CB 2356,235-CB	.7512
167	245,345-CB		.7814
156	2345,34-CB	2346,234-CB 2356,2356-CB	.8105
157	234,345-CB	23456,23-CB 2346,2356-CB	.8184
169	345,345-CB		.8625
189	2345,345-CB		.9142

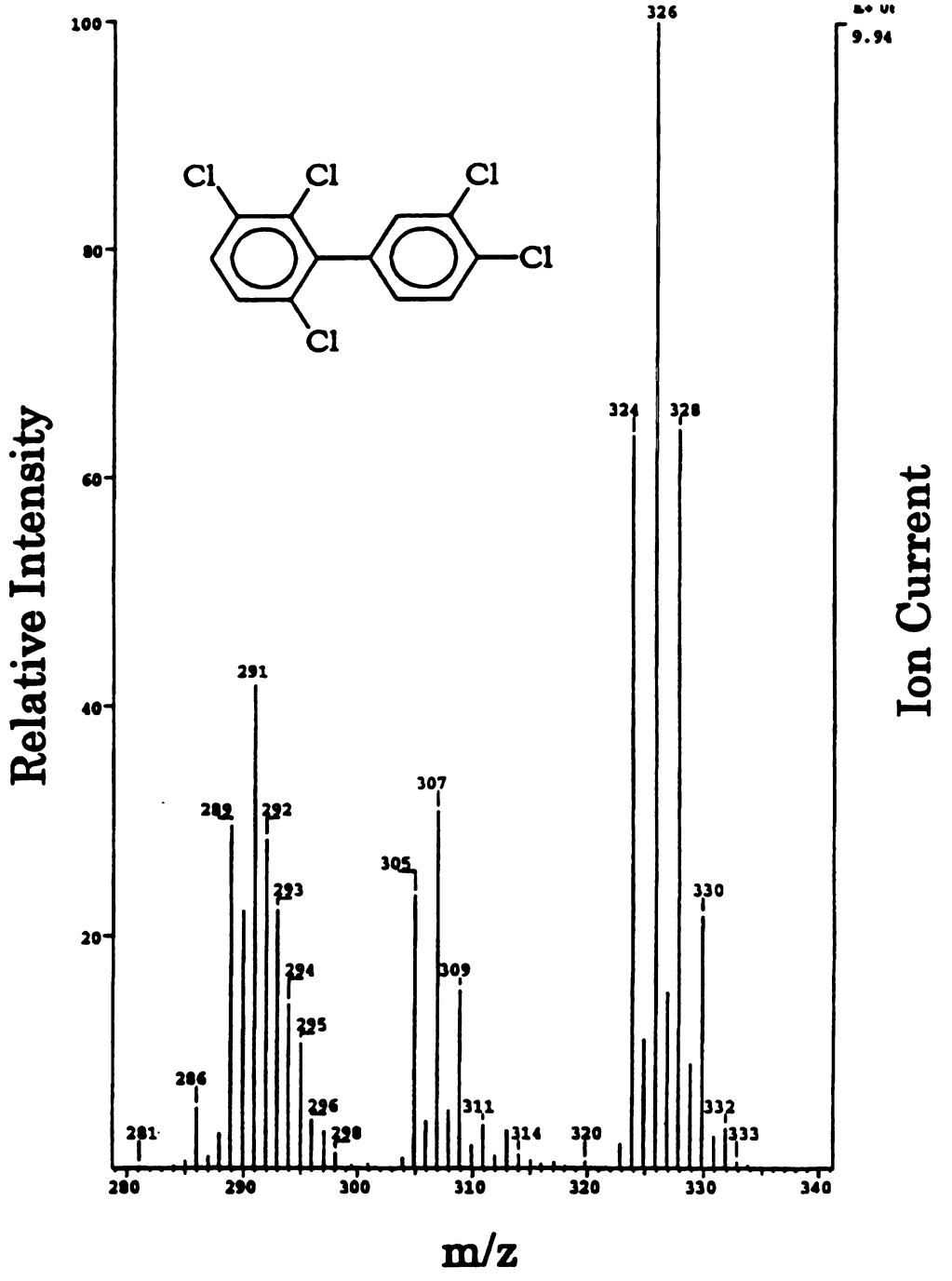

Figure 5.6. The ECNI mass spectrum of a pentachlorobiphenyl which co-elutes with a coplanar congener in a GC analysis. This congener exhibits the loss of 35u in the source as opposed to the loss of 34u observed with the coplanar congeners.

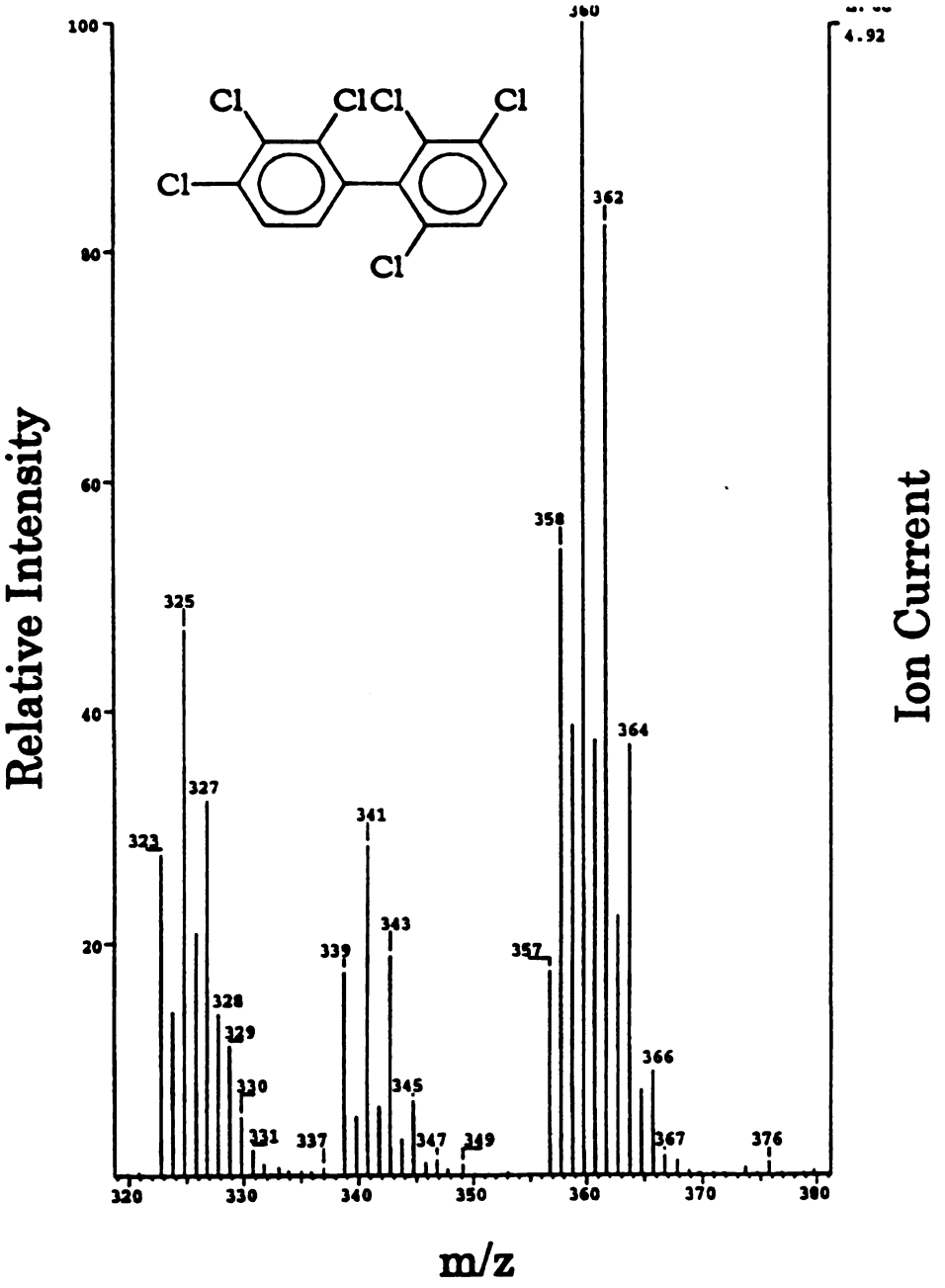

Figure 5.7. The ECNI mass spectrum of a hexachlorobiphenyl which co-elutes with a coplanar congener in a GC analysis. This congener exhibits the loss of 35u in the source as opposed to the loss of 34u observed with the coplanar congeners.

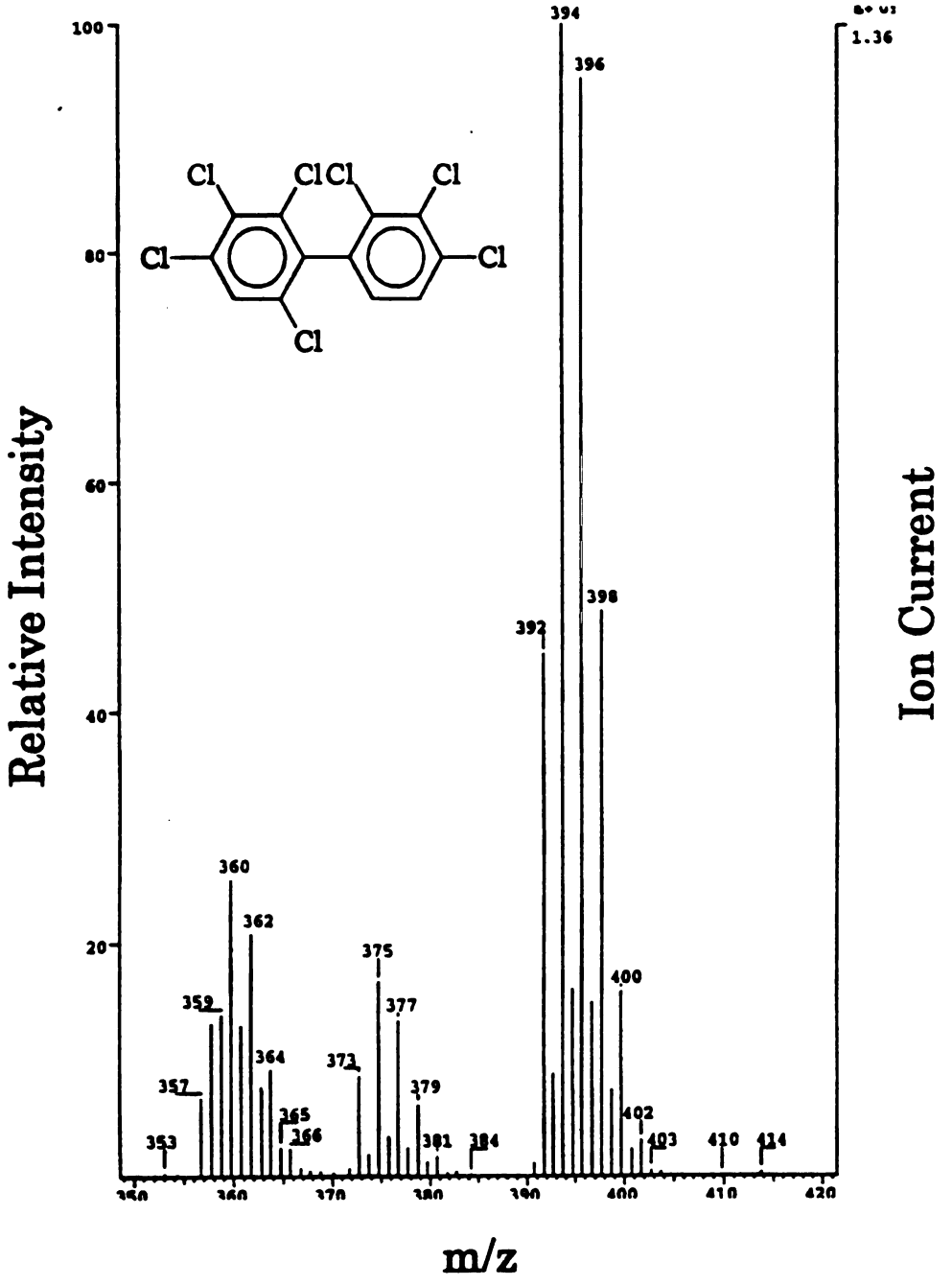

Figure 5.8. The ECNI mass spectrum of a heptachlorobiphenyl which co-elutes with a coplanar congener in a GC analysis. This congener exhibits the loss of 34u in the source, and as such, interferes in a GC/MS/MS analysis of coplanar congeners.

lose 35u *(23)*. When these congeners are analyzed in a GC/MS/MS experiment by monitoring the products of reactions with oxygen, the ions that result from a loss of chlorine in the source do not react while those ions that result from a net loss of 34u in the source do react. These phenomena are represented in the results shown in Figure 5.9. As is shown, the heptachlorobiphenyl, which loses 34u in the source, exhibits the products of several reactions. Each ion from a subsequent loss of chlorine and gain of hydrogen readily undergoes reaction with molecular oxygen. The penta- and hexachlorobiphenyl congeners, however, produce only odd-electron molecular anions and even-electron fragmentation products in the source, and as such, only undergo one reaction.

Selected Reaction Monitoring. In MS/MS selected reaction monitoring (SRM) is analogous to selected ion monitoring (SIM) in MS. These modes of detection are generally used for quantitation with chromatographic experiments. For MS analyses, SIM provides a significant improvement in signal-to-background with a loss in selectivity. For MS/MS, either full-scan or SRM, a reduction in selectivity may not always be the case since the MS/MS reaction may be specific for a given analyte.

