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The determination of selected trace organics in clams from the Lake St. Clair area.

Taras William. Obal
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THE DETERMINATION OF SELECTED TRACE ORGANICS IN CLAMS FROM THE LAKE ST. CLAIR AREA

by

Taras William Obal

A Thesis
submitted to the Faculty of Graduate Studies
through the Department of Chemistry
in Partial Fulfillment of the
requirements for the Degree
of Master of Science at
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Windsor, Ontario, Canada
1983

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ABSTRACT

THE DETERMINATION OF SELECTED TRACE ORGANICS IN CLAMS FROM THE LAKE ST. CLAIR AREA

by

Taras William Obal

Levels of octachlorostyrene (OCS) and polychlorinated biphenyls (PCBs) were determined in clams (*Lampsilis radiata siliquoidea*) taken from 93 sites in the Lake St. Clair area. Authentic OCS was prepared and methods were developed for the extraction and clean-up of these samples. OCS was observed in all samples analyzed (0.2-32.3 ppb, wet weight), while the levels of Aroclors 1254 and 1260 ranged from not detected to 105 ppb. The method established for the quantitation of Aroclors 1254 and 1260 was based on the levels of two specific isomers found in the commercial mixtures.

To My Family

ACKNOWLEDGEMENTS

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The work of a biology task group of the Great Lakes Institute, under the direction of Dr. Chris Pugsley deserves special acknowledgement. Without their assistance and cooperation, much of our research would not have been possible. Their work in sample collection, speciation of the clams, and the development of the sampling program is a major component of the larger project which provides the framework for our analytical study.

Contract UP-G-175: A Case Study of Selected Toxic Substances in the Essex Region.

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF APPENDICES

LIST OF ABBREVIATIONS

BCF	bioconcentration factor
CCIW	Canadian Centre for Inland Waters
GC	gas chromatography
GC/ECD	gas chromatography/electron capture detection
GC/MS	gas chromatography/mass spectrometry
HCB	hexachlorobenzene
HCS	heptachlorostyrene
IR	infrared
LOD	limit of detection
LOQ	limit of quantitation
MS	mass spectrometry
m/z	mass to charge ratio
NMR	nuclear magnetic resonance
OCS	octachlorostyrene
PB	phenobarbital
PCB	polychlorinated biphenyl
ppb	parts per billion (ug/kg)
ppm	parts per million (mg/kg)
ppt	parts per trillion (ng/kg)
PVC	polyvinyl chloride
RI	retention index
RRT	relative retention time
σ	standard deviation
t_R	retention time
U.S. EPA	United States Environmental Protection Agency
UV	ultraviolet

CHAPTER I

INTRODUCTION

The presence of persistent environmental contaminants in the Great Lakes has been an issue of major concern since the early 1960s[1]. Among the compounds listed by the Ontario Ministry of the Environment[2] and the U.S. Environmental Protection Agency[3], chlorinated derivatives are of foremost interest because of their high stabilities, which allow them to bioaccumulate in aquatic biota, and also because of their high toxicities. Recent studies[4,5] show octachlorostyrene (OCS) and polychlorinated biphenyls (PCBs) as major chlorinated contaminants in the Great Lakes area.

First observed in 1969 as an unknown compound (containing 8 chlorines and having molecular weight 376) in the tissues of eider ducks (*Somateria mollisima*) and sandwich sterns (*Sterna sandvicensis*) from the Rhine River and the Netherlands coastal area[6], the structure was determined to be C_8Cl_8 in cormorant (*Phalacrocorax carbo*) extracts using gas chromatographic/mass spectrometric (GC/MS) analyses[7]. Although OCS is not commercially produced, nor is it used by anybody, high levels have been reported for the Lake Huron, Lake St. Clair, lower Detroit River, Lake Ontario and Ashtabula River areas[8].

PCBs are among the most abundant of the chlorinated aromatic pollutants in the ecosystem[9]. They have been wide-

ly used commercially as industrial chemicals since the early 1930s. In 1966, Jensen[10] first reported levels of PCBs in fish and birds. By 1969, it had been realized that PCB contamination had become global in extent. Because of their widespread popularity in uncontrolled, high volume applications over the past 40 years, large amounts of PCBs have become potential environmental hazards[11].

The study of OCS and PCBs is of interest not only because of their environmental impact, but because their analysis poses a formidable problem in terms of isolation, detection and quantitation of trace amounts.

CHAPTER II

BACKGROUND

A. Octachlorostyrene

1. Levels and Areas of Occurence

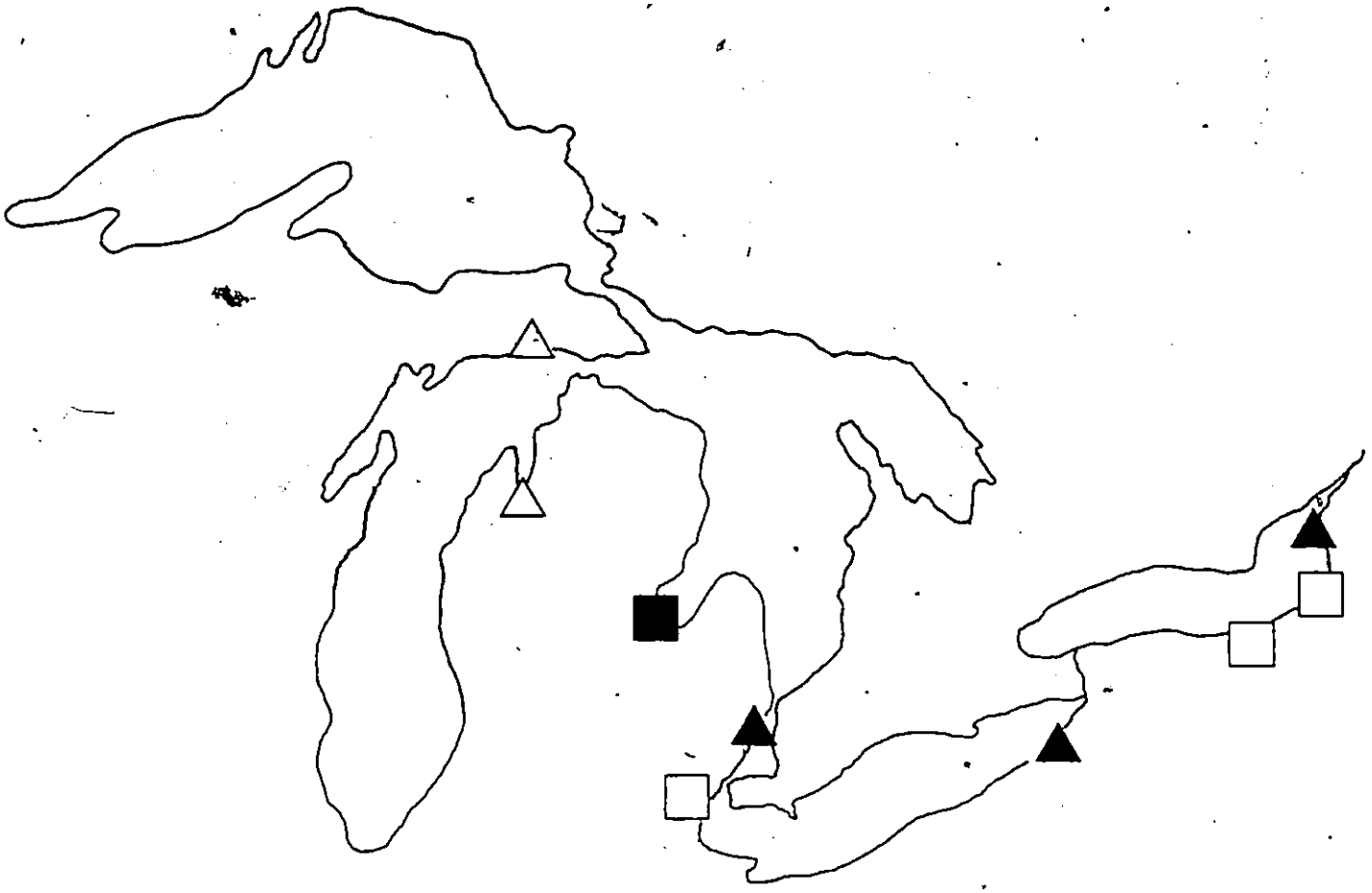
Octachlorostyrene is a unique chlorinated contaminant, in that high levels have been reported in only two areas of the world. Major OCS contamination has been described only in the Frierfjord region of southeastern Norway and the lower Great Lakes[8], namely Lakes Huron, Erie and Ontario, Lake St. Clair and the Ashtabula River.

In Norway, OCS was first identified in the tissues of seals (*Phoca vitulina*) and the common roach (*Leuciscus rutilus*). Further studies[12] of sprat (*Clupea sprattus*) tissues indicated levels of OCS ranging from less than 0.1 mg/kg (ppm) in the Sandefjord, to 11.2 ppm in the Frierfjord. Later analyses[13,14] of marine organisms from the Frierfjord indicated considerably increased levels of OCS. Sea star (*Asteroidea*) carcasses were reported to contain up to 11 ug/kg (ppb) (wet weight) of OCS, while cod (*Gadus morhua*) liver tissues contained up to 675 ppm. Furthermore, OCS levels in the bottom sediments were reported between 32 and 64 ppb. Summarizing these results, invertebrates generally contained between 2 and 400 times, and fish between 205 and 600 times, as much OCS as was present in the sediments.