Using an Aroclor 1242 standard, the oxygen-for-chlorine exchange reaction was monitored for all 10 homologous series in separate experiments *(Figures 5.10-5.16)*. No reactions were observed for the mono-, dichloro- and decachlorobiphenyls and very little for the trichlorobiphenyls. This also supports the premise that these congeners form almost exclusively $[M-H]^-$ in the ion source, and being even-electron

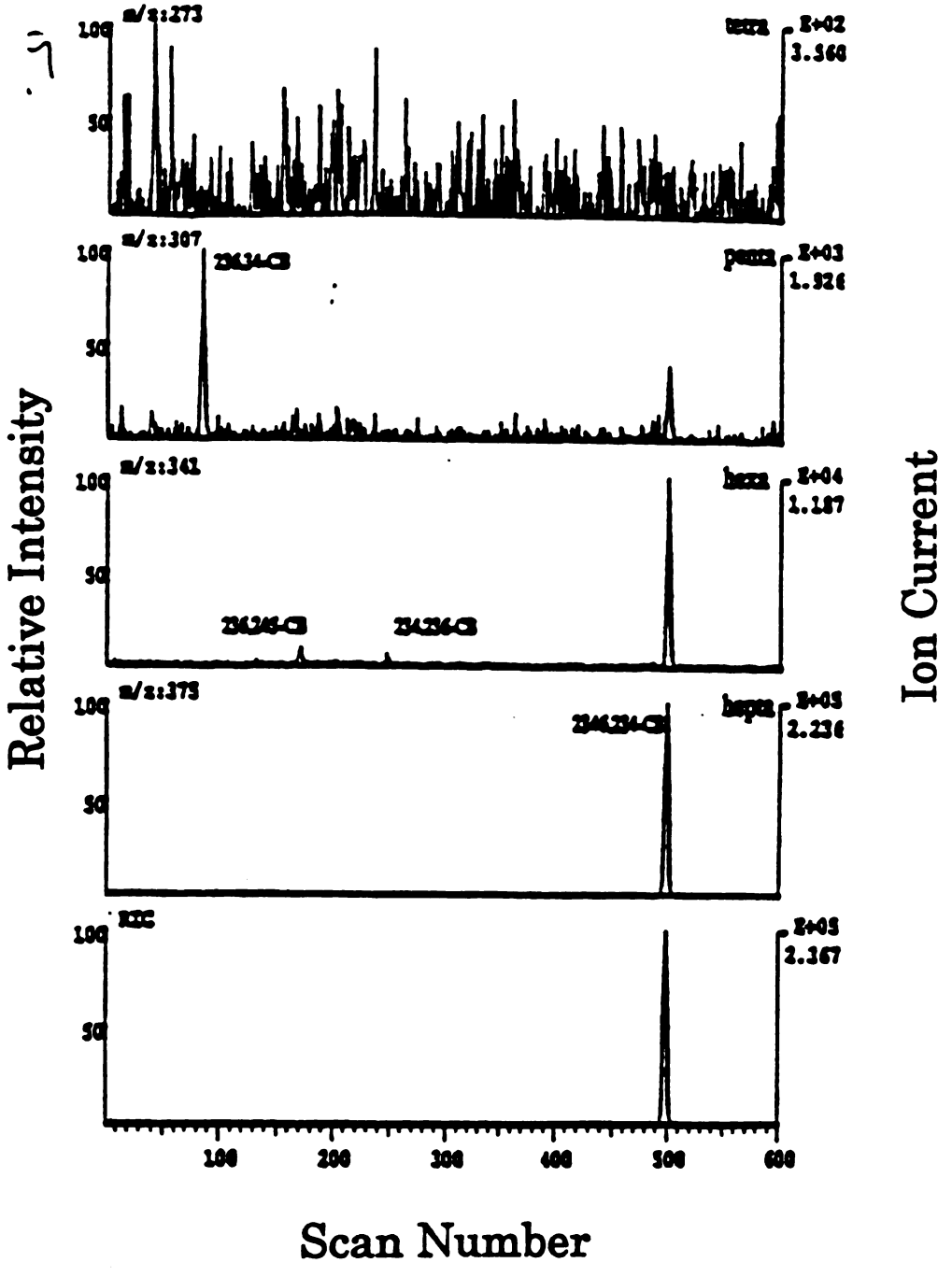

Figure 5.9. Mass chromatograms at m/z 273, 307, 341 and 375 and a reconstructed total ion chromatogram (RIC) that represent the analysis by GC/MS/MS of four PCB congeners that co-elute with coplanar congeners.

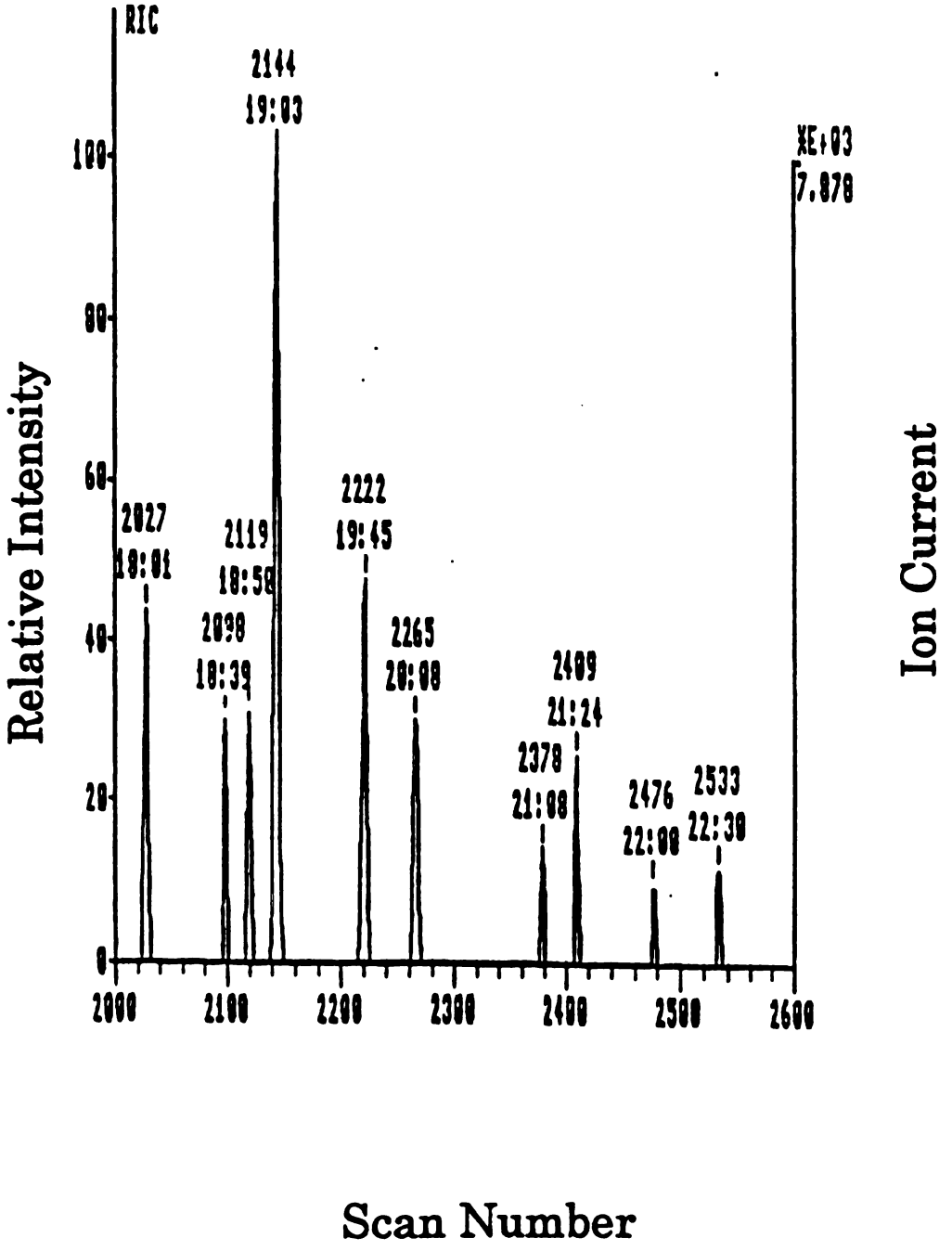

Figure 5.10. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for trichlorobiphenyls in an Aroclor 1242 standard.

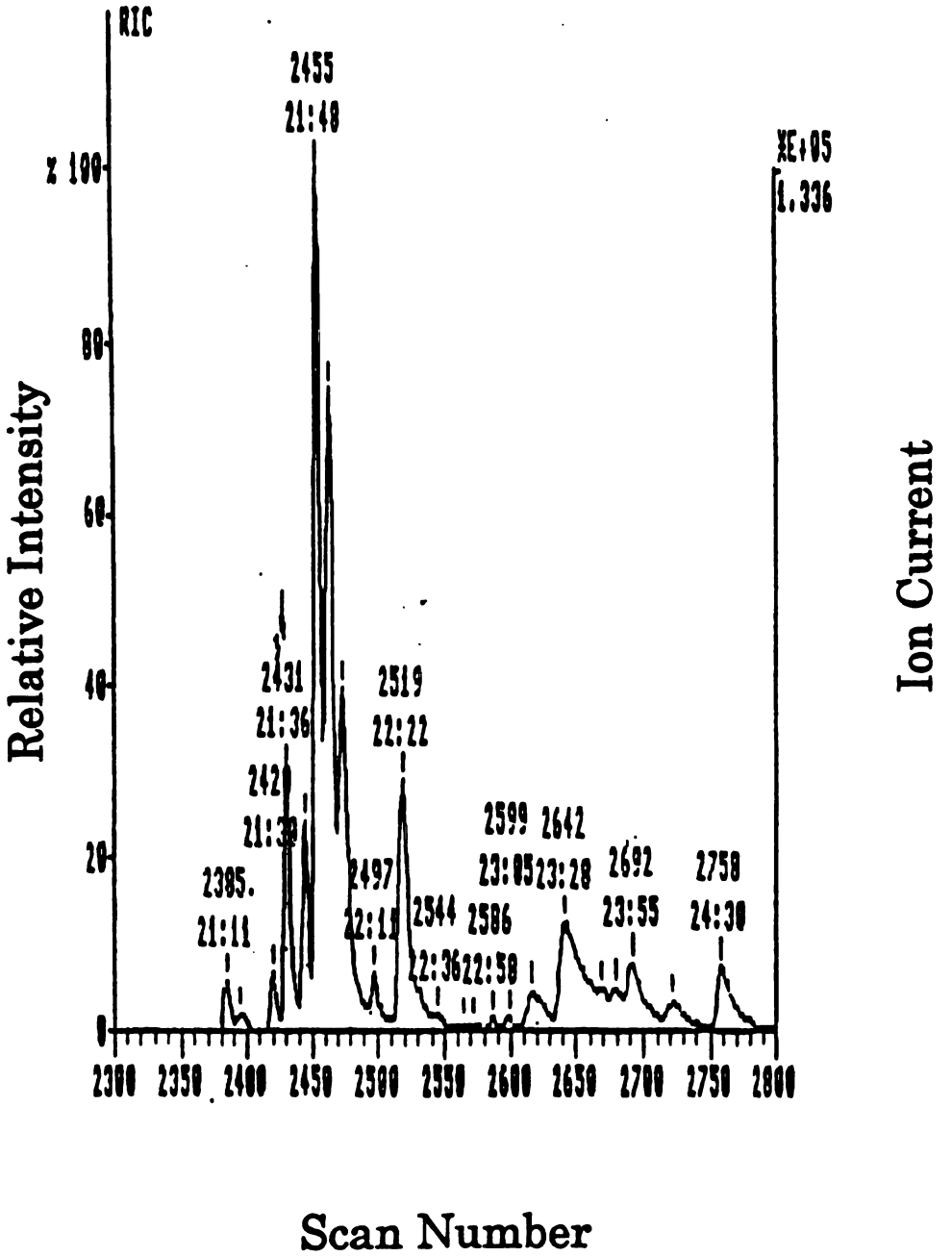

Figure 5.11. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for tetrachlorobiphenyls in an Aroclor 1242 standard.

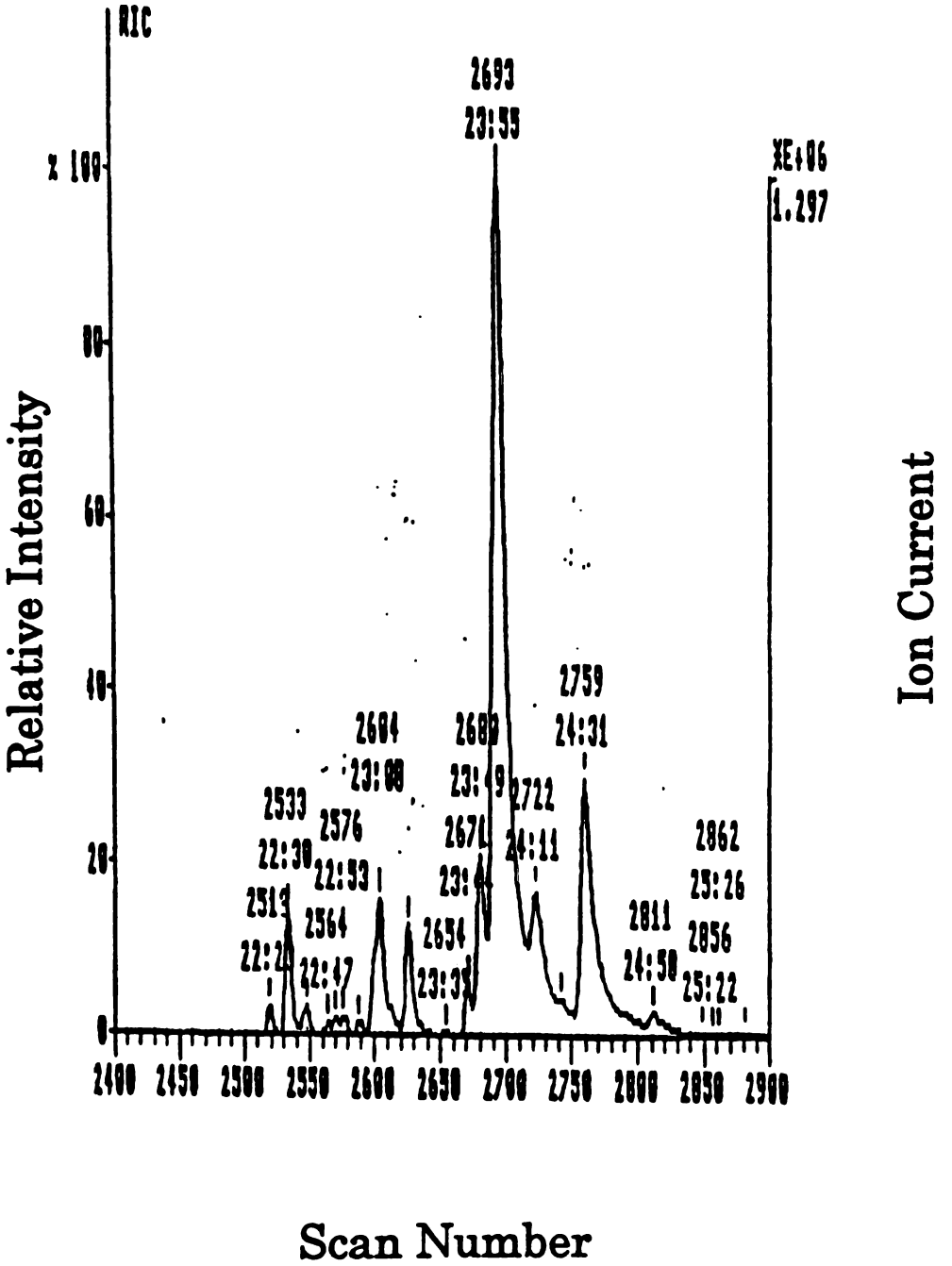

Figure 5.12. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for pentachlorobiphenyls in an Aroclor 1242 standard.

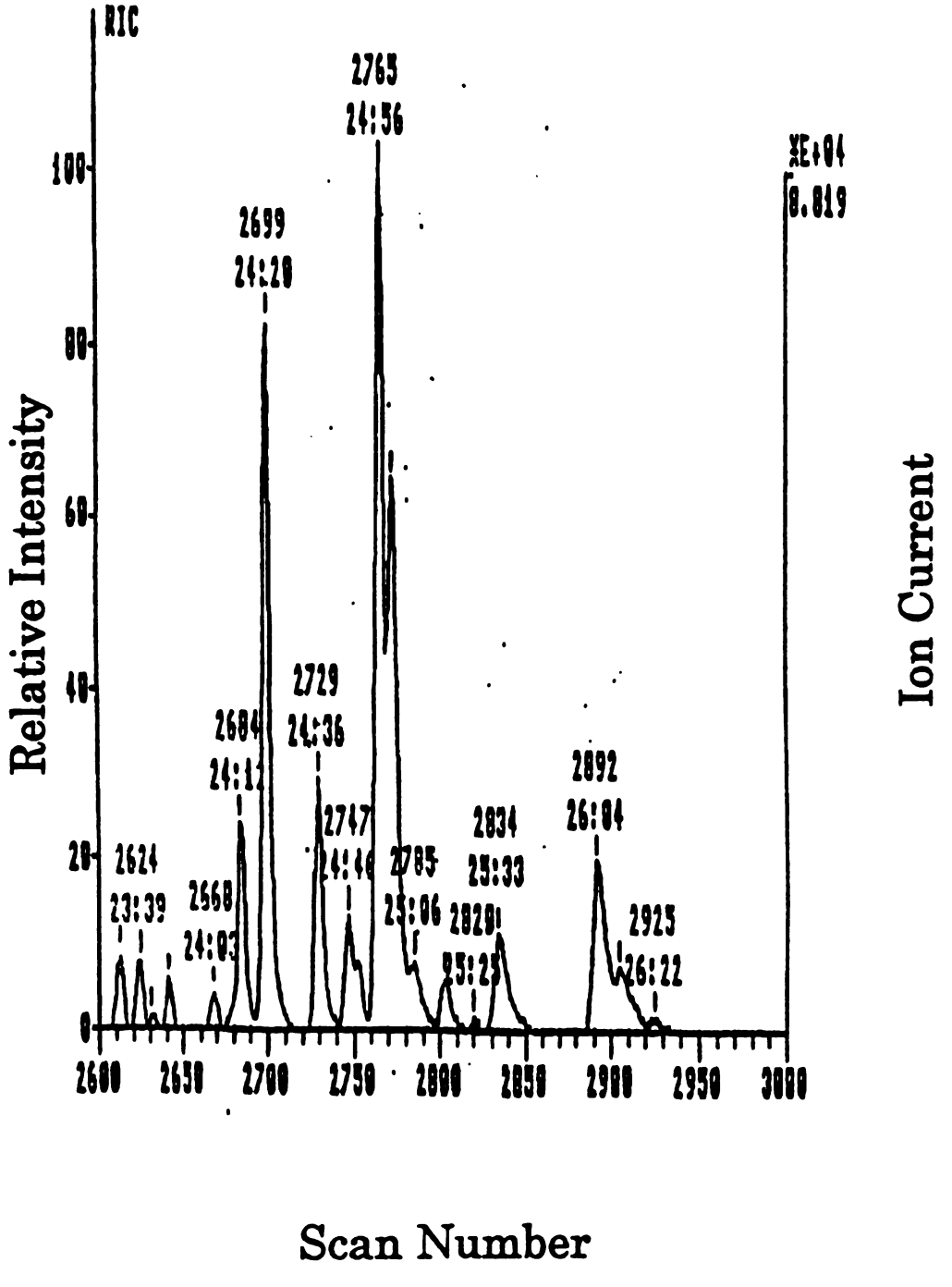

Figure 5.13. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for hexachlorobiphenyls in an Aroclor 1242 standard.

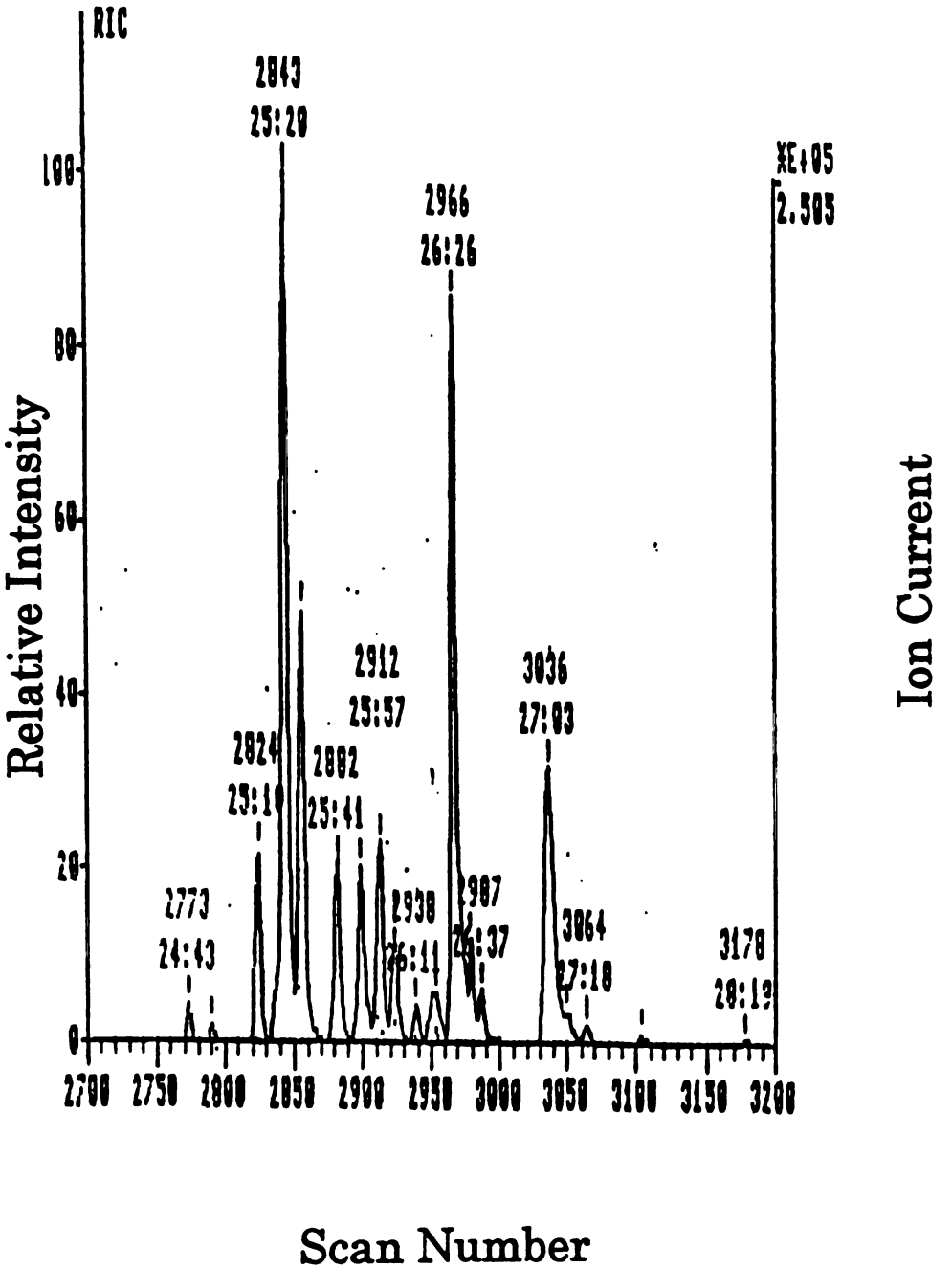

Figure 5.14. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for heptachlorobiphenyls in an Aroclor 1242 standard.

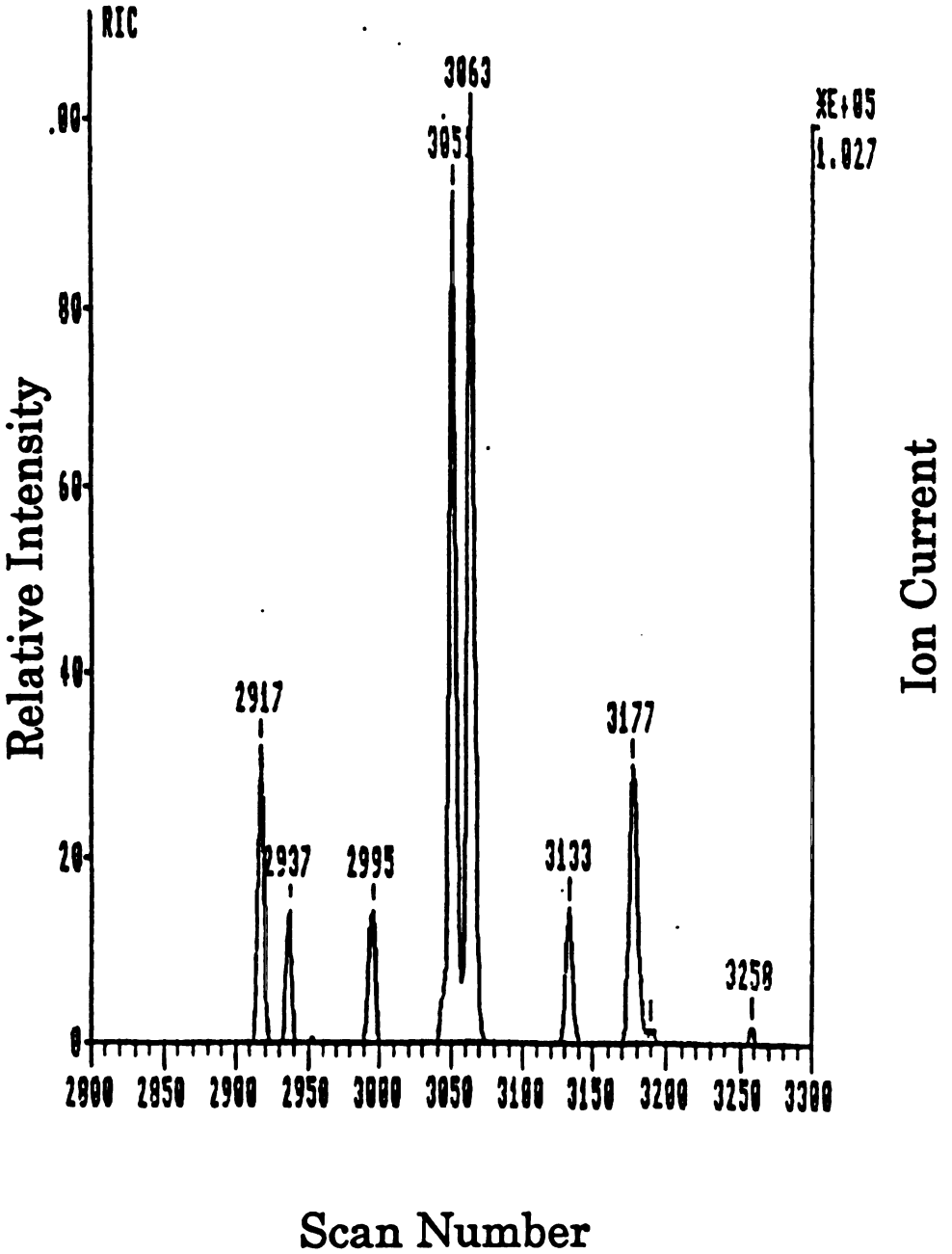

Figure 5.15. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for octachlorobiphenyls in an Aroclor 1242 standard.

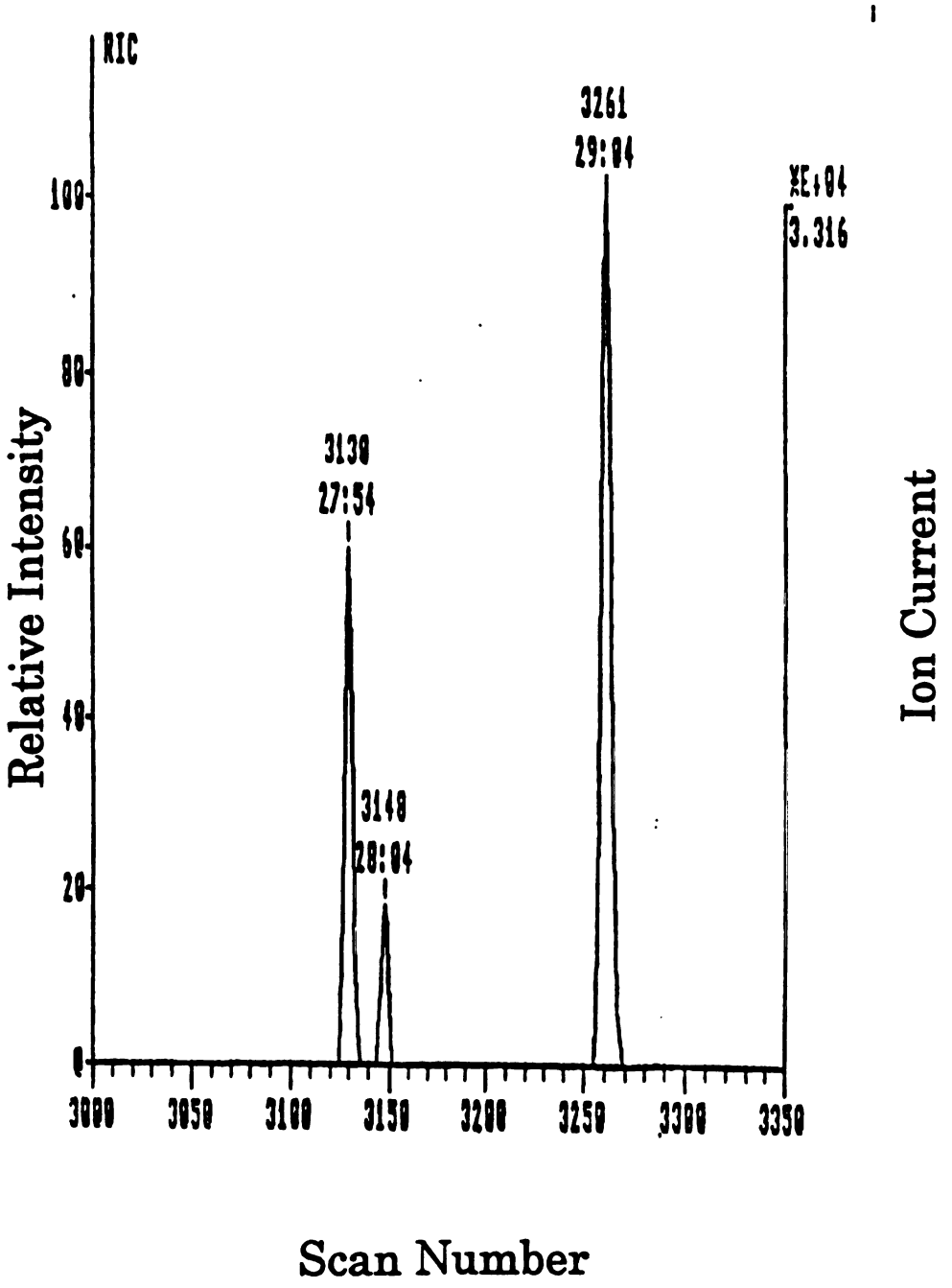

Figure 5.16. The mass and reconstructed total ion current (RIC) chromatograms from a GC/MS/MS experiment where the exchange reaction of oxygen for chlorine was monitored for nanochlorobiphenyls in an Aroclor 1242 standard.

anions, do not undergo this reaction. In addition, the presence of the three nanochlorobiphenyls is observed with a substantial amount of reaction product. These congeners are typically not able to be detected, much less quantitated, using ECD or MS modes of detection with this Aroclor.

Since the two choices in MS/MS are using each analyzer to either select a specific ion (set mass) or to scan, there are four modes of MS/MS —— product scans, precursor scans, constant neutral loss (or gain) scans and SRM. The TSQ-700 is under total digital control, and as a result, unlike many analog-controlled instruments, the instrument must be scanned in order to function. Thus, for SRM experiments, the TSQ-700 is operated in one of the other three modes but scanned over a very narrow mass range. This narrow mass range scanning may or may not actually involve analyzer voltages changing depending on the width of the scan window and the resolution of the DAC control of the voltages.