Reported OCS levels in the Great Lakes biota have been considerably lower than those reported for Norway. OCS residues in the Great Lakes were first reported[15] in the tissues of the Great Blue Heron (*Ardea herodias*). Up to 430 ppb was found in the carcass, and up to 230 ppb was reported in the heron egg. OCS has been confirmed[16] in walleye (*Stizostedion vitreum vitreum*) from Lake Huron and alewife (*Alosa pseudoharengus*) from Lake Ontario. In the Ashtabula River, OCS is the chlorinated compound of second highest concentration. Levels up to 500 ppb have been reported[17] in composite fish samples. OCS contamination in the Great Lakes has recently been summarized[8] (Figure 1). Levels vary from 2.0 ppb in Lake Huron lake trout to 405 ppb in northern pike from the Ashtabula River. It must be noted, that all the samples containing OCS also contained levels of hexachlorobenzene (HCB). Similarly, three isomers of heptachlorostyrene (HCS) were consistently found in samples containing high levels of OCS.

A study[18] was carried out to determine occupational exposure to persistent organochlorine compounds by Norwegian workers. Levels of OCS up to 4.96 ppb were reported in blood samples from workers in a magnesium plant and up to 0.39 ppb in workers from a polyvinyl chloride plant. However, it should be noted, that levels up to 0.43 ppb were reported in workers not exposed to persistent chlorinated compounds.

Fig. 1: Incidence of OCS in Great Lakes Fish between 1974-1980[8]



△ NOT DETECTED

□ < 100 ppb

■ 100 - 200 ppb

▲ > 200 ppb

2. Sources

Various sources of OCS have been proposed. It has been suggested[14] that OCS and other chlorinated contaminants are produced as wastes and by-products of high temperature industrial processes involving chlorine and organic material. These include bleaching in the paper industry and chloride disinfection in water treatment. Likewise, the incineration of polyvinyl chloride (PVC)[19], the electrolytic production of chlorine gas using carbon electrodes[4] and the commercial production of magnesium[18] are all suggested to produce OCS as a by-product.

The incineration of PVC was found to produce 2.3 mg of OCS per kg of PVC, yet it was concluded[19], that this was not a major source of the chlorinated hydrocarbon in the environment. The commercial production of magnesium has been confirmed as a source of OCS in Norway, as shown by the results obtained in a study of OCS levels in the blood of various plant workers (see Chapter II, A.1.). In this case, magnesium oxide (MgO) is chlorinated to give anhydrous magnesium chloride ($MgCl_2$). The $MgCl_2$ is further reduced electrolytically, using graphite electrodes, to give magnesium. In both situations, high temperatures, carbon and chlorine are involved.

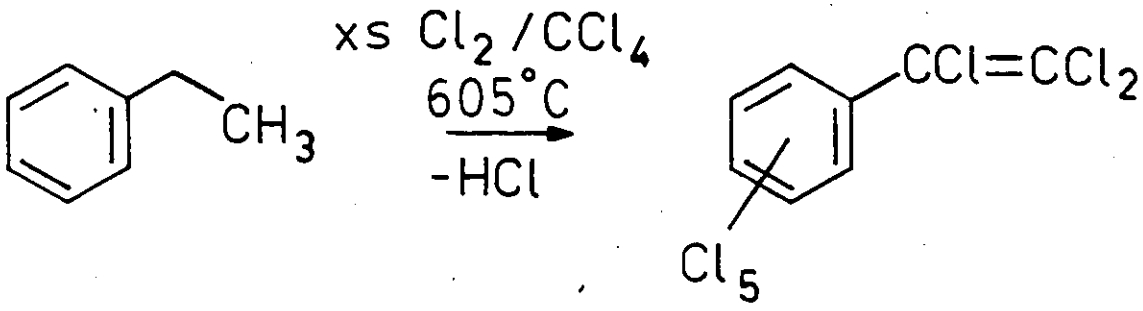
No other sources of OCS have been identified in either Norway or the Great Lakes, although the distribution of OCS levels in Great Lakes fish[8] indicates several sources of OCS in this area, and that they are of differing industrial

processes.

3. The Chemistry of OCS

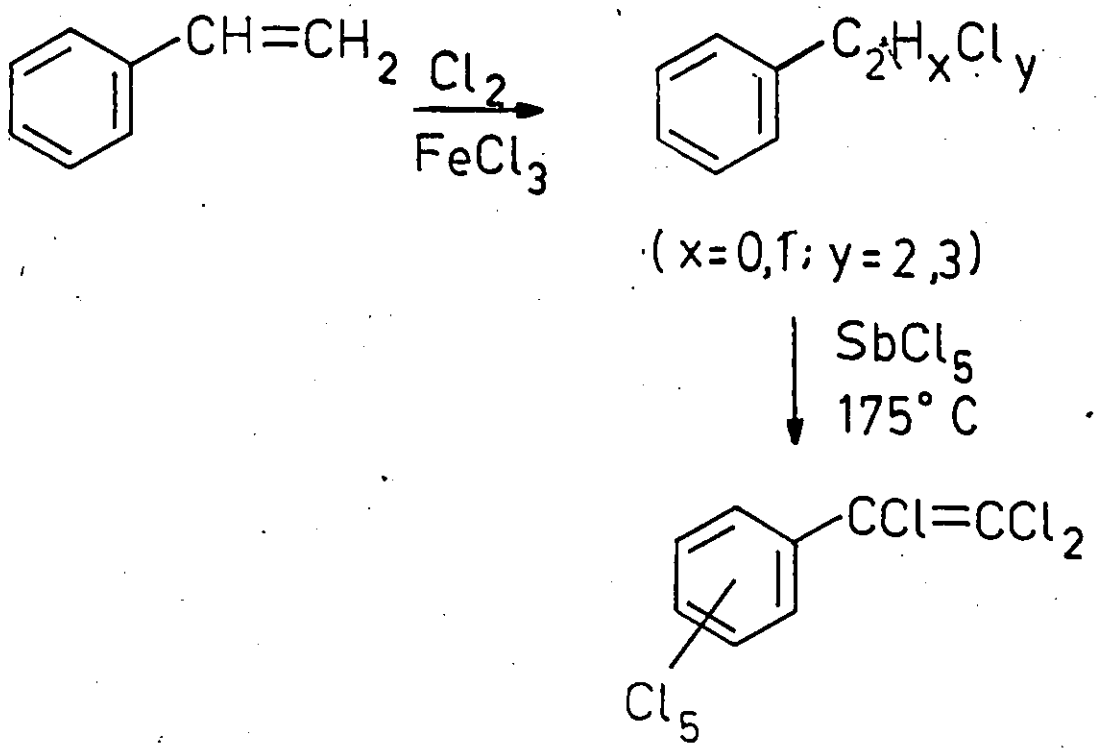
a. Syntheses

Since 1973, several synthetic methods for OCS have been published. The first[20] involved a straightforward chlorination of ethylbenzene with chlorine (Cl_2) gas at a high temperature and a subsequent loss of HCl.



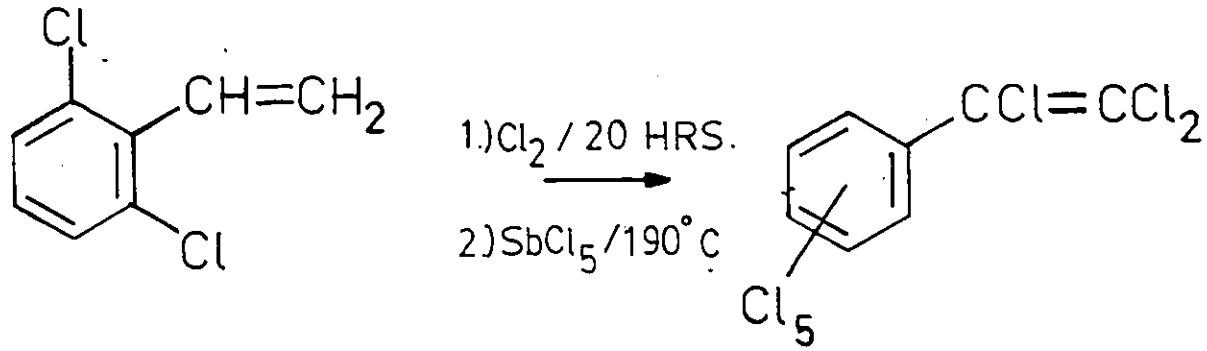
This process gave an 84% yield of OCS.

Kuehl *et al*[16] developed a procedure whereby styrene is chlorinated with Cl_2 gas, using ferric chloride ($FeCl_3$) as a catalyst. This produced a mixture of dichloro- and trichloroethylbenzenes. This mixture was further reacted with antimony pentachloride ($SbCl_5$), at 175° C to give the final product.



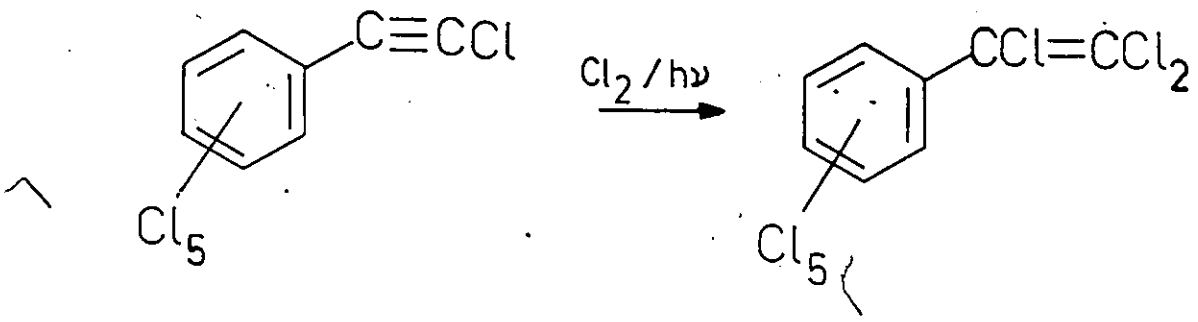
The yields and purity of product were not elaborated upon in this example, nor were any mechanisms postulated.