In order to determine which mode of MS/MS scanning —— product, precursor or neutral loss —— offered best sensitivity and precision, several scans of each mode were acquired for the given oxygen-chlorine exchange reactions. In all cases, regardless of the instrument tune used, the product scan mode was shown to exhibit maximum sensitivity *(Figure 5.17)*. There is no inherent reason that one mode should necessarily be more sensitive than the other two for SRM experiments. However, in almost all cases, the product analyzer (Q3) on a TSQ-700 is more sensitive than the precursor quadrupole (Q1). This is generally due to the better quadrupole rod

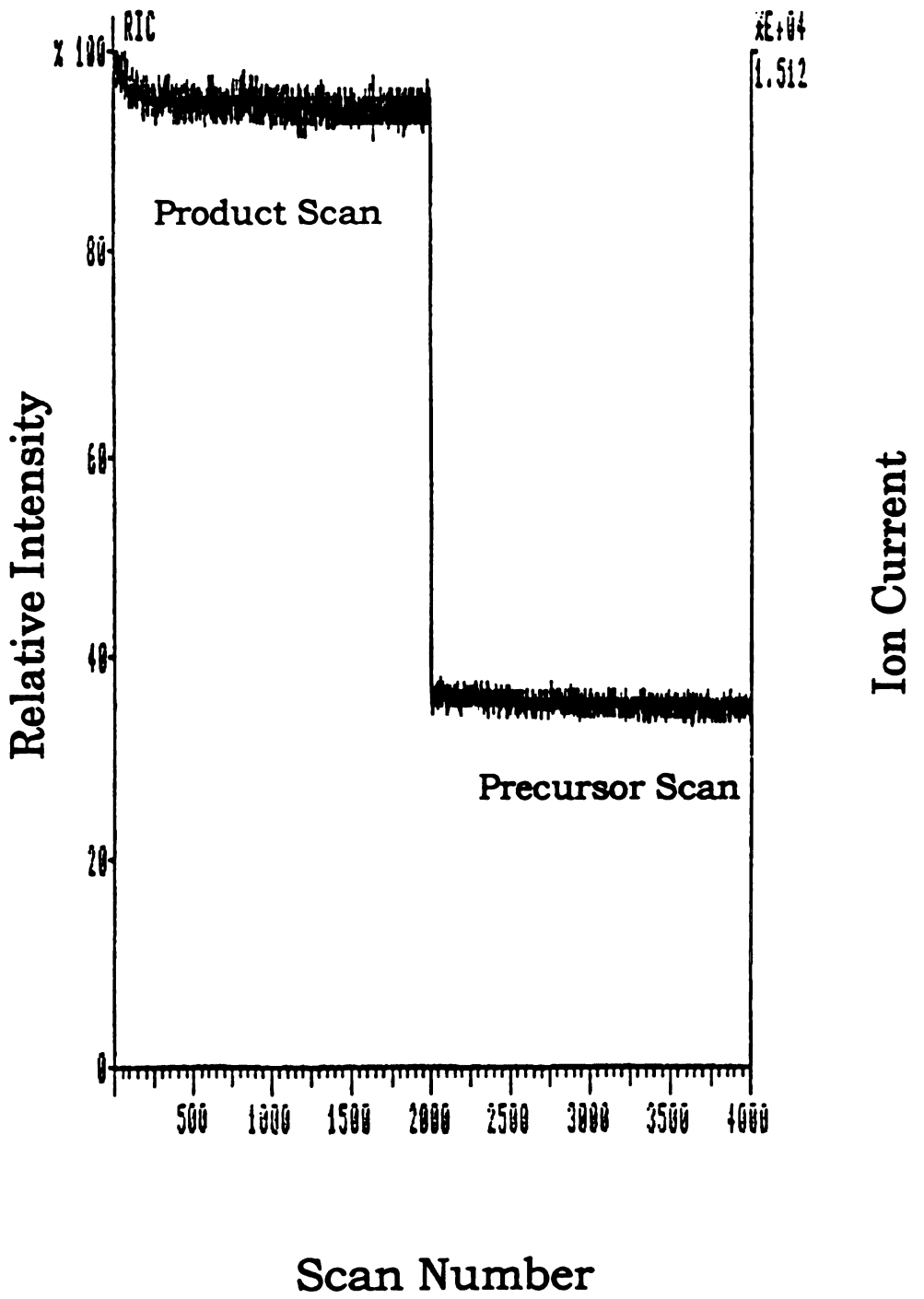

Figure 5.17. The reconstructed total ion current (RIC) chromatogram of a GC/MS/MS experiment using product and precursor scanning of the instrument for the same reaction indicating the product scan mode to be the more sensitive.

assembly being used as Q3. This is desired since most MS/MS experiments are product scans, and a better analyzer is required for scanning than for a set mass filter. Also, Q3 is always less contaminated than Q1.

Since product scans were used for SRM, it was necessary to select the size of the Q3 scan window. Generally, to a limit, as the size of the window is narrowed, an enhancement in signal to background is observed. The limit is a function of the stability of the instrument calibration. The specifications of this stability for the TSQ-700 is ±10 mmu over 8 hours. This is also supported from experiments that showed best signal-to-background when the scan window was maintained from 50 to 100 mmu. Smaller windows resulted in a significant increase in signal noise due to scan-to-scan variation in the observed mass of the peak. Due to the method that is used by the TSQ-700 to sample and sum to registers the data, the apparent signal actually decreases as the scan window is narrowed. Also, the stability of the instrument calibration required that the precursor set mass be defined to the nearest 10 mmu.

The other variable studied was the SRM scan time. On a TSQ-700, the scan time for SRM is the actual time spent sampling ion current. The inter-scan time is then the sum of the scan time plus the voltage settling time plus the time of the electronic overhead. As stated previously, with SRM very rapid scan cycling is possible such that the compromise between chromatographic sampling and MS sampling is not an issue. Using scan times of 10 to 50 ms and voltage settling times of 5 to 10 ms

provided optimum signal to background with adequate sampling of both even when cycling rapidly between reaction products. Faster scan times require the voltage settling to be increased to eliminate memory or carry-over in the signal such that the overall duty cycle suffers. Also, with very rapid scanning, ion flight time corrections become an issue. The capability exists to correct for this, but this is only necessary when chromatographic sampling is an issue in SRM such that extremely rapid scanning of the instrument is necessary.

Quantitation. Many PCB analyses are performed chromatographically using octachloronaphthalene as an internal standard. For ECD or MS routines, octachloronaphthalene is a reasonable choice since it is chromatographically resolvable from PCB congeners and exhibits a response factor of the same order of magnitude as that of the PCB congeners. This or any single internal standard may not be the best choice, however, for the oxygen-chlorine exchange reaction. While octachloronaphthalene will undergo chlorine exchange with oxygen, response factors for PCB congeners vary more than two orders of magnitude with respect to this internal standard.

Relative Response Factors. The relative response factors with respect to octachloronaphthalene for several congeners that undergo chlorine exchange with oxygen have been determined, and these vary from approximately one to five hundred *(Table 5.2)*.

Table 5.2. The response factors of several PCB congeners with respect to octachloronaphthalene for a GC/MS/MS experiment where the products of the reaction of molecular ions with oxygen are monitored.

IUPAC	CONGENER	RRF
77	34,34-CB	1.6
85	234,24-CB	290
105	234,34-CB	18
109	236,34-CB	88
118	245,34-CB	11
126	345,34-CB	14
132	234,236-CB	94
149	236,245-CB	190
153	245,245-CB	130
156	2345,34-CB	14
169	345,345-CB	6.0
176	2346,236-CB	48
182	2346,245-CB	500
197	2346,2346-CB	130
199	2345,2356-CB	580
205	23456,345-CB	160
206	23456,2345-CB	230
207	23456,2346-CB	220
208	23456,2356-CB	120

Internal Standards. Since the response factors vary dramatically with the degree and position of chlorine substitution, it was of interest to explore using other or multiple internal standards for these analyses. It was desired to find at least one chlorinated aromatic compound that undergoes this reaction, does not co-elute with any of the PCB congeners of interest, and exhibits a response to this reaction, two to three orders of magnitude greater than that of octachloronaphthalene.

Chlorinated Analogs. The first species chosen as an additional internal standard was pentachlorobenzene. This standard elutes in a PCB chromatographic analysis between the solvent (hexane) and the monochlorobiphenyls, and undergoes the oxygen-chlorine reaction with a response approximately 500 times that of octachloronaphthalene. Thus, this standard meets all of the desired criteria and would seem to be an ideal choice. However, on both of the columns generally used for PCB analyses —— 5% phenyl and 5% phenyl/1% vinyl methyl silicones —— pentachlorobenzene exhibits excessive tailing for many GC temperature programs. This was thought to be the result of some condensation phenomenon occurring in the injection port or transfer line; and some improvement in peak shape was observed when the temperatures of the injection port and transfer line were both increased to 300C. The tailing was not decreased sufficiently, though, to allow for reproducible responses to be observed. Since this is the upper temperature limit of these stationary phases, increases in temperature were not feasible. By using lower initial temperatures, tailing was reduced significantly, but extended analysis times were necessary. This would indicate that instead of condensation occurring, thermal

breakdown of the molecule was occurring in the injection port.

The next molecule chosen as a candidate for use as an additional internal standard for a multiple internal standard scheme was aldrin. This is a hexachloro-, tetracyclic compound that meets all of the aforementioned desirable chromatographic and response properties. In addition, under the conditions favorable for PCB analyses, chromatographic peak shapes are acceptable. Unfortunately, this molecule for the most part undergoes dissociative electron capture ionization, and as such, not enough $[M]^{-\cdot}$ is produced to allow for its use as an internal standard. Other chlorinated species examined were dieldrin, heptachlor, and heptachlor epoxide. These species also were unusable due to the same problems associated with aldrin.

Isotope Dilution. Many MS methods of analyses for PCBs utilize isotopic dilution as a means of providing for an internal standard for quantitation *(24)*. It was decided that this would also be the best choice for this method. Since the response of most congeners to the oxygen/chlorine exchange reaction generally increases with the amount of chlorine substitution, three ^{13}C-labelled congeners (34,34-CB, 345,34-CB and 345,345-CB) were chosen to be used as internal standards. The responses of the twelve coplanar congeners with respect to these labelled standards are listed in Table 5.3.

Summary. The aforementioned experiments resulted in the following observations for setting instrumental parameters to observe the oxygen-chlorine exchange

Table 5.3. The responses of the twelve coplanar PCB congeners with respect to the three ^{13}C-labelled standards.

		Relative Response Factors		
IUPAC #	Congener	^{13}C-#77	^{13}C-#126	^{13}C-#169
81	345,4-CB	1.7	0.15	0.097
77	34,34-CB	0.82	0.087	0.055
123	345,24-CB	7.7	0.77	0.48
118	245,34-CB	7.1	0.71	0.45
114	2345,4-CB	8.1	0.77	0.49
105	234,34-CB	7.7	0.76	0.48
126	345,34-CB	11	1.1	0.72
167	245,345-CB	55	5.5	3.5
156	2345,34-CB	56	5.5	3.5
157	234,345-CB	62	6.1	3.8
169	345,345-CB	15	1.5	0.95
189	2345,345-CB	56	5.5	3.5

reaction as a chromatographic detection scheme for quantitative purposes:

- The mass resolution may be decreased with the instrument tune offering an improvement in signal but still allowing for mass specificity.

- Product scanning is the more sensitive scan mode for SRM.

- Scan windows in SRM of 50 to 100 mmu offer optimum signal to background.

- Scan times of 10 to 50 ms allow for adequate chromatographic and mass spectral sampling.

- Voltage settling times of 5 to 10 ms allow for the elimination of memory effects with a minimum compromise in duty cycle.

- Using isotopically-labelled PCBs as internal standards met all the desirable characteristics of internal standards. The only disadvantage as a general method may be in some cases price and availability.

Chapter 6

Application of the MS/MS Method using the Oxygen-Chlorine Exchange Reaction for the Analysis of Polychlorinated Biphenyls in River Sediment Samples

The analytical method using the oxygen-chlorine exchange of PCBs was applied to the quantitative analysis of river sediment samples. These samples were studied for the purpose of determining the amount of dechlorination that had occurred under anaerobic experimental conditions. The dioxin-like toxicity of these samples was determined for those samples that had been innoculated with dechlorinating microbes and those that had been autoclaved. The toxicity levels were calculated based on the concentrations of the twelve PCB congeners that had been shown to exhibit dioxin-like toxicity. This method was used since the dioxin-like toxicity of PCB-containing samples had been shown to derive almost solely from these congeners *(100)*.

Introduction. Those parameters that were studied for the application of this method to the analysis of PCB-containing sediment samples included the preparation and

analysis of PCB standards using ^{13}C-labeled internal standards, the precision and accuracy of the method and the presence of possible analytical interferences. The quantitative results for several sediment samples of varying sources are also presented. As a preface to this discussion, the experimental parameters used for sample preparation and instrumental analysis are provided.

Experimental. The Aroclor standards were provided by John Quensen, and all other individual congeners and standards were provided by George Frame. All standards were prepared by dilution with Organic Residue Analysis grade hexane (J.T. Baker). River sediment samples spiked with Aroclor 1242 (ppm levels) were extracted with 50/50 acetone/hexane. Each extract was subjected to Florisil clean-up and then shaken with mercury for sulfur removal and finally concentrated to ten milliliters. While these clean-up procedures are not necessary for sample analysis by GC/MS/MS, this was done such that these samples could also be analyzed by GC/ECD.

Gas Chromatography. All gas chromatographic methods were performed with a Varian 3400 gas chromatograph equipped with a 30 meter x 0.250 mm column of 0.25 mm DB-1 phase (methyl silicone, J & W Scientific). The GC was temperature programmed —— 40 C for two minutes, 40 C to 160 C at 20 C per minute, 160 C to 270 C at 5 C per minute and held at 270 C for seven minutes. Helium was used as the GC carrier gas. The Varian 3400 was equipped with an injector body that accommodates both split and splitless modes of injection. All experiments were

performed using the splitless mode of injection with the split valve closed for the initial two minutes and open for thirty minutes. The injector was held at 270 C and the transfer line held at 275 C.

Mass Spectrometry. Early MS and MS/MS experiments were done with a Finnigan TSQ-70B mass spectrometer operated in a negative ion mode. Later, the TSQ instrument was upgraded to a TSQ-700. The pertinent changes included the installation of an octapole as a collision cell (the TSQ-70 instruments use a quadrupole) and replacing the PDP-11/73 data system with a DEC Station. Ammonia was used as the chemical ionization reagent gas. The CI reagent gas pressure was set to approximately 9500 mTorr as measured by the source Convectron gauge and was optimized daily using perfluorotributylamine. Pure oxygen was introduced into the collision cell. The source was held at 150 C and the manifold at 70 C. All reactions were generated using electron energies of 50 eV, collision offset energies of 1-2 eV (laboratory), and collision pressures from 0.5 mTorr to 1.5 mTorr.

Using perfluorotributylamine (PFTBA), two instrumental tunes were generated, one at unit resolution and one with open resolution. The open resolution tune was generated with Q1 being approximately twice as under-resolved (percent valley) as Q3. All MS experiments were performed in the Q1MS mode using the baseline-resolved tune, and all MS/MS experiments performed with the open-resolution tune.

The instrument utilizes a 20-kV conversion dynode that may be set from 3-20 kV, and an electron multiplier that may be set from 0-3000 volts. For ions less than approximately 200 u, the gain in the observed signal as the dynode is increased becomes non-linear. For the ions of interest for the current work (>250 u), though, increases in dynode and/or multiplier voltage causes a significant increase in the number of ADC counts observed as the ion current. In order to provide optimum precision for the analytical method, these voltages are set for maximum signal for the most concentrated standard but such as not to saturate the data register (268,000,000 counts). Using PFTBA anions, in separate experiments the dynode and multiplier voltages were varied and the relative standard deviation of the observed signal plotted *(Figures 6.1 and 6.2)*. It was determined that best precision occurred when the conversion dynode was operated from 8 to 18 kV and the electron multiplier from 1000 to 2000 volts. As such, for the current work, the dynode was set to 10 kV and the multiplier to 1500 volts.

Quantitation. Using the oxygen-chlorine exchange reaction as an analytical method involved setting all parameters affecting the scan of the instrument. Scan times and scan ranges were set to accommodate the simultaneous monitoring of up to four specific reactions at one time but still allow for adequate chromatographic sampling. The voltage settling time was set to 6 milliseconds to prevent unwanted memory effects. For this method, lowest detection limits, best precision and good chromatographic sampling occurred when the instrument was operated in a product scan mode of operation with a 2u scan range (see below) about the chosen product

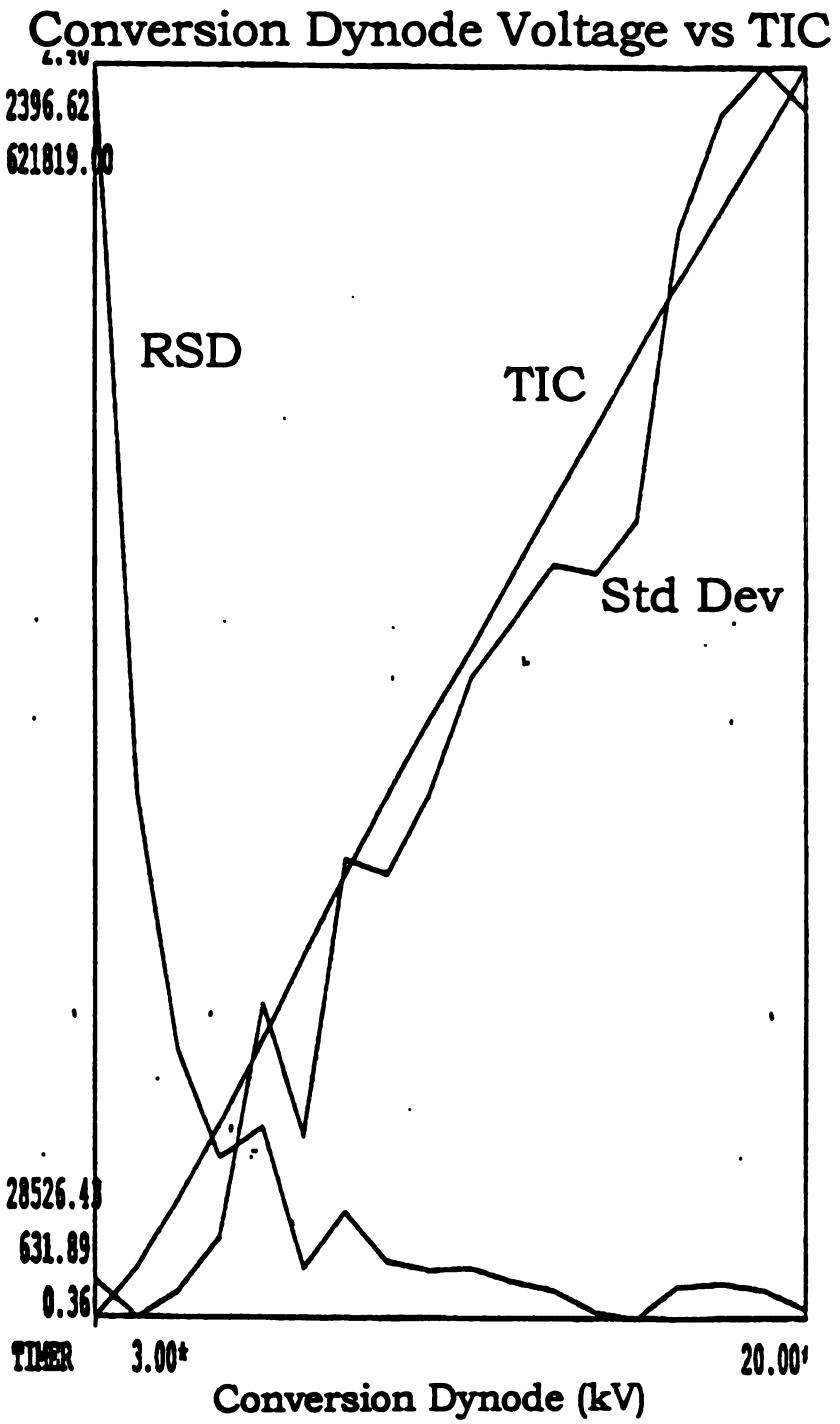

Figure 6.1. The plot of the ion current, standard deviation and relative standard deviation as the conversion dynode voltage was varied from 3 to 20 kV.

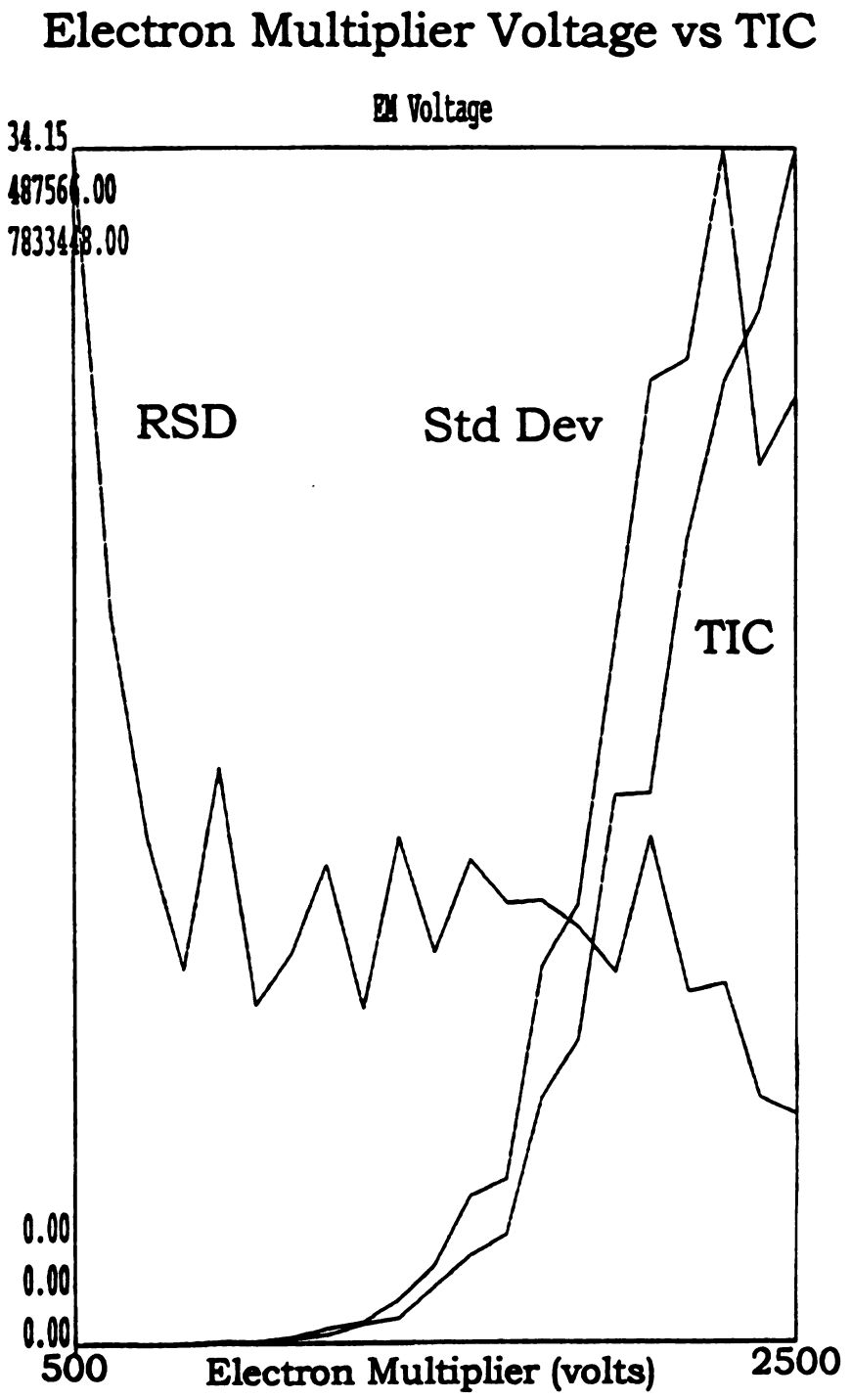

Figure 6.2. The plot of the ion current, standard deviation and relative standard deviation as the electron multiplier voltage was varied from 500 to 2500 volts.

and a scan rate of 20 scans per second. This allowed for one PCB reaction product to be monitored at any given time, and quantitation was achieved by using peak areas of mass chromatograms. The product chosen could be changed over the course of a chromatographic run allowing for the analysis of all the congeners of interest in a single GC run. Since there are never more than three PCB congeners of differing degrees of chlorination eluting in given region of the chromatograms produced with the stationary phase used, all possible PCB exchange reactions could be monitored in a single GC run by monitoring three reactions in each scan. For quantitation, four reactions were monitored to include the ^{13}C-labelled internal standards. This raised detection limits by less than an order of magnitude, which may or may not be detrimental in the analysis of the coplanar congeners in some samples. For the coplanar congeners, detection limits ranged from low to mid pg/μl levels.

For the analysis of the sediment samples, only the coplanar congeners and the ^{13}C-labeled analogs of three of these were of interest. Therefore, for this specific method, only two reactions were monitored at one time. The reactions monitored were changed according to chromatographic retention time to accommodate all four coplanar homologs and ^{13}C-labelled analogs of three of these homologs.

Precision. The precision of such a selective reaction method is influenced by several factors. It was apparent early in the development of this method that gas flows and/or pressures must be maintained in a relatively steady state over fairly long periods of times. Thus, great care was exerted in setting and monitoring these

parameters. The valves controlling both the moderating and the CID gases were replaced with fine high precision metering valves. Even so, in order to achieve the best precision, the use of gas flow controllers may be required.

In addition to constant gas flows, the instrument needs to be tuned in such a fashion that the tune does not drift significantly over a period of several hours or even days. This requires that the instrument (source, quadrupoles and lenses) be very clean. Also, the voltages and parameters used to tune the instrument were set not for maximum transmission, but for best stability over long periods of time. This was particularly important since this was a chromatographic experiment, and injections were made no more frequently than every 45 minutes. Without the availability of an autosampler, a typical analysis took up to three days for all injections including samples, standards and blanks. This was the reason that, even with a good stable instrument tune, scan windows for SRM were maintained at 2u. Better signal-to-noise could be achieved with smaller scan windows but only if the instrument was recalibrated daily which was counterproductive.

Interferences. Many of the inherent problems with ECD and MS modes of chromatographic detection for the analysis of PCB-containing samples are eliminated by using this MS/MS method. Much of the sample cleanup such as Florisil column elution and sulfur removal with mercury may not be necessary for MS/MS detection. This is often done, however, to keep the GC injector liner and column and the MS ion source clean. With MS detection it is difficult to reliably

quantitate an individual PCB congener that co-elutes with a congener containing one more chlorine, since that congener generally loses a chlorine in the source. That ion's ^{13}C isotope $[M-34]^-$ has the same mass as the molecular anion of the co-eluting congener. As such, the precision and accuracy of monitoring that anion quantitatively in a SIM experiment suffers drastically.

As discussed in Chapter 5, the primary interference with this MS/MS method is in the case where a higher homolog co-eluting PCB congener loses a chlorine and gains a hydrogen in the source for a net loss of 34u. Fortunately, many of the congeners that co-elute with the coplanar PCB congeners do not exhibit this loss. It would be advantageous, therefore, to explore other GC stationary phases that may offer worse chromatographic resolution overall, but allow all twelve coplanar PCB congeners to be resolved from higher homolog congeners that lose 34u in the ion source.

Accuracy. The accuracy of the quantitative results of this method were compared to those recently reported by Schulz *(14)* for the analysis of several commercial mixtures of PCBs including Aroclor 1242 by means of multiple GC with electron capture detection. The results are listed in Table 6.1. The discrepancies between the two methods may be due to differences in the two Aroclor 1242 mixtures used. A major problem with determining accuracy, then, is that there is no generally agreed upon standard Aroclor mixture.

Results. As mentioned previously, the focus of this research was to develop a

Table 6.1. The results of the analysis of an Aroclor 1242 standard for the twelve coplanar congeners compared to values reported in literature.

IUPAC NUMBER	CONGENER STRUCTURE	RESULTS [a]				
		AROCLOR 1242		LITERATURE [b]		PERCENT DEVIATION
		AMOUNT µg/L	PERCENT TOXIC	AMOUNT µg/L	PERCENT TOTAL	
81	345,4-CB	200	0.80			
77	34,34-CB	4 200	17	3 500	0.45	19
123	345,24-CB	500	2.0			
118	245,34-CB	12 000	46	13 000	1.6	-8.4
114	2345,4-CB	560	2.2			
105	234,34-CB	7 700	31	6 700	0.86	15
126	345,34-CB	36	0.14			
167	245,345-CB	40	0.16			
156	2345,34-CB	140	0.57	700	0.09	-80
157	234,345-CB	35	0.14			
169	345,345-CB	0.38	<0.002			
189	2345,345-CB	1.4	0.005			
TOTALS		25 000	100	24 000	3.0	-55

[a] The amounts from literature were estimated for an identical sample based on the amounts given for all Aroclor 1242 congeners.
[b] The literature percents are from Reference 14.

method of analysis for PCB congeners to aid in toxicity assessment and in dechlorination studies. Recent work with PCB-contaminated river sediment samples has shown evidence for anaerobic dechlorination of individual PCB congeners. It was therefore important to develop a method of analysis for those congeners that may be involved in these processes. In particular, the congeners of interest are those twelve congeners that have been shown to exhibit dioxin-like toxicity. The results of the analysis of two river sediment samples spiked with Aroclor 1242, one of which was autoclaved and the other inoculated under anaerobic conditions, are shown in Table 6.2. Two of the more important and more abundant congeners are 34,34 CB and 234,34 CB. Both of these co-elute with other congeners in a GC method. Due to the high specificity of the oxygen-chlorine exchange reaction, however, these can be quantitated using this method. It is apparent that these results support the premise that dechlorination is occurring under anaerobic conditions.