A variation of this procedure was developed by Bieniek and Korte[21]. In this case, 2,6-dichlorostyrene is chlorinated with Cl_2 gas for 20 hours with no catalyst present. The product is perchlorinated with $SbCl_5$ at 190° C to yield OCS.

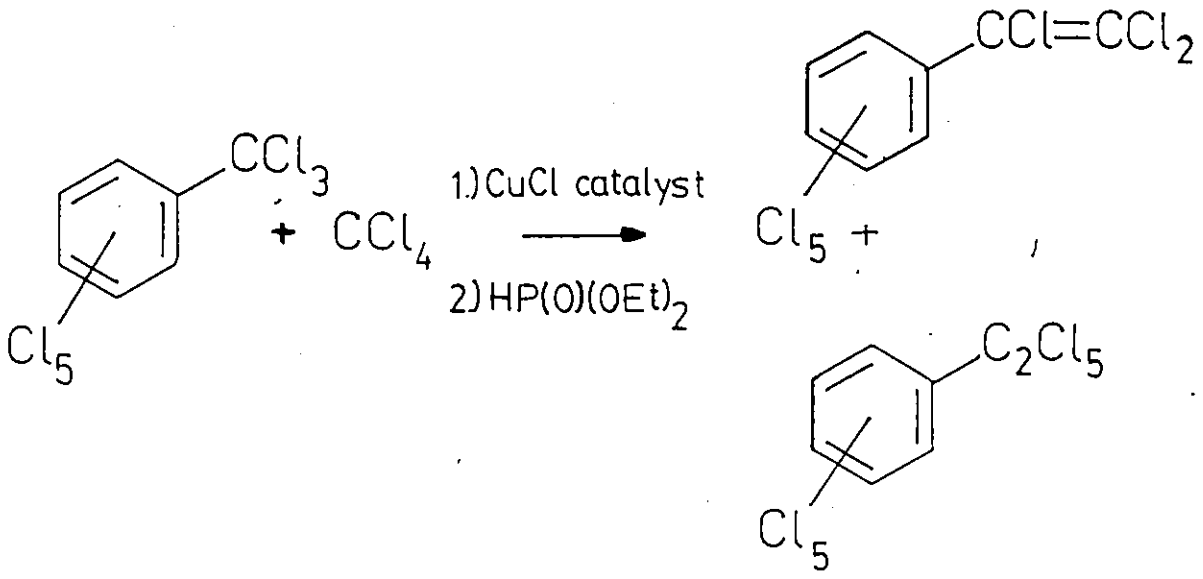


The yield of OCS was cited as 60%. No mechanism for the reaction was given.

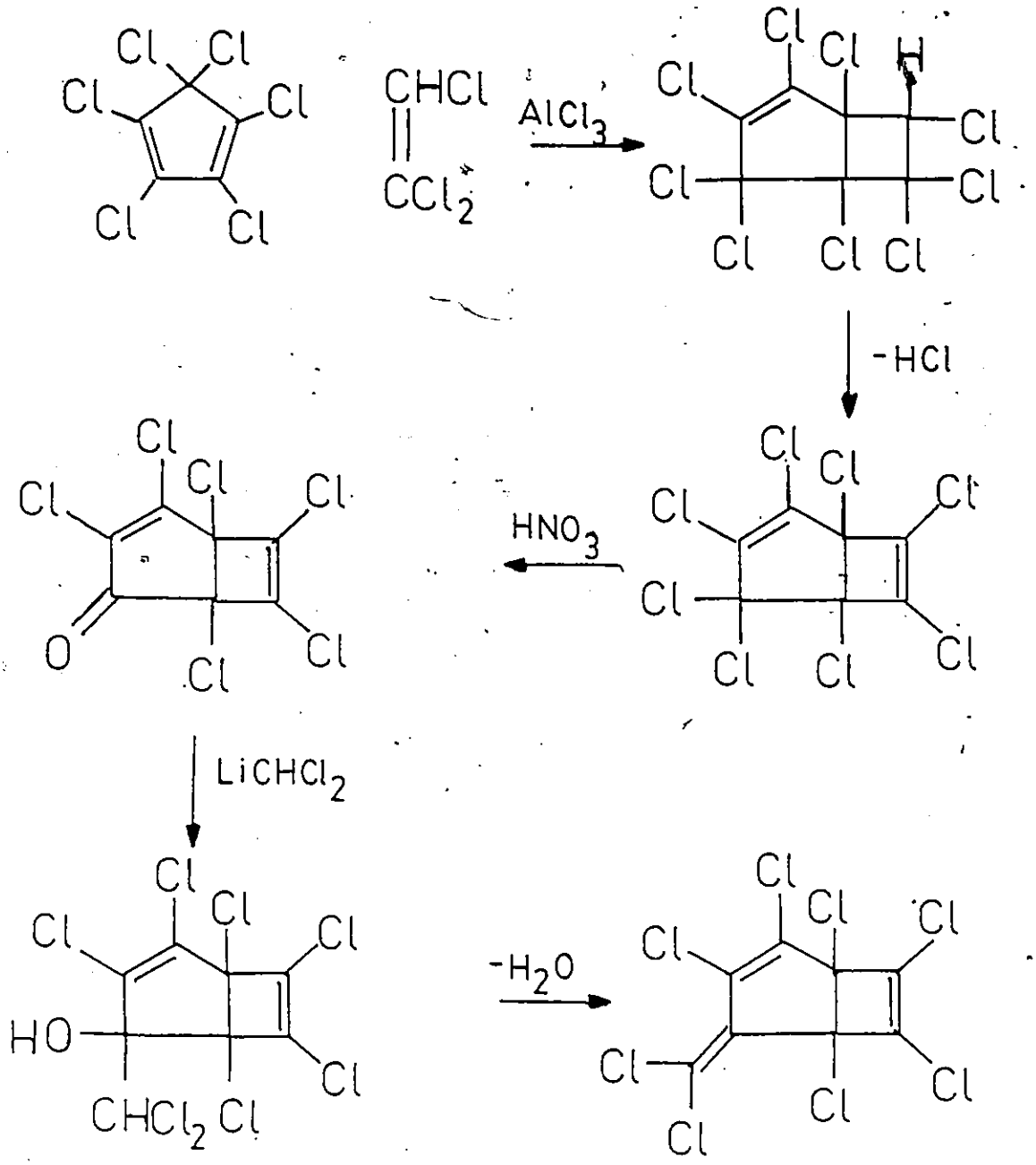
Other, more elaborate OCS syntheses have been developed[22-24]. Ballester *et al*[22], while synthesizing perchloroacetylenes, used perchlorostyrene as a starting material. The final product, perchlorophenylacetylene, was found to revert back to OCS in the presence of sunlight and chlorine gas.

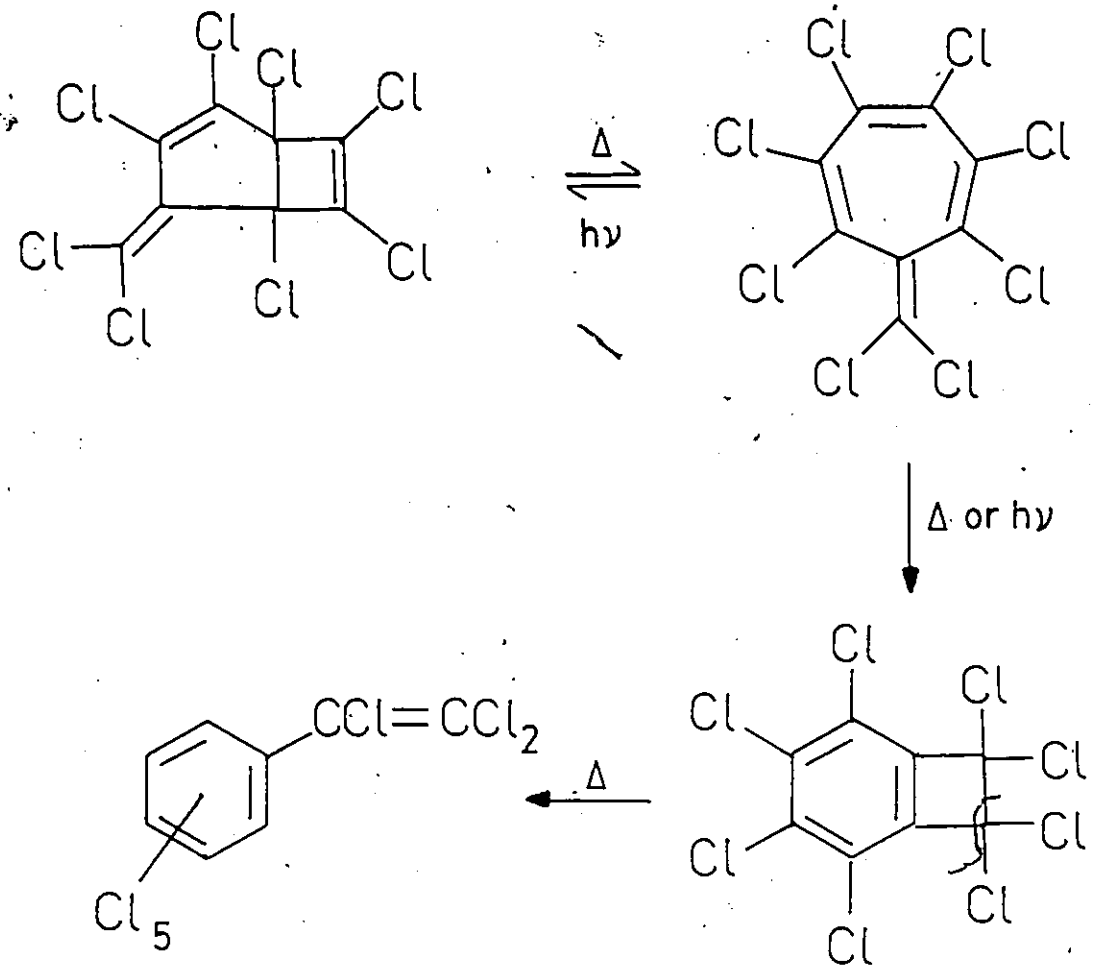


Further work by Ballester[23] led to a procedure where perchlorotoluene and carbon tetrachloride (CCl_4) are converted to OCS (55% yield) and decachloroethylbenzene (27.1% yield).