Table 6.2. The results of the quantitation by GC/MS/MS of two Aroclor 1242 samples, one of which was autoclaved and the other inoculated and incubated under anaerobic conditions.

IUPAC NUMBER	CONGENER STRUCTURE	RESULTS [a]							
		SEDIMENT SAMPLE #1				SEDIMENT SAMPLE #2			
		AUTOCLAVED		INOCULATED		AUTOCLAVED		INOCULATED	
		AMT	PER CENT	AMT	PER CENT	AMT	PER CENT	AMT	PER CENT
81	345,4-CB	170	0.68	93	0.37	290	1.1	2400	0.09
77	34,34-CB	2500	9.9	370	1.5	3300	13	1200	4.4
123	345,24-CB	440	1.8	74	0.29	2300	8.5	750	2.8
118	245,34-CB	13000	52	4400	17	12000	45	2200	8.3
114	2345,4-CB	660	2.6	360	1.4	610	2.3	150	0.57
105	234,34-CB	8000	32	2300	9.3	7900	30	1100	4.2
126	345,34-CB	18	0.07	2.6	0.01	7.5	0.03	11	0.04
167	245,345-CB	42	0.17	23	0.09	36	0.13	18	0.07
156	2345,34-CB	150	0.60	80	0.32	130	0.50	67	0.25
157	234,345-CB	40	0.16	22	0.08	32	0.12	16	0.06
169	345,345-CB	<16	<0.06	0.13	0.00	<11	<0.04	1.1	0.00
189	2345,345-CB	2.0	0.01	0.71	0.00	2.1	0.01	1.7	0.01
TOTALS		25000	100	7800	31	27000	100	5600	21

[a] All amounts are in μg/L. All percents are based on the total amount of the autoclaved samples.

Chapter 7

Application of the MS/MS Method using the Oxygen-Chlorine Exchange Reaction to the Analysis for Polychlorinated Dibenzodioxins and Dibenzofurans in Samples containing Polychlorinated Biphenyls

The analytical method using the oxygen-chlorine exchange reaction was used for detection of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in PCB-containing sediment samples. Early experiments had indicated the presence of furans but not dioxins, and as such, PCDF standards were prepared for purposes of comparison but not quantitation.

Introduction. The main focus of this research was to develop MS/MS and GC/MS/MS methods for the quantitation of individual PCB congeners, and in particular those congeners that have been shown to exhibit dioxin-like toxicity, in sediment samples. It has been shown, though, that the overall toxicity of PCB-containing samples is quite often due not to the PCB content of these samples but to the presence of PCDFs *(7)*. A PCDF mixed standard was prepared and analyzed

by monitoring the exchange of oxygen for chlorine with the molecular anions of tetra- through octachloro dibenzofurans in a GC/MS/MS experiment. This experiment was also done with an autoclaved and inoculated Aroclor 1254 standard and with two PCB-containing sediment samples.

The toxicity of chlorinated aromatics is usually determined relative to that of 2,3,7,8-TCDD (tetrachlorodibenzodioxin) *(10-12)*. In the case of PCB congeners, it has been shown that 34,34 CB (chlorobiphenyl) is the most toxic of all 209 congeners. It is also speculated that this is as expected from the fact that this congener in the coplanar (the two aromatic rings in the same plane) conformation has the four chlorine nuclei in almost the exact geometric configuration as that of the 2,3,7,8 TCDD molecule. As stated in the previous chapter, those PCB congeners that have been shown to exhibit dioxin-like toxicity are only those congeners of at least tetra substitution that can exist in the coplanar conformation. This conformation is stericly possible only with congeners with one or no chlorines ortho to the biphenyl linkage.

In the environment, the fate of individual PCB congeners includes chlorine uptake, photo-induced dechlorination and dechlorination due to microbial activity. The oxidation of PCB congeners to dioxins, furans and ethers has been demonstrated in the laboratory. There is evidence that these processes may also be photo-induced in the environment. It was therefore expedient to not only develop a method to screen for these possible metabolites, but to also investigate the possibility of these compounds interfering in the analysis of individual PCB congeners.

Experimental. The Aroclor and sediment samples were provided by John Quensen, and the PCDF standards were purchased from AccuStandard, New Haven, CT. All standards were prepared by dilution with Organic Residue Analysis grade hexane (J.T. Baker). River sediment samples spiked with Aroclor 1242 were extracted with 50/50 acetone/hexane. Each extract was subjected to Florisil clean-up and then shaken with mercury for sulfur removal and finally concentrated to ten milliliters. While this extensive clean-up procedure is not necessary for sample analysis by GC/MS/MS, this was done such these samples could also be analyzed by GC/ECD.

Gas Chromatography. All gas chromatographic methods were performed with a Varian 3400 gas chromatograph equipped with a 30-meter x 0.250-mm column of 0.25-mm DB-1 phase (methyl silicone, J & W Scientific). The GC was temperature programmed —— 40 C for two minutes, 40 C to 160 C at 20 C per minute, 160 C to 270 C at 5 C per minute and held at 270 C for seven minutes. Helium was used as the GC carrier gas. The Varian 3400 was equipped with an injector body that accommodates both split and splitless modes of injection. All experiments were performed using the splitless mode of injection with the split valve closed for the initial two minutes and open for thirty minutes. The injector was held at 270 C and the transfer line held at 275 C.

Mass Spectrometry. All experiments were done with a Finnigan TSQ-700 mass spectrometer operated in a negative ion mode. Ammonia was used as the chemical ionization reagent gas. The CI reagent gas pressure was set to approximately 9500

mTorr as measured by the source Convectron gauge. Pure oxygen was introduced into the collision cell. The source was held at 150 C and the manifold at 70 C. All reactions were generated using electron energies of 50 eV, collision offset energies of 1-2 eV (laboratory), and collision pressures from 0.5 mTorr to 1.5 mTorr.

Isotopic Distribution. The isotopic distribution of molecular anions of polychlorinated aromatics provides for several anions as candidates as precursor ions in an MS/MS experiment. In the case of any polychlorinated aromatic, the most abundant anion in the molecular cluster is the $[M]^-$ (or $[M-H]^-$) anion for congeners comprised of less than four chlorines, the $[M+2]^-$ anion for congeners comprised of four to seven chlorines and the $[M+4]^-$ anion for congeners comprised of over seven chlorines. This is indicated for PCBs in Figure 7.1.

The isotopic distribution of six tetrachlorinated aromatics (*Figure 7.2)* of particular environmental importance are shown in Figures 7.3 (spectra) and 7.4 (text listing of exact masses). These distributions are theoretical and were calculated using the natural abundances of ^{12}C, ^{13}C, ^{1}H, ^{2}H, ^{16}O, ^{18}O, ^{35}Cl and ^{37}Cl. With a perfectly calibrated instrument, the molecular anion region of an ECNI spectrum of these analytes should appear as shown for those compounds not exhibiting any hydrogen stripping ($[M-H]^-$).

Molecular Anions. The molecular anions (chlorine isotopes only) of six chlorinated compounds are indicated in Table 7.1. The six compounds chosen include PCBs,

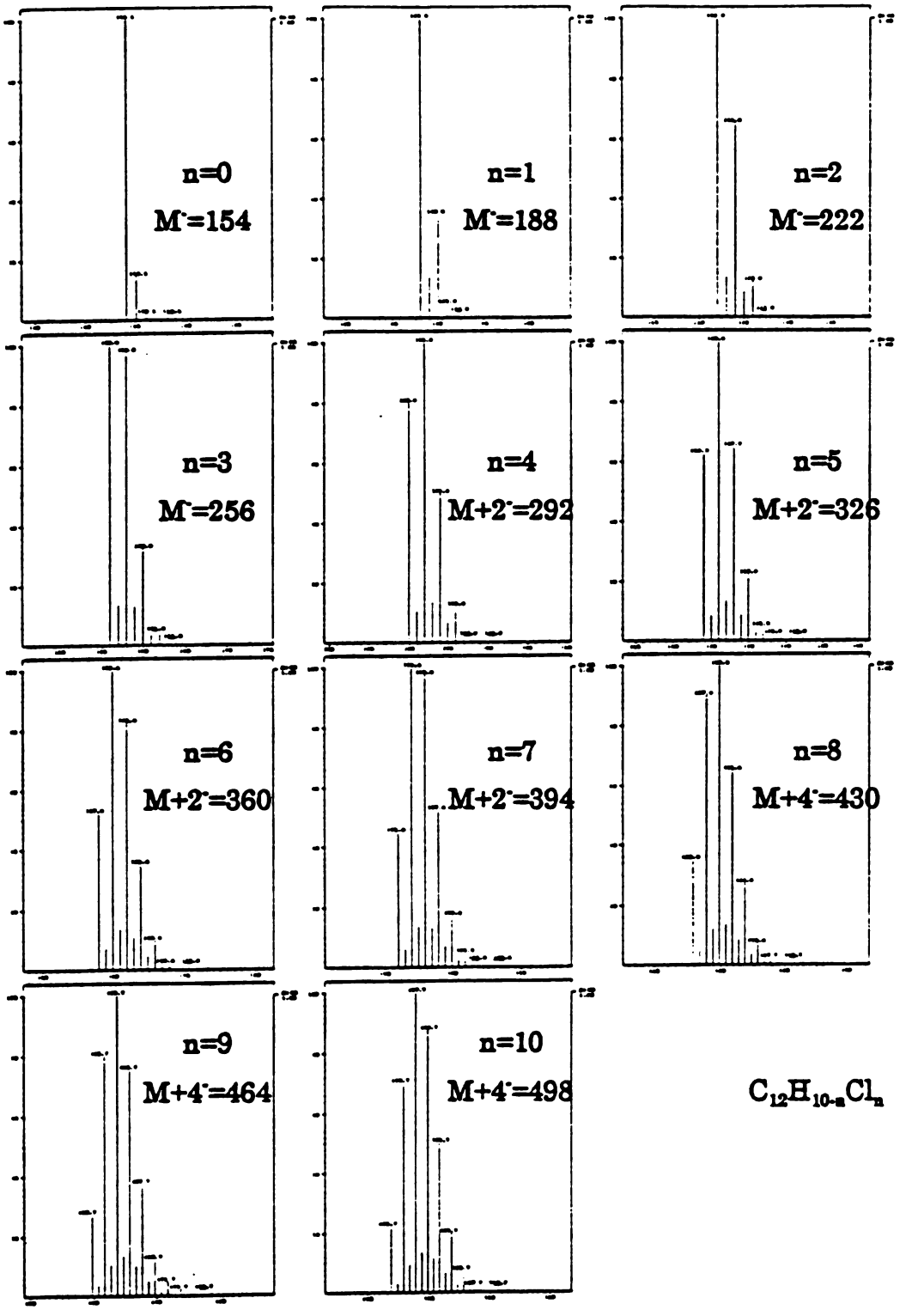

Figure 7.1. The molecular anion clusters of polychlorinated biphenyls calculated using the natural abundances of carbon, chlorine and hydrogen.

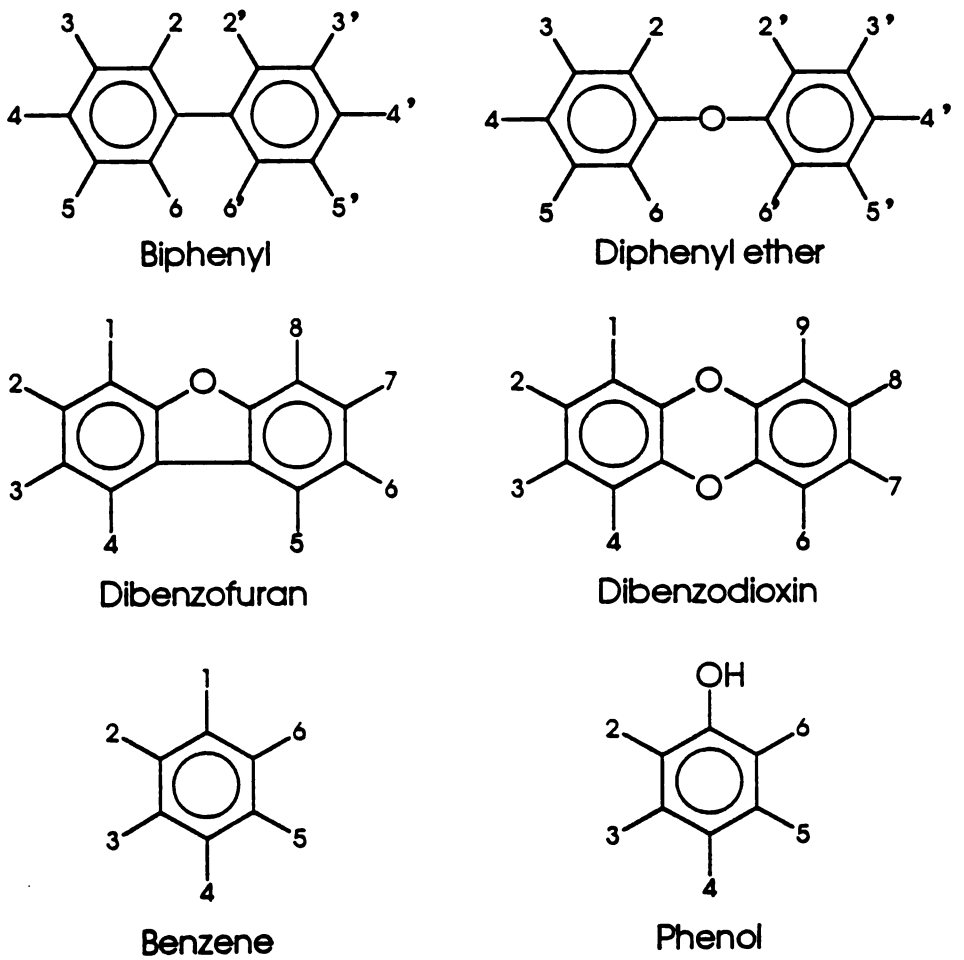

Figure 7.2. The structures and numbering conventions for six compounds whose chlorinated analogs are of environmental significance.

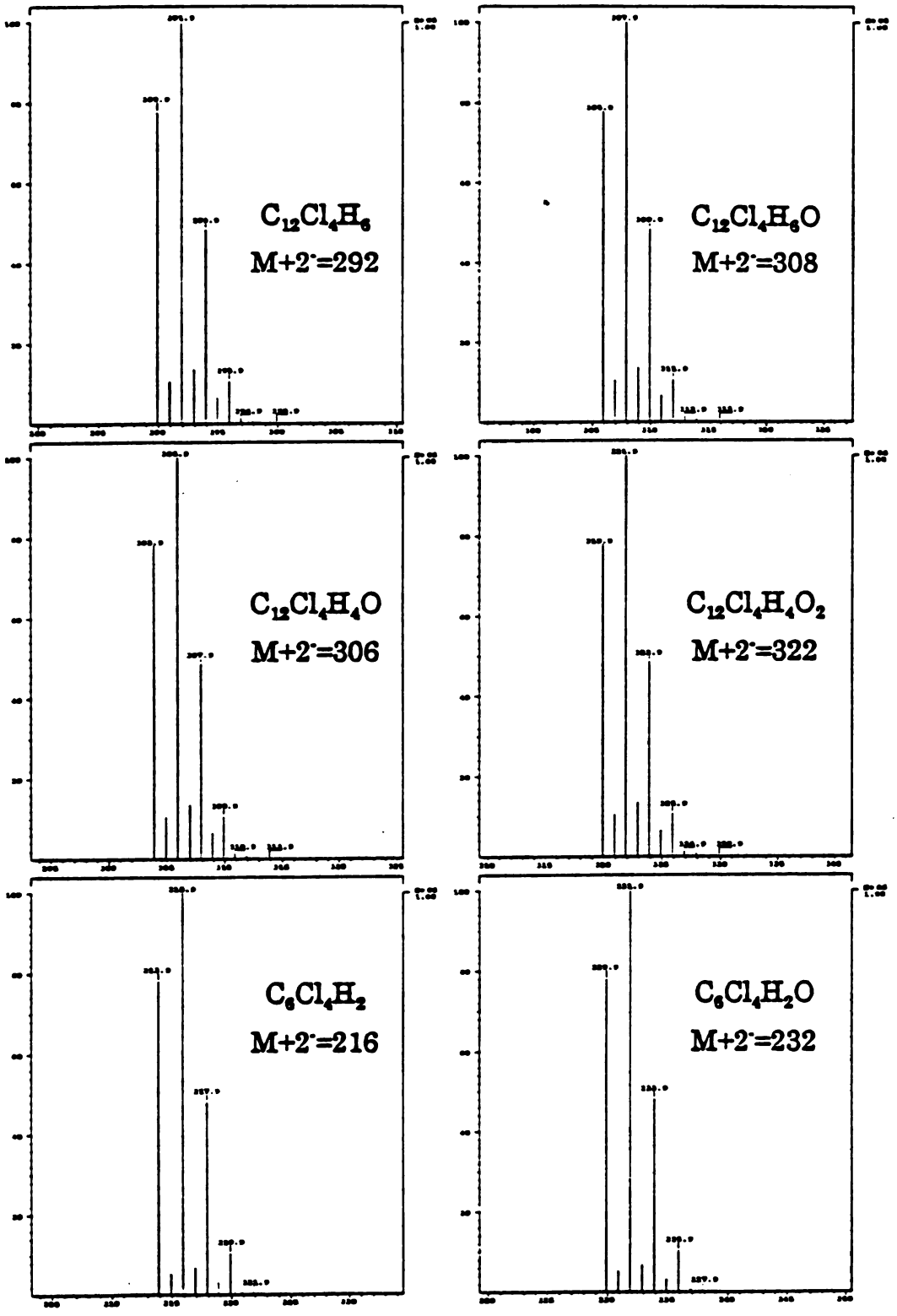

Figure 7.3. Generated spectra from isotope calculations of the molecular ion region of six tetrachlorinated aromatics.

Tetrachlorobiphenyl $C_{12}H_6Cl_4$		
n	M+n	% Abundance
0	289.922	77.67
1	290.926	10.53
2	291.919	100.00
3	292.923	13.50
4	293.917	48.49
5	294.920	6.49
6	295.914	10.56
7	296.917	1.39
8	297.911	0.90
9	298.914	0.11
10	299.917	0.01

Tetrachlorophenyl ether $C_{12}H_6Cl_4O$		
n	M+n	% Abundance
0	305.917	77.54
1	306.921	10.54
2	307.914	100.00
3	308.918	13.53
4	309.912	48.62
5	310.915	6.53
6	311.909	10.64
7	312.912	1.41
8	313.907	0.92
9	314.909	0.12
10	315.912	0.01
11	316.915	⟨0.01

Tetrachlorodibenzofuran $C_{12}H_4Cl_4O$		
n	M+n	% Abundance
0	303.901	77.54
1	304.905	10.52
2	305.899	100.00
3	306.902	13.50
4	307.896	48.62
5	308.899	6.51
6	309.893	10.64
7	310.896	1.40
8	311.891	0.92
9	312.894	0.12
10	313.896	0.01
11	314.899	⟨0.01

Tetrachlorodibenzodioxin $C_{12}H_4Cl_4O_2$		
n	M+n	% Abundance
0	319.896	77.42
1	320.900	10.53
2	321.894	100.00
3	322.897	13.54
4	323.890	48.75
5	324.894	6.55
6	325.888	10.73
7	326.891	1.42
8	327.886	0.94
9	328.889	0.12
10	329.891	0.01
11	330.893	⟨0.01

Tetrachlorobenzene $C_6H_2Cl_4$		
n	M+n	% Abundance
0	213.891	78.06
1	214.894	5.28
2	215.888	100.00
3	216.891	6.76
4	217.885	48.09
5	218.889	3.24
6	219.882	10.30
7	220.886	0.69
8	221.879	0.84
9	222.883	0.06
10	223.886	⟨0.01

Tetrachlorophenol $C_6H_2Cl_4O$		
n	M+n	% Abundance
0	229.886	77.94
1	230.889	5.30
2	231.883	100.00
3	232.886	6.79
4	233.880	48.31
5	234.883	3.27
6	235.877	10.38
7	236.881	0.70
8	237.875	0.86
9	238.878	0.06
10	239.880	⟨0.01

Figure 7.4. The molecular anion clusters of six tetrachloro- aromatics calculated using the natural abundances of carbon, chlorine and hydrogen.

chlorobenzenes, PCDFs, PCDDs, chlorophenols and chlorodiphenyl ethers. These compounds are of significance environmentally and have been shown to be the products of microbial and photo-induced reactions of PCBs, and as such, are often present in PCB-containing samples. The molecular anions of these compounds are indicated in Table 7.1 as *n,x* where *n* is indicative of the isotope $[M+n]^-$ and *x* is the chlorine number. The Table includes the molecular anions of isomers that are of at least trichloro- substitution. Many of these anions, such as $[M+n]^-$ where *n* is greater than 10 for *x* less than 8, are of such low natural abundance that their presence is never observed. This is the reason that a ^{13}C isotopically-labeled standard may be used for a hexachlorobiphenyl.

Some of the anions listed in Table 7.1 do not exist as radical anions, and as such, are not amenable to the oxygen-for-chlorine exchange reaction. For example, the anion cluster at m/z 222 for PCBs is associated with the dichloro congeners which exist with ECNI only as $[M-H]^-$. For all six compounds, M^- and the associated isotopes are observed with at least tetrachloro substitution. By reading across the Table for a particular anion, it is apparent which compounds may interfere with the analysis of any chlorinated aromatic compound. It is also of use in choosing a precursor anion for the MS/MS exchange reaction experiment.

The appropriate choice of a precursor anion must be made with regard to abundance and the possibility of interferences from chlorinated metabolites. For example, monitoring the reaction of m/z 304 to m/z 285 for TCDFs would be free of

Table 7.1. The molecular anions $[M+n]^-$ observed in the primary mass spectra of polychlorinated aromatics designated as *n,x* where *x* indicates the chlorine number.

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{8-x}$	$C_{12}O_2Cl_xH_{8-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
180		0,3				
182		2,3				
184		4,3		0,0		
186		6,3				
188	0,1					
190	2,1					
192						
194						
196					0,3	
198					2,3	
200					4,3	
202			0,1		6,3	
204			2,1			0,1
206						2,1
208						
210						
212						
214		0,4				
216		2,4				
218		4,4		0,1		
220		6,4		2,1		
222	0,2	8,4				
224	2,2					
226	4,2					
228						

Table 7.1 (continued).

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{8-x}$	$C_{12}O_2Cl_xH_{8-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
230					0,4	
232					2,4	
234					4,4	
236			0,2		6,6	
238			2,2		8,4	0,2
240			4,2			2,2
242						4,2
244						
246						
248		0,5				
250		2,5				
252		4,5		0,3		
254		6,5		2,3		
256	0,3	8,5		4,3		
258	2,3	10,5		6,3		
260	4,3					
262	6,3					
264					0,5	0,3
266					2,5	2,3
268					4,5	4,3
270			0,3		6,5	6,3
272			2,3		8,5	
274			4,3		10,5	
276			6,3			
278						

Table 7.1 (continued).

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{8-x}$	$C_{12}O_2Cl_xH_{6-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
280						
282		0,6				
284		2,6				
286		4,6		0,3		
288		8,6		2,3		
290	0,4	10,6		4,3		
292	2,4	12,6		6,3		
294	4,4					
296	6,4					
298	8,4					
300						
302						
304			0,4			
306			2,4			0,4
308			4,4			2,4
310			6,4			4,4
312			8,4			6,4
314						8,4
316						
318						
320				0,4		
322				2,4		
324	0,5			4,4		
326	2,5			6,4		
328	4,5			8,4		

Table 7.1 (continued).

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{8-x}$	$C_{12}O_2Cl_xH_{8-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
330	6,5					
332	8,5					
334	10,5					
336						
338			0,5			
340			2,5			0,5
342			4,5			2,5
344			6,5			4,5
346			8,5			6,5
348			10,5			8,5
350						10,5
352						
354				0,5		
356				2,5		0,5
358	0,6			4,5		2,5
360	2,6			6,5		4,5
362	4,6			8,5		6,5
364	6,6			10,5		8,5
366	8,6					10,5
368	10,6					
370	12,6					
372			0,6			
374			2,6			0,6
376			4,6			2,6
378			8,6			4,6

Table 7.1 (continued).

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{8-x}$	$C_{12}O_2Cl_xH_{8-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
380			10,6			6,6
382			12,6			8,6
384						10,6
386						12,6
388				0,6		
390				2,6		
392	0,7			4,6		
394	2,7			6,6		
396	4,7			8,6		
398	6,7			10,6		
400	8,7			12,6		
402	10,7					
404	12,7					
406	14,7		0,7			
408			2,7			0,7
410			4,7			2,7
412			6,7			4,7
414			8,7			6,7
416			10,7			8,7
418			12,7			10,7
420			14,7			12,7
422				0,7		14,7
424				2,7		16,7
426	0,8			4,7		
428	2,8			6,7		

Table 7.1 (continued).

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{8-x}$	$C_{12}O_2Cl_xH_{8-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
430	4,8			8,7		
432	6,8			10,7		
434	8,8			12,7		
436	10,8			14,7		
438	12,8					
440	14,8		0,8			
442	16,8		2,8			0,8
444			4,8			2,8
446			6,8			4,8
448			8,8			6,8
450			10,8			8,8
452			12,8			10,8
454			14,8			12,8
456			16,8	0,8		14,8
458				2,8		16,8
460	0,9			4,8		
462	2,9			6,8		
464	4,9			8,8		
466	6,9			10,8		
468	8,9			12,8		
470	10,9			14,8		
472	12,9			16,8		
474	14,9					
476	16,9					0,9
478	18,9					2,9

Table 7.1 (continued).

$[M+n]^-$	$C_{12}Cl_xH_{10-x}$	$C_6Cl_xH_{6-x}$	$C_{12}OCl_xH_{9-x}$	$C_{12}O_2Cl_xH_{8-x}$	$C_6OCl_xH_{5-x}$	$C_{12}OCl_xH_{10-x}$
480						4,9
482						6,9
484						8,9
486						10,9
488						12,9
490						14,9
492						16,9
494	0,10					18,9
496	2,10					
498	4,10					
500	6,10					
502	8,10					
504	10,10					
506	12,10					
508	14,10					
510	16,10					0,10
512	18,10					2,10
514	20,10					4,10
516						6,10
518						8,10
520						10,10
522						12,10
524						14,10
526						16,10
528						18,10

interference from the M^- of tetrachlorodiphenyl ethers even though choosing m/z 306 as a precursor would be warranted from its abundance. Also, monitoring the reaction of m/z 458 to m/z 439 would be specific for octachlorodibenzo dioxins (the $[M+16]^-$ is not abundant enough to be observed in a routine analysis) even though m/z 460 is more abundant but is also observed with nonachlorobiphenyls. While using the exchange reaction method for targeted analyses with direct introduction into the ion source (such as solids probe), the choice of precursor is of paramount importance in preserving the specificity of the method. With the increased specificity of chromatographic detection, this may not be necessary for some analytes.

Dioxins and Furans. As stated previously, much of the toxicity of PCB-containing samples can often be attributed to the PCDF content of the sample. This may be true even when the amount of total PCDF concentration is much less than that of total PCBs. It is obvious that with these concentrations, GC/ECD or GC/MS will not suffice for identification and quantitation for these samples. The oxygen-for-chlorine exchange reaction has been used in the past to determine the presence of PCDDs in sediment samples, and none have been observed at a quantifiable level. The GC/MS/MS analysis of a standard mixture of 5 tetrachlorodibenzo dioxins and 1 pentachlorodibenzo dioxin is shown in Figure 7.5. Indicated in the chromatograms are the reactions for tetra-, penta- and hexachlorodibenzo dioxins. Notice that in this "pure' standard of only Cl_4 and Cl_5 dibenzo dioxins, there is a significant response at approximately scan number 875 for a Cl_6 dibenzo dioxin.

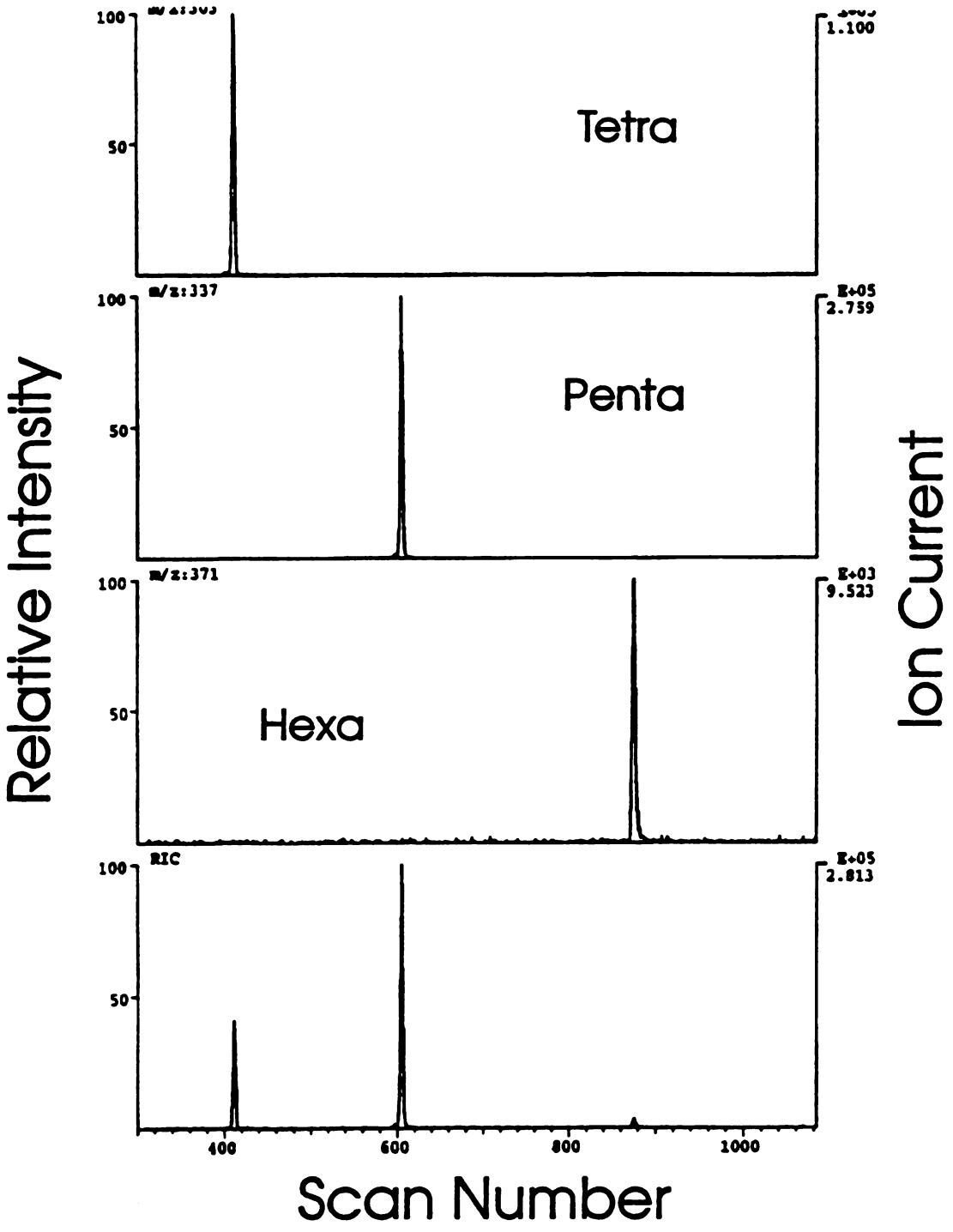

Figure 7.5. The reconstructed total ion current (RIC) chromatogram generated by monitoring the oxygen-for-chlorine exchange reaction of the molecular anions [M+2]- of PCDDs in a GC/MS/MS experiment.