The most elaborate method was developed by Roedig[24], while synthesizing halogenated heptafulvenes. Through a series of steps, perchlorocyclopentadiene and trichloroethylene are eventually converted to perchloroheptafulvene and consequently OCS.





b. Physical and Chemical Properties

OCS occurs as colourless needles, having a melting point of 99-100° C when recrystallized from a mixture of acetone/ethanol (1:1)[20] or 93.5-97° C when recrystallized from methanol[21]. OCS shows high chemical stability, low water solubility and high solubility in organic solvents. Reports indicate its solubility in water as 7.1 ppb[25] and 2.5 ppb[13].

OCS is reported[25] to have a bioconcentration factor (BCF) of 33000 in the flathead minnow (*Pimephales promelas*). A BCF is indicative of a particular organism's ab-

ility to accumulate a certain compound. The value for OCS is comparable to those reported for other persistent chlorinated contaminants. Similarly, OCS is shown to have an n-octanol-water partition coefficient (P) of 1.9×10^6. The logarithm of P is commonly used as an indicator of the lipophilic nature of chemicals.

Characterization of OCS is generally carried out using mass spectrometric techniques[21]. Mass spectrometry is one of the most useful qualitative indicators of OCS. The mass spectra show characteristic chlorine clusters due to the natural occurence of two chlorine isotopes (^{35}Cl and ^{37}Cl). The molecular ion peak occurs at m/z= 376,378,380,..., 392. The most common fragmentation pattern, as with most other organochlorine compounds, involves subsequent losses of chlorine (eg: $[M-Cl]^+$, m/z= 341,343,345,...,355; $[M-2Cl]^+$, m/z= 306,308,310,...,318).

^{13}C nuclear magnetic resonance (NMR) spectroscopy is also useful for qualitative studies of OCS[21]. The ^{13}C-NMR spectra of OCS show peaks at 135.74 ppm, 134.13 ppm (C-1,4); 132.60 ppm, 132.66 ppm (C-2,6; C-3,5); 124.42 ppm, 125.29 ppm (C-7,9). The infrared (IR) and ultraviolet (UV) spectra for OCS have not been reported.

c. Methods of Determination and Quantitation

Increased interest in the types and levels of persistent organochlorine contaminants in the ecosystem has led to a greater demand for more accurate, sensitive and selec-

tive forms of analysis[26]. Improved chromatographic instrumentation has fulfilled many of these requirements. The two most common forms of detection available are the gas chromatograph combined with an electron capture detector (GC/ECD) and the gas chromatograph interfaced to a mass spectrometer (GC/MS). The former, because of its high selectivity and high sensitivity[27], is used more commonly for quantitation, whereas the latter, although it may be used quantitatively[8], is more often used as a confirmational technique[28].

For the quantitation of OCS, GC/ECD is the generally accepted technique[12-14,17,18]. The technique involves injection of a sample through a packed or capillary column GC and comparison of the ECD response to that of an external standard. The internal standard technique may also be used to quantitate OCS, provided the internal standard has a similar detector response to that of OCS. Commonly used internal standards are hexachlorobenzene[14] and *p*-diiodobenzene[8].

The ECD is well suited for the analysis of halogenated compounds[29]. A "standing current" is formed in the detector by a radioactive source, generally ^{63}Ni or ^{3}H. Compounds which have an affinity for free electrons deplete this background current as they pass through the detector cavity. The magnitude of this current depletion is indicative of the amount of capturing species and thus proportional to the detector response. Because of the degree of chlorination in OCS, the ECD is ideal for its analysis.

A GC/MS is used mostly for the confirmational anal-

ysis of OCS[30]. Because of the presence of two chlorine isotopes, OCS yields very characteristic mass spectra (see Chapter II, A.3.b.).

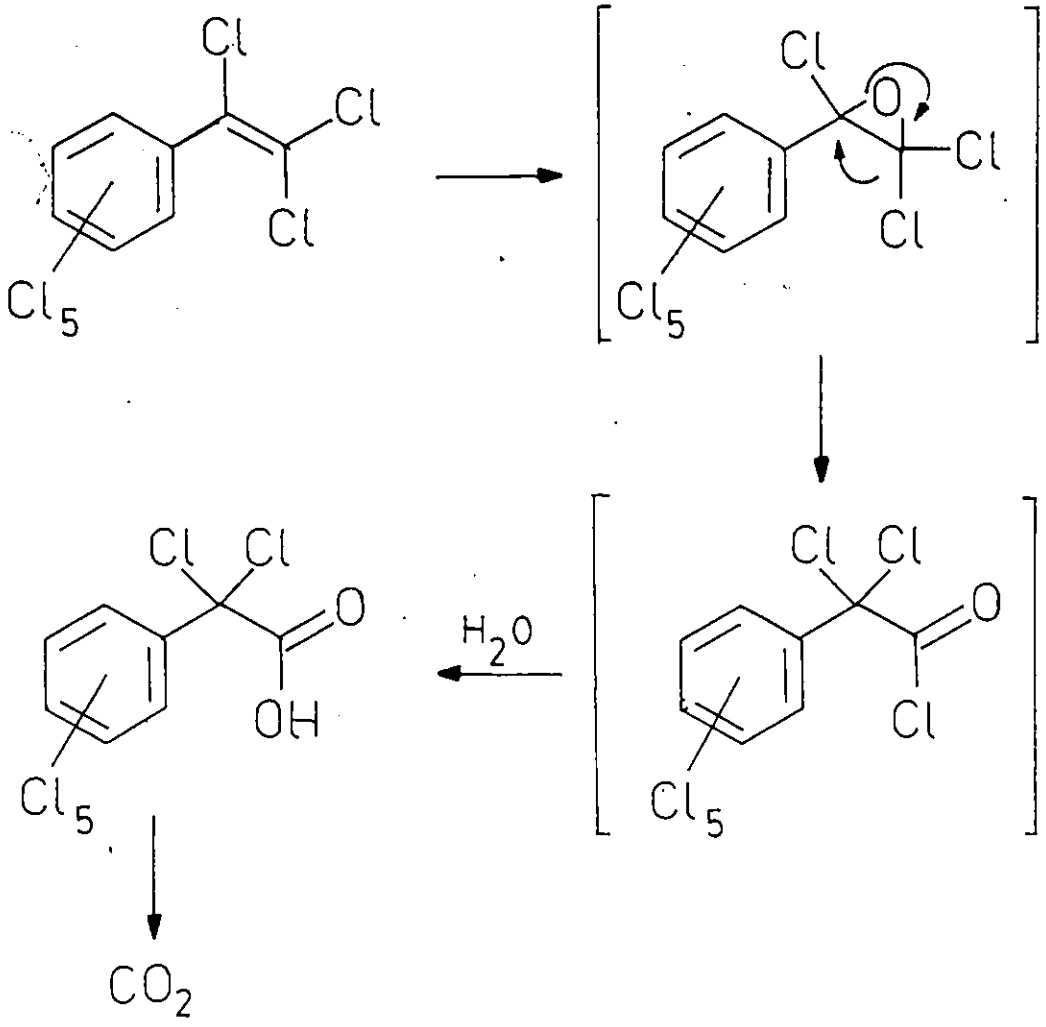
d. Toxicity

Until recently, little research on the toxicity of OCS has been carried out. Its appearance as one of the predominant chlorinated environmental contaminants in the regions mentioned led to a greater interest in the possible hazards OCS may present.

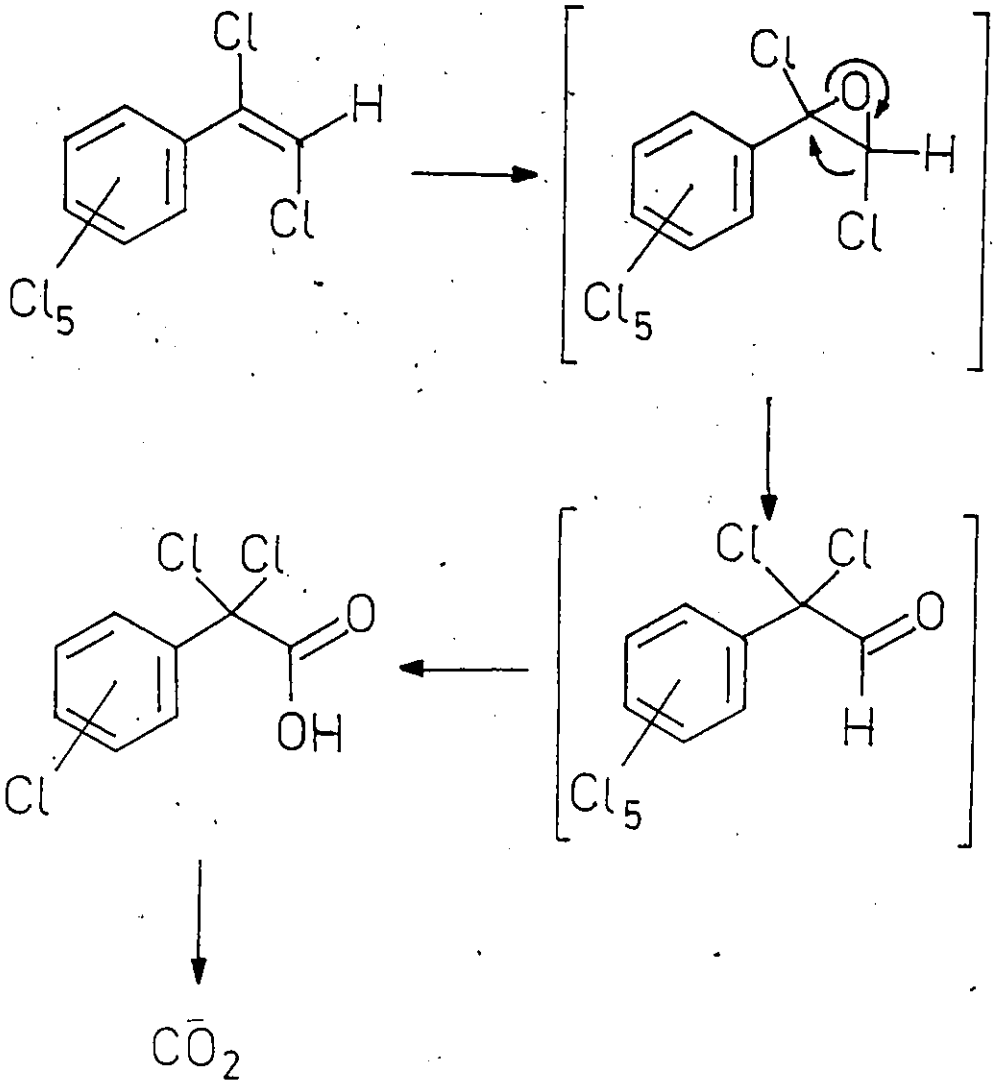
Holme and Dybing[31] have shown that the inducing effect of OCS on the microsomal cytochrome P-450 monooxygenase system in rats is practically identical to that of HCB. After the administration of single intraperitoneal doses of OCS in the rats, the authors reported increases in microsomal protein and cytochrome P-450 content, cytochrome P-450 reductase, ethylmorphine N-demethylase, 4-nitroanisole O-demethylase and acetanilide 4-hydroxylase activities. Similar patterns of induction were observed after 14 days of intraperitoneal or oral dosing. The authors also reported protein increases, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), similar to those found with phenobarbital (PB) induction, and thus concluded that OCS is a PB-type inducer. Evidence also shows[31] that OCS is a potent porphyrinogen in rats and Japanese quail.

In more recent studies, carried out by Chu *et al*[33,34], OCS was found to be lipophilic, resistant to metabolic deg-

radation and toxic in rats. Acute and subacute effects of OCS in rats included hepatomegaly, hepatic microsomal enzyme induction and pathological changes. The symptoms were observed after dosages as low as 5-50ppm. The apparent maximum no effect level of OCS in rats is 0.5 ppm. A unique finding was high levels of OCS in the lungs of the experimental animals, making it different from other organochlorine compounds which preferentially accumulate in fat and liver tissues. OCS was also found to exhibit slow metabolic degradation to pentachlorophenyldichloroacetic acid, heptachlorostyrene and carbon dioxide.



Heptachlorostyrene is also a possible intermediate metabolite. It is proposed that HCS is metabolized in the same manner as OCS, with the formation of an intermediate aldehyde. HCS is more susceptible to epoxidation than OCS, because the lower degree of chlorination on the double bond decreases the electron withdrawing effect of its substituents, and consequently, increases its electron density.



The results obtained show OCS to be a stable chemical which is slowly eliminated and/or metabolized, and which shows similar pharmacokinetic features to many other organochlorine contaminants.

B. Polychlorinated Biphenyls

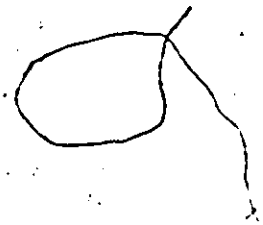
1. Structure and Nomenclature

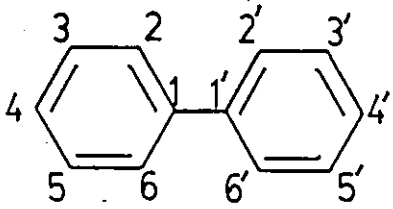
In a biphenyl molecule, there are ten possible positions for chlorination (Figure 2). This results in 209 possible chlorinated isomers. PCBs are produced and sold commercially as crude mixtures of these isomers[35]. They are marketed under various tradenames, for example, Aroclor (Monsanto, North America), Clophen (Bayer, Germany), Phenoclor (Prodelec, France), Kanechlor (Kanegafuchi, Japan), etc. Most information is available regarding the Aroclor variety. The Aroclors are characterized by a four digit number. The first two digits represent the molecular type. For example, 12- represents a chlorinated biphenyl and 54- a chlorinated terphenyl mixture. 25- and 44- are blends of chlorinated biphenyls and terphenyls. The last two digits represent the weight percent of chlorine in the preparation. Thus, Aroclor 1254 is a chlorinated biphenyl mixture containing 54% chlorine.

2. Overall PCB Contamination

The widespread use of PCBs as industrial chemicals since the early 1930s, has led to environmental contamination

Fig. 2: Distribution of, and Numbering System for, PCB Isomers[9]





Chlorine Substitution	Number of Isomers
mono-	3
di-	12
tri-	24
tetra-	42
penta-	46
hexa-	42
hepta-	24
octa-	12
nona-	3
deca-	1
	209

which is practically global in its extent[36]. The ubiquitous nature of PCB contamination is indicated by levels being reported[37] in human milk, adipose tissue, brain and the liver. As well, PCBs have been reported[6] in most forms of wildlife. It is clear that the properties which make PCBs indispensable to industries are the same properties which cause them to be such persistent environmental contaminants.

The highest incidence of PCB contamination appears to occur in highly industrialized and urbanized areas[36]. It has been reported[38] that PCB concentrations in the atmosphere range from less than 1 ng/m^3 to 50 ng/m^3. PCB concentrations in non-polluted waters are generally less than 0.5 ppt (ng/kg), compared to moderately polluted waters, which contain approximately 50 ppt. Highly polluted waters may contain as much as 500 ppt of PCBs[38]. Various PCB levels have been reported for different species of wildlife. These range from 0.1 ppm in fish and blue crabs from Charleston, South Carolina[39] to as much as 14000 ppm in white tailed eagles[40].

3. PCB Contamination in the Essex County Region

Many industrial chemicals, such as PCBs, have been entering the ecosystem for many years. While extensive studies were being carried out to determine DDT levels in the Great Lakes, additional compounds were being detected, which interfered with the DDT analyses. The interfering compounds were recognized as PCBs and were found to be present at levels up to 25 ppm[41]. The introduction of PCBs and other per-

sistent chlorinated compounds into the Great Lakes basin has caused contamination of nearly every component of the aquatic ecosystem[42].

At present, the major pathway of PCBs into the Great Lakes is through atmospheric deposition[43]. It has been reported[44] that the total PCB concentration in air samples from the Great Lakes area ranges up to 7.95 ng/m^3. It has further been reported[45,46], that the total PCB concentration in wet precipitation for the Windsor area occurs at levels less than 20-30 ppt (mean range).

Levels of PCBs in the waters of the St. Clair River, Lake St. Clair and Detroit River vary. Frank *et al*[47] reported PCB levels in the suspended solids ranging from 30-395 ppb. The sediments in these regions have been shown[48] to contain up to 5.3 ppm, with an average of 0.3 ppm of PCBs in the St. Clair River and 0.05 ppm in the Detroit River.

PCB levels in the aquatic biota from these regions vary according to species. Spottail shiners (*Notropis* sp.) were reported[49] to contain PCB concentrations ranging from not detected to 337 ppb (wet weight). Coho salmon (*Oncorhynchus kisutch*) from the Detroit River contained total PCB levels up to 1.5 ppm[42].

4. Uses of PCBs

PCBs found widespread application in industry because of their chemical and physical characteristics. PCBs show high boiling points, low water solubilities and high

dielectric constants. Commercial preparations are highly compatible with many types of polymers. PCB mixtures are extremely stable. They are not hydrolyzed by water, acid or alkali. They are so thermally stable, that some mixtures are used as fire retardants. Generally, PCBs are stable during prolonged heating at 150° C.

These properties and others (see Chapter II, B.6.a.) led to the incorporation of PCBs in adhesives, paints, carbonless reducing paper, printing inks, varnishes, elastomers and general fillers. Their high dielectric constants led to their use in electric insulators and coolant insulators in transformers. PCBs have been incorporated into lubricants, liquid seals and vacuum diffusion pump oils because of their high stabilities and low vapour pressures. Other uses for PCBs include formulations into ballasts for fluorescent fixtures, heat transfer fluids, plasticizers, impregnation of cotton and asbestos for the braided insulation of electrical wiring, high pressure hydraulic fluids, machine tool cutting oils and fire retardants (see Table 1).

5. Sources of PCBs

Because of their early, uncontrolled and widespread use, an exact figure cannot be assigned to the amount of PCBs present in the environment. Estimates for worldwide PCB production vary. In North America, it has been estimated[51] that PCB production between 1930 and 1975 amounted to 570 million kilograms, with an additional 1.4 million kilograms being

Table 1
Some Uses of PCBs[50]

Material with which Aroclor is combined	Aroclor (weight%)	Use
Polyvinyl chloride	1248, 1254, 1260 (7-8%)	Secondary plasticizer to improve flame retardance and chemical resistance
Epoxy resins	1221,1248 (20%)	Increase chemical and oxidative resistance, and adhesive qualities
Polystyrene	1221 (2%)	Plasticizer
Chlorinated rubber	1254 (5-10%)	Enhances resistance, flame retardance and improves electrical insulating properties
Styrene-butadiene copolymer	1254 (8%)	Improves chemical resistance
Neoprene	1268 (40%) 1268 (1-5%)	Fire retardant Injection moldings

Table 1 (cont.)

Material with which Aroclor is combined	Aroclor (weight%)	Use
Crepe rubber	1262 (5-50%)	Plasticizer in paint compositions
Varnish	1260 (25% of oil)	Improves water and alkali resistance
Wax	1242 (5%)	Increases moisture and flame resistance

imported. From these estimates, it is assumed[38], that over one half of this amount has entered dump and landfill sites, where it is likely to be stable and slowly released into the environment.

There are four possible pathways by which PCBs may enter the environment[34]. These are summarized as follows:

i) Because of their numerous industrial applications, PCBs may be flushed as wastes into river and lake waters. Their low water solubilities allow them to accumulate in sediments and aquatic biota thus reaching high concentrations.

ii) Similarly, PCBs enter the atmosphere through industrial smoke created by aircraft exhaust systems, and the incineration or combustion of PCB containing materials. These include plasticizers and objects coated with PCB formulated coatings.

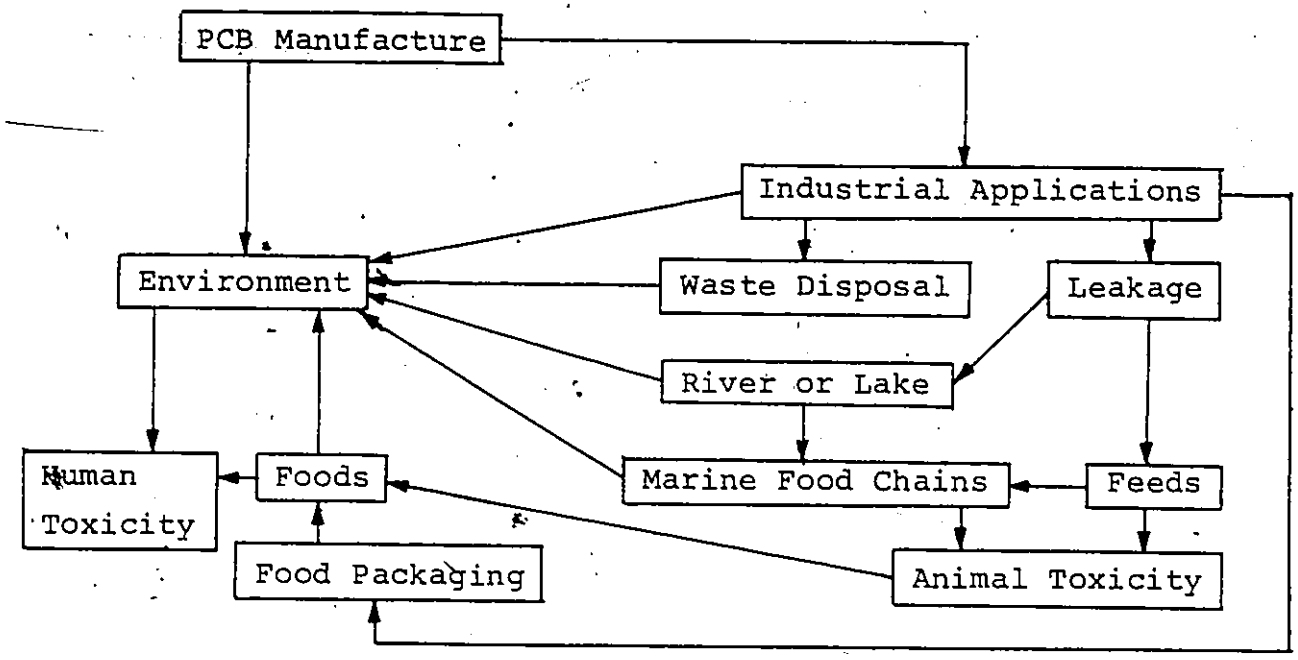
iii) PCBs have been shown[52] to increase the insecticidal properties of certain pesticides. Being thus incorporated into the pesticidal formulations, PCBs find their way into the environment.

iv) The direct leakage of PCB containing heat transfer fluids into foods and foodstuffs leads to the contamination of certain commercial products.

A major source of PCB contamination is leakage into the waterways and atmosphere directly from their point of manufacture[36].

It is reported[44], that one of the major factors affecting PCB distribution both locally and on a worldwide

Fig. 3: Schematic Representation of PCB Routes into the Environment[36]


PCB Manufacture
Industrial Applications
Environment
Waste Disposal
Leakage
River or Lake
Human Toxicity
Foods
Marine Food Chains
Feeds
Food Packaging
Animal Toxicity

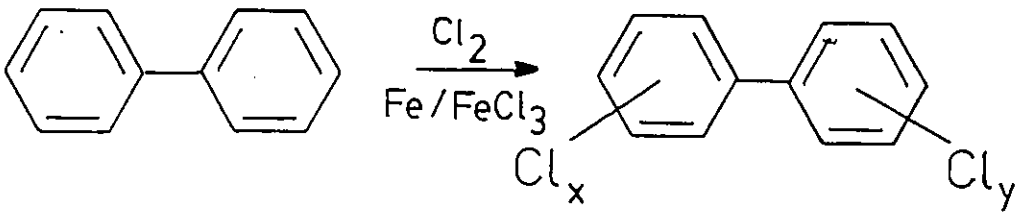
basis is atmospheric transport. It has been estimated[53] that up to 2500 kg of PCBs enter Lake Michigan through precipitation each year.

The U.S. E.P.A.[54] has shown that the major source of exposure to PCB contamination by humans occurs through the consumption of contaminated fish. A study[55] of blood serum PCB levels of Michigan residents revealed elevated levels compared to control populations. The Great Lakes are representative of one of the areas in North America contaminated by PCBs. Serum PCB levels in human tissues were the highest in the test population consuming sport fish from the Lake Michigan area.

6. The Chemistry of PCBs

a. Physical and Chemical Properties

Commercial PCB mixtures are prepared industrially by the chlorination of biphenyl with anhydrous chlorine, in the presence of iron filings or a ferric chloride catalyst[56,57].



(x,y = 1-5)

The process produces a mixture of chlorinated biphenyls with different numbers of chlorine atoms per molecule, and their isomers. The degree of chlorination of a particular mixture accounts for its physical and chemical properties.

The viscosity of PCBs increases in direct proportion to the chlorine content of the mixture. Commercial PCB mixtures may occur as mobile oils (Aroclors 1221, 1232, 1242 and 1248), viscous liquids (Aroclor 1254) or sticky resins (Aroclors 1260 and 1262), due to the mutual depression of the melting points of the components. At room temperature, the individual isomers generally occur as solids[58].

From an environmental standpoint, the major physical properties of PCBs of concern are solubility and vapour pressure. The water solubility of commercial Aroclor preparations decreases with increasing chlorine content[58]. In general, PCBs show low water solubility, but high solubility in hydrocarbon solvents. Water solubility values may vary from as much as 200 ppb for Aroclor 1242 to 25 ppb for Aroclor 1260[59]. These values may vary because of the selective solubilization of the lower chlorinated components. Similarly, it is difficult to determine the water solubility of PCBs because of their tendency to rapidly adsorb to various surfaces, especially glass. Specific chlorobiphenyl isomers show similar trends in water solubility.

The vapour pressures of commercial PCB preparations show similar trends to that of water solubility. The vapour pressures of the mixtures are related to the chlorine content.

Increased chlorine content causes a decrease in the vapour pressure of the PCB mixture[58]. Again, these values may vary due to the presence of lower chlorinated components which show increased volatilities.

The different Aroclor mixtures show a wide range of other physical properties, some of which are summarized in Table 2, for Aroclors 1242, 1254 and 1260. Generally, most Aroclors do not crystallize, but show a pour point, below which they become a resinous substance.

One of the properties of PCBs which led to their extensive use as transformer fluids is their high dielectric constant[60], which is indicative of their conductivity. A high dielectric constant implies low conductivity.

PCBs, in general, show low reactivities. The reactivity appears to decrease as the degree of chlorination increases[58]. The various isomers are stable to industrial oxidative and reductive conditions.

Oxidation of PCBs may be carried out by reacting the mixture with boiling nitric acid (HNO_3) for 100 hours to yield the corresponding chlorobenzoic acid[61].

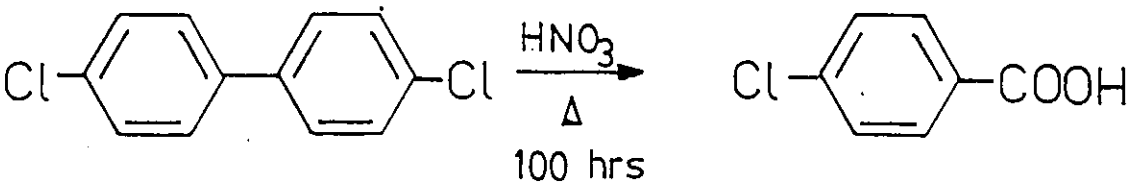
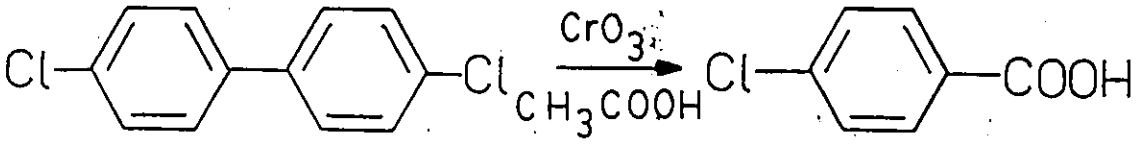


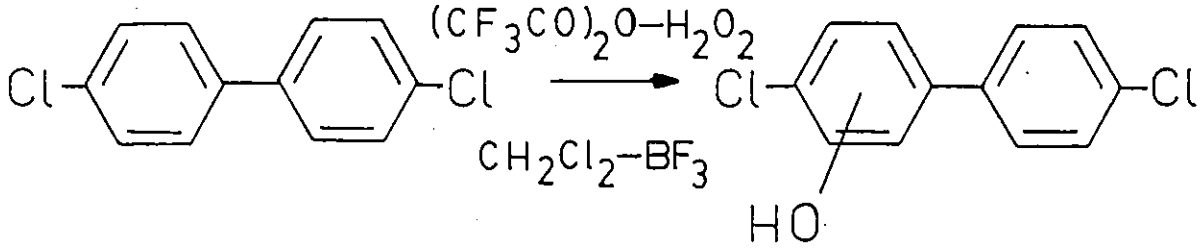
Table 2
Physical Properties of
Aroclors 1242, 1254, 1260[58]

	Aroclor 1242	Aroclor 1254	Aroclor 1260
Appearance	Clear, mobile oil	Light yellow, viscous liquid	Light yellow, soft, sticky resin
Density (gm/ml, 25°C)	1.38	1.55	1.62
Distillation Range (°C, corrected)	325-366	365-390	385-420
Pour Point (°C)	-19	10	31
Dielectric Constant (at 1000 cycles)	5.8 (25°C) 4.6 (100°C)	5.0 (25°C) 4.3 (100°C)	4.3(25°C) 3.7(100°C)

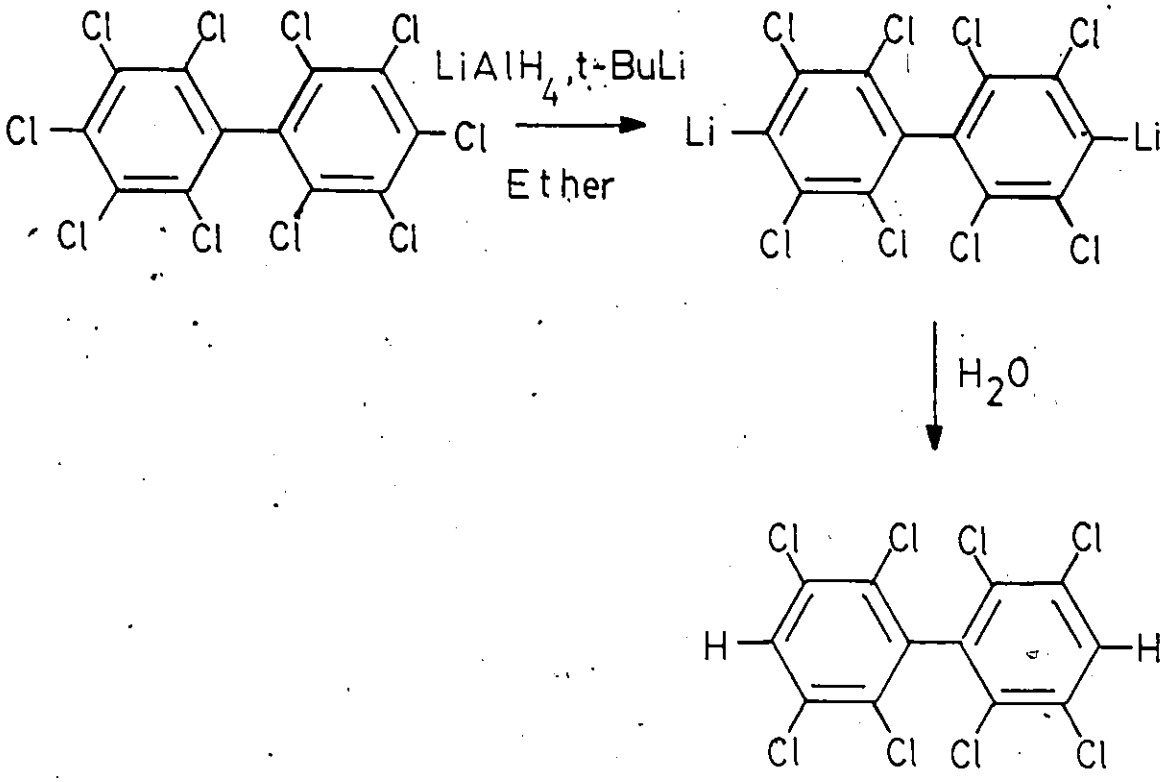
Biphenyls containing an average of 5-7 chlorine atoms per molecule are resistant to oxidation with potassium permanganate ($KMnO_4$), chromic acid (H_2CrO_4) and nitric acid. The mono-, di- and trichlorinated congeners are readily oxidized by chromic anhydride and acetic acid[62].



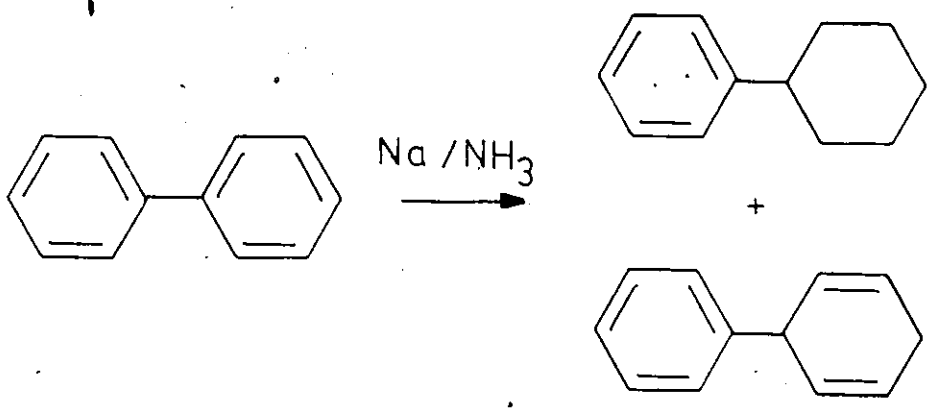
Chlorobiphenylols are readily formed when a chlorinated biphenyl is treated with an appropriate hydroxylating agent such as peroxytrifluoroacetic acid and boron trifluoride[63].



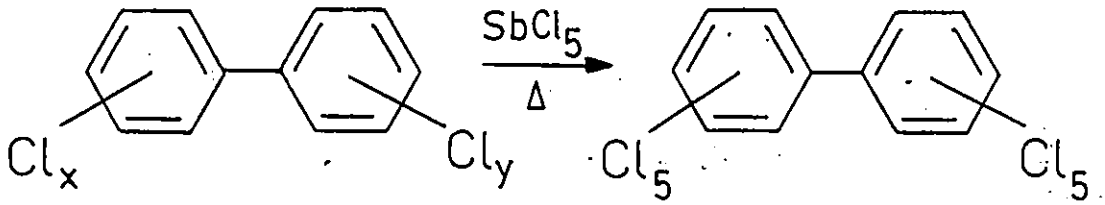
Reductive dechlorination of PCBs may be carried out using lithium aluminum hydride ($LiAlH_4$), butyl lithium (t-BuLi) and water, or the appropriate Grignard reagent and water[64].



Other reagents reported to effect reductive dechlorination include Raney nickel[65], Na_2Te[66], sodium in liquid ammonia[67] and others. If carried out using an alkali metal and liquid ammonia, though, this reaction causes the further reduction of the biphenyl moiety to phenylcyclohexane[68] and phenyl-2, 5-cyclohexadiene[69] via a Birch reduction.



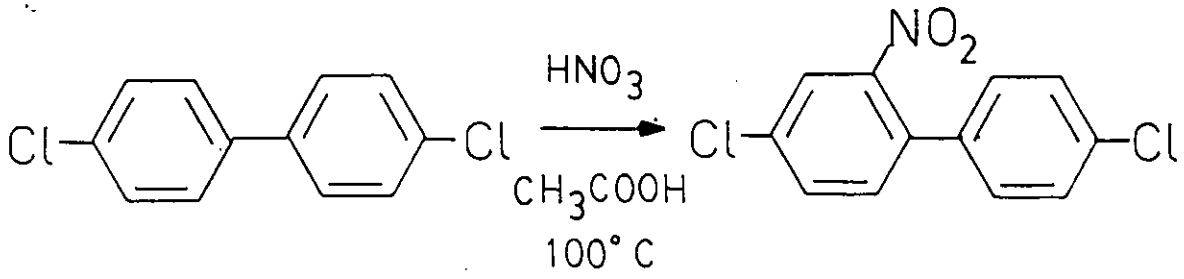
The complete chlorination of a PCB congener may be accomplished using antimony pentachloride ($SbCl_5$)[70,71].



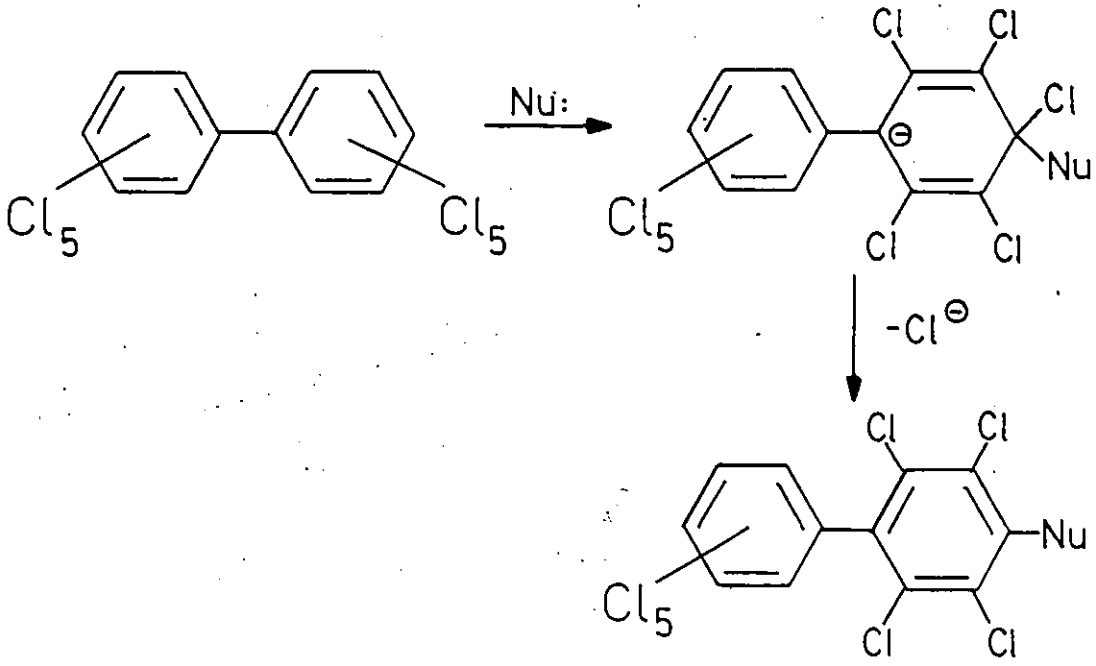
(x,y=0-5)

Similar results have been reported when the starting material is reacted with antimony pentachloride-iodine or the reagent BMC (SO_2Cl_2, $AlCl_3$, S_2Cl_2)[72], and trichlorosulfur tetrachloroaluminate (SCl_3AlCl_4)[73].

Depending on the conditions used, nitration of chlorobiphenyls has been reported[58] to yield well defined products, in substantial yields, of even the highly chlorinated PCB congeners.

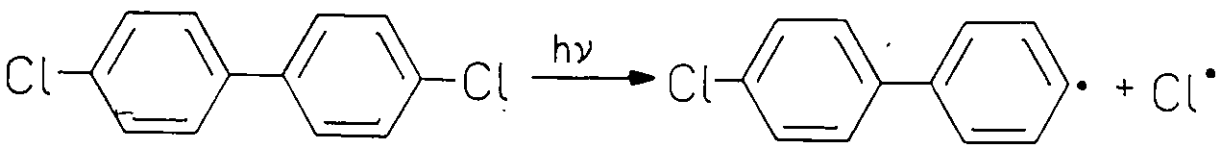


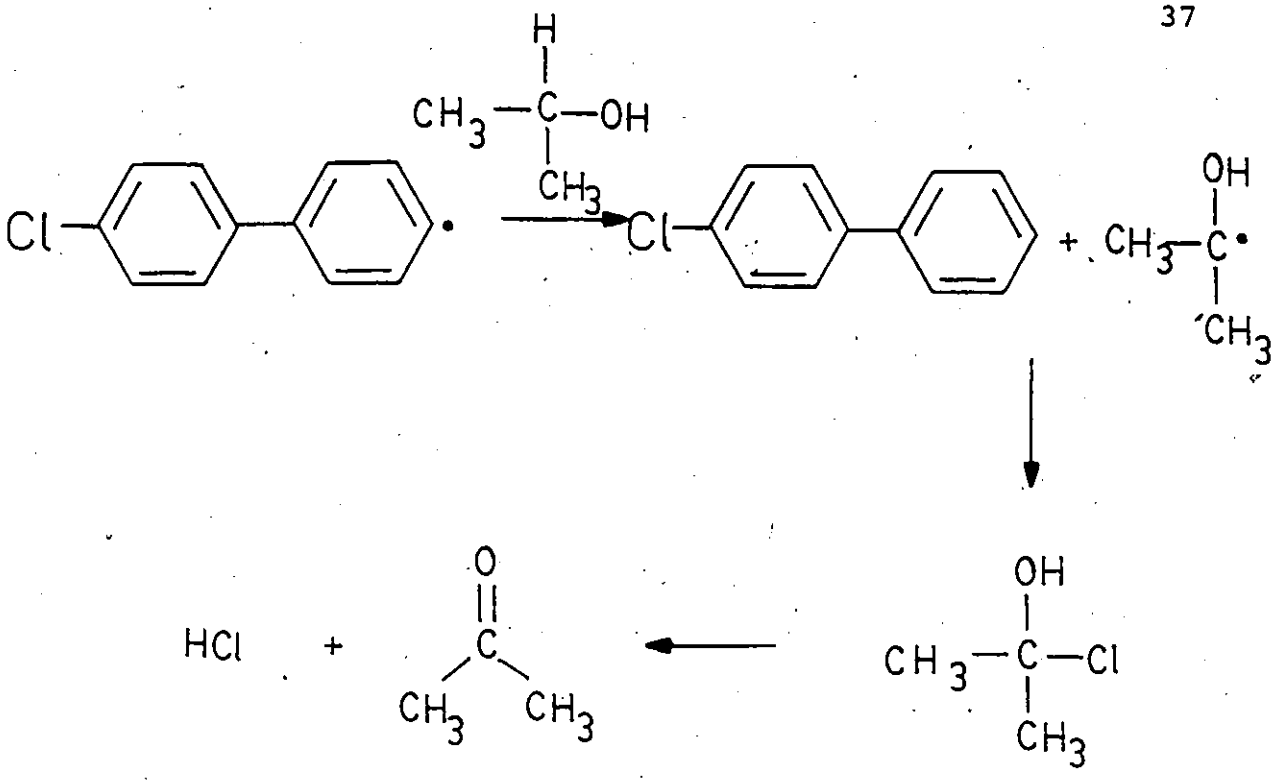
Nucleophilic displacement of chlorines in the more highly chlorinated isomers occurs readily[58]. The substitution is reported to occur preferentially at the 4 and 4' positions due to the generation of a para-quinonoid intermediate.



Recently, the photochemical degradation of PCBs has been of great interest because of the environmental impact it may have. Photochemical degradation causing the cleavage of aromatic C-Cl bonds has been shown[58] to be a major route for the environmental breakdown of not only PCBs, but many other organochlorine compounds. The photochemical degradation of PCBs results in:

i) Dechlorination[74-77], as proposed by the following mechanism



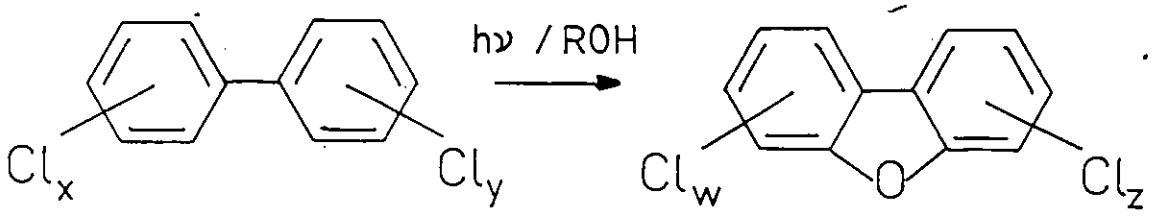


ii) Possible isomerization[74], though no structures have been assigned.

iii) Condensation[58] to chlorinated terphenyls and quaterphenyls.

iv) Chlorination to higher chlorinated congeners.

v) The formation of oxygenated species[58], when irradiated in a hydroxylic organic solvent.



(x, y = 1-5)

(w, z = 1-4)

Chlorodibenzofurans are themselves photolabile[78] and therefore their accumulation due to the photodegradation of PCBs is not known.

vi) Polymerization[75].

b. Characterization of PCBs

The characterization of specific PCB isomers may be done using an assortment of analytical techniques. These include mass spectrometry (MS), infrared (IR) spectrometry, ultraviolet (UV) spectrometry and nuclear magnetic resonance (NMR) spectroscopy.

Mass spectrometry is the most extensively used analytical technique for the confirmational analysis of PCBs[79]. Most chlorobiphenyls give relatively intense molecular ion and $[M-70]^+$ ion peaks. Another important feature of the mass spectra is the appearance of characteristic chlorine isotope clusters (see Chapter II, A.3.b.), which greatly facilitates the determination of the degree of chlorination.

The IR spectra of a biphenyl molecule have been discussed in detail[80]. The major absorption bands occur in three specific regions of the spectrum. These include 4000-2000 cm^{-1} (C-H stretching frequencies), 2000-1250 cm^{-1} (C=C stretching frequencies) and 1250-250 cm^{-1} (bending and deformation frequencies). Webb and McCall[81] studied the IR spectra of different commercial PCB preparations. The IR spectra of specific PCB isomers are characteristic, especially in the 1200-300 cm^{-1} range. Bands in this region of the spectrum

indicate C-H bending and C-Cl stretching vibrations, which are useful in characterizing PCB congeners[82,83].

The UV spectra of specific chlorobiphenyl isomers appear to be more indicative of the molecular structures. A biphenyl has two major UV absorption bands[58]. The main band occurs at λ_{max}= 202 nm (ϵ = 44000) and the lesser, "k" band occurs at λ_{max}= 242 nm (ϵ = 17000). The shifts in λ_{max} are affected by four main factors:

i) The type of substituent.

ii) The positions of the substituents.

iii) The number of substituents.

iv) The degree of substitution at the positions ortho to the Ph-Ph bond.

In general, the main UV band for PCB isomers ranges from λ_{max}= 199nm (ϵ= 43300) for 4-monochlorobiphenyl to λ_{max}= 216 nm (ϵ= 108000) for the fully chlorinated isomer[58].

NMR spectroscopy is probably the most diagnostic of the techniques described, for the precise structural determination of specific PCB isomers. The chemical shifts of the protons are influenced by a number of factors:

i) The position of the proton relative to the chlorines present.

ii) The number of chlorine atoms present, particularly at the positions ortho with respect to the Ph-Ph bond.

iii) The electronic effect of the chlorine substituents on the Ph-Ph bond.

The presence of chlorine substituents has a profound effect

on the chemical shifts of the aromatic protons. Relative to the protons