The tetra- through octachloro-substituted PCDFs were monitored for an autoclaved and an inoculated sample spiked with Aroclor 1254 standard (congeners at the ppm level) *(Figure 7.6)* and two sediment samples from natural sites contaminated with PCBs *(Figure 7.7)*. The chromatograms for the Aroclor 1254 samples indicate that microbial action may have resulted in a slight decrease in the overall PCDF content (39,000 ADC counts versus 77,000 counts), but there is virtually no difference in the overall pattern. This is not generally the case with sediment samples showing apparent dechlorination (see Chapter 6). These results indicate that even with a pure Aroclor standard, there is a significant amount of other chlorinated aromatics present. This is not surprising, since, as stated previously (Chapter 2), the toxicity of many PCB-containing samples is quite often due to the PCDF-content of these samples.

For the two sediment samples, a significant amount of PCDFs is indicated from the chromatographic data. These samples are from two different environmental sites that have been found to be PCB-contaminated. As the dechlorination of PCB-containing samples is monitored, it may be worthwhile to also monitor other chlorinated aromatics. This data also demonstrates the inadequacies of other detection methods such as ECD or MS for low levels of analytes in the presence of high levels of similar compounds.

Conclusions. The data from the analysis of PCB-containing samples for PCDDs and PCDFs indicates that using the oxygen-for-chlorine exchange reaction in a

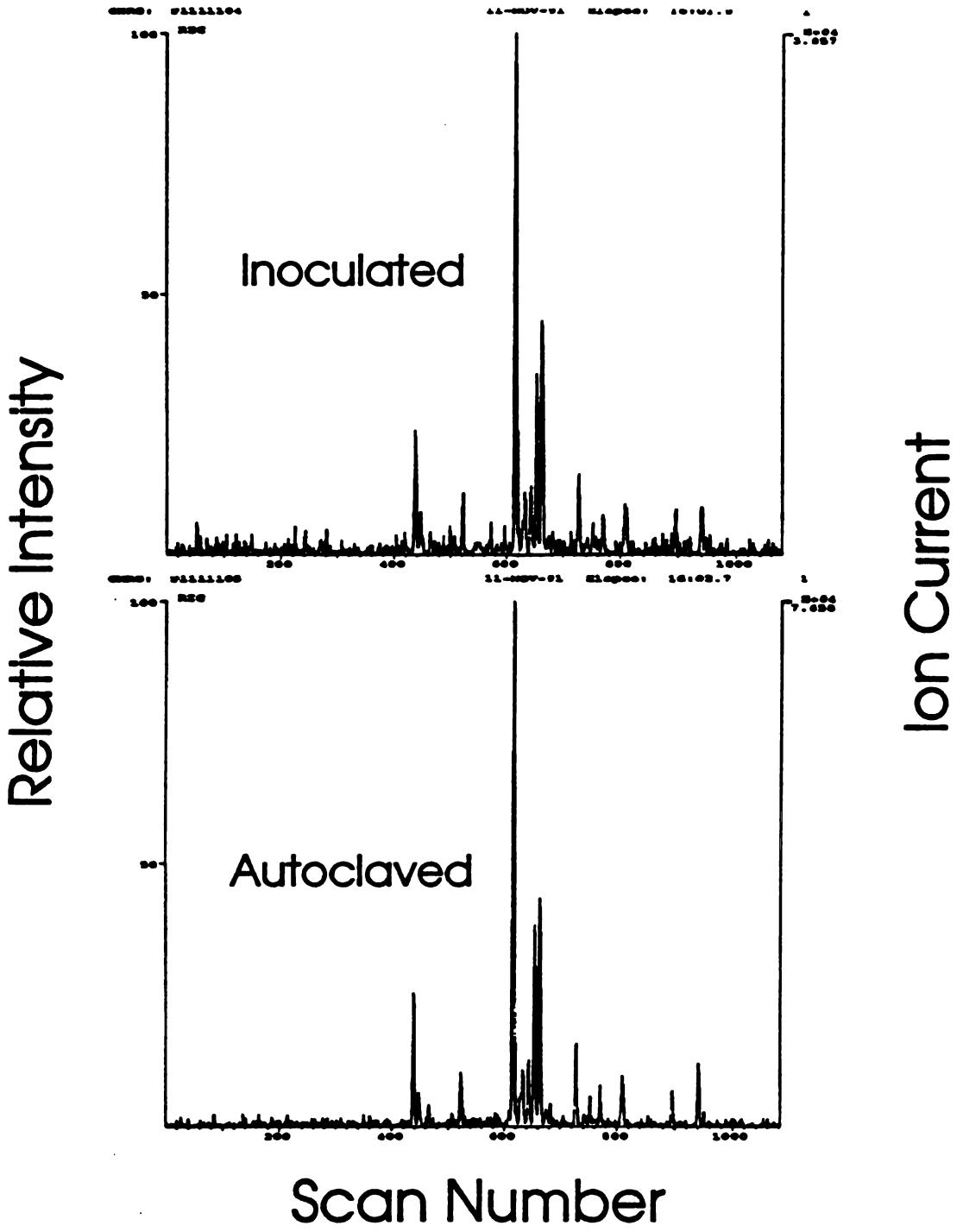

Figure 7.6. The reconstructed total ion current (RIC) chromatogram generated by monitoring the oxygen-for-chlorine exchange reaction of the molecular anions [M+2]- of tetrachlorodibenzofurans in a GC/MS/MS experiment.

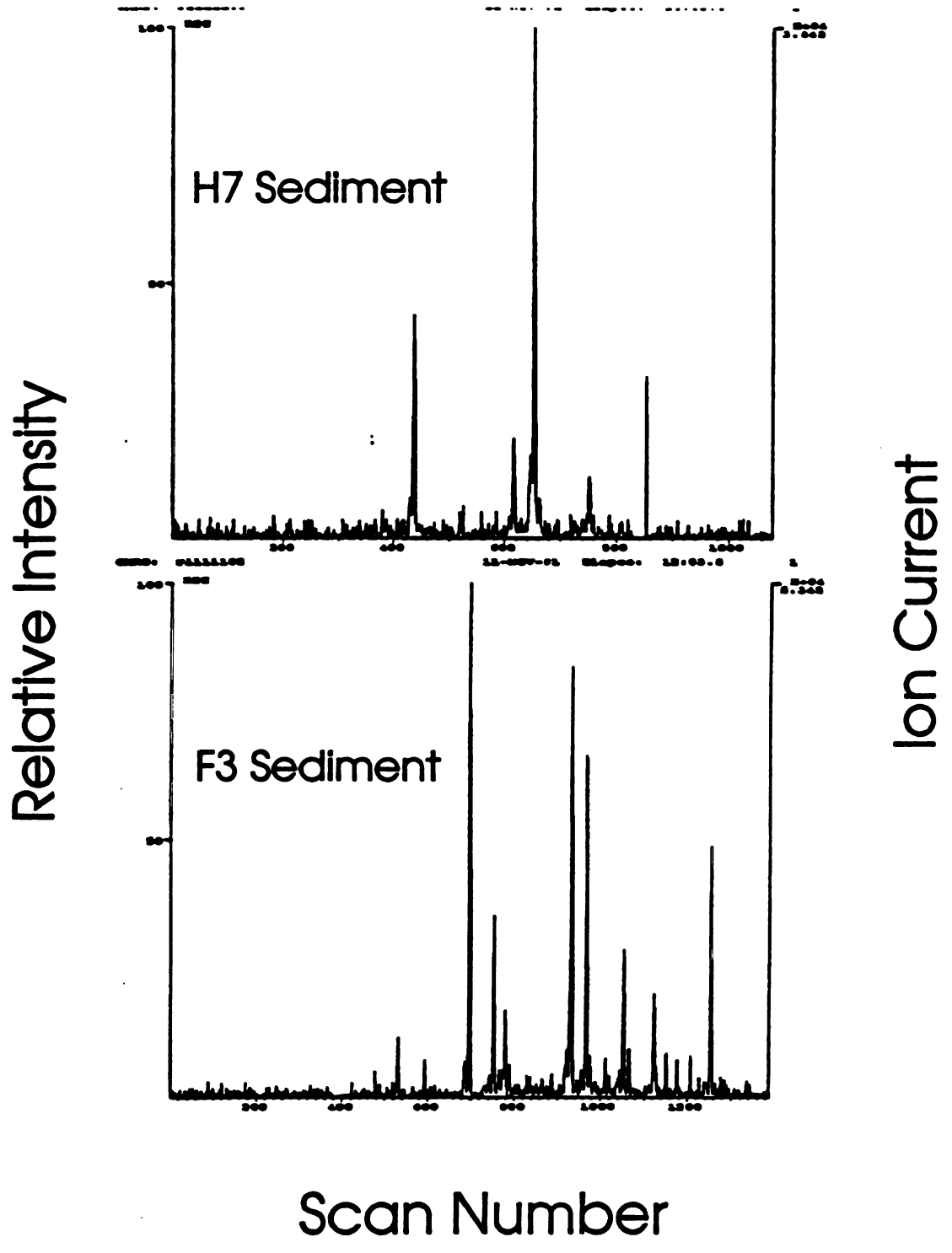

Figure 7.7. The reconstructed total ion current (RIC) chromatogram generated by monitoring the oxygen-for-chlorine exchange reaction of the molecular anions [M+2]- of tetrachlorodibenzofurans in a GC/MS/MS experiment.

GC/MS/MS experiment may be of utility for the analysis of many chlorinated aromatics. Several issues remain to be investigated. One, each of the chlorinated compounds studied needs to be further analyzed to determine which if any exhibit the loss of 34 u in the source as discussed for PCBs in previous chapters. Also, for some samples, other chlorinated compounds such as naphthalenes, anthracenes, phenanthrenes and biphenylenes should be studied analytically. Furthermore, for each of the chlorinated aromatics studied including the PCDDs and PCDFs, these methods need to be developed with individual quantitative standards.

Chapter 8

Conclusions

Due to the problems inherent with ECD and MS methods of detection for PCB samples, an MS/MS method was developed for the chromatographic detection of samples containing PCBs and other chlorinated aromatics. This method employs monitoring the products of ion-molecule reactions generated in a triple-stage quadrupole using oxygen as a reagent. The products observed were the result of an exchange of an oxygen atom for a chlorine atom for a net loss of 19 u. The method development included instrument optimization for production of the precursor ions (molecular anions) and for the MS/MS reaction products. The method was also used to detect the presence of other chlorinated aromatics (specifically polychlorinated dibenzofurans and polychlorinated dibenzodioxins) in samples containing significant amounts of PCBs. Also studied were possible interferences with this method.

Instrumental Parameters. The instrumental parameters studied that affect the formation of molecular anions in the MS ion source included source temperature, type and pressure of moderating gas and filament electron energy. It was determined that molecular anion production was optimum with ammonia as a moderating gas

and at a pressure of 8×10^{-6} Torr as measured by the manifold ion gauge. The ion source was operated at 150 C, and the filament emission was generated with an electron energy of 50 eV. Instrumental tunes were generated with baseline resolution for MS experiments and with slightly less resolution (<25% valley) for MS/MS experiments.

The significant instrumental parameters controlling the generation of ion-molecule reaction products in an MS/MS experiment include type and pressure of collision gas, reaction energy and translational kinetic energy correction. Both ambient air and pure oxygen were used as the collision gas, but the use of oxygen provides for greater yields in reaction products. Unlike higher-energy CID experiments, the use and/or amount of translational kinetic energy correction is not critical for product ion transmission into the detector with this ion-molecule reaction. Clearly the most critical parameter controlling the amount of reaction product observed in this MS/MS experiment is the collision energy. The optimum energy for this reaction was determined to be approximately 1 eV.

In applying this reaction as an analytical method for the determination of PCBs in sediment samples, up to two orders of magnitude of improvement in signal is realized using the selected-reaction monitoring (SRM) mode of detection instead of full-scan acquisition. Also, with the instrumental tunes and set masses used, the product scan mode is more sensitive than precursor or neutral loss scan modes for SRM. Chromatographic conditions were used that allowed for comparison with

GC/ECD methods for the same samples. Due to the selectivity of the SRM method, GC run times could be shortened considerably for routine analysis of PCB samples.

Other Chlorinated Aromatics. The oxygen-for-chlorine reaction was also applied as a detection method for polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs). This was particularly useful for detecting these compounds in samples containing significant amounts of PCBs. In almost all sediment samples analyzed, the presence of PCDDs was not observed. In almost all sediment samples analyzed, however, the presence of significant amounts of PCDFs was observed.

Future Studies. With chromatographic detection for some coplanar PCB congeners, congeners that co-elute and lose 34 u in the ion source interfere with this MS/MS method. Each congener that may interfere, then, must be analyzed as a pure standard in order to determine which of these congeners lose 34 u and which lose 35 u. Also, it may be possible to use other GC stationary phases that offer overall less chromatographic resolution for PCB congeners, but might provide for fewer interferences with the GC/MS/MS method.

Since the MS/MS method is applicable for many chlorinated compounds even in the presence of significant amounts of PCBs, it may be possible to determine the fate of chlorinated compounds in samples subjected to microbial action. These results could also be compared to other types of analysis such as total organic chlorine content

and toxicity assessment. These methods could also be used to determine the inadequacies of methods such as GC/ECD where PCDFs and PCDDs are possible interferences.

Due to the high selectivity offered by MS/MS detection, particularly with the use of ion-molecule reactions, other reagent gases should be explored. These would include other radical molecules that may undergo the same type of reaction or reactions as the oxygen-for-chlorine exchange. In addition, other reagents may prove to be even more selective than oxygen and other free radicals. Ideally, these reagents could also be selective for the differences in the steric conformations exhibited by various PCB congeners. Specifically, an optimum reagent may be one that reacts only with those congeners that form the coplanar conformation, particularly since these are the congeners that have been shown to exhibit dioxin-like toxicity.

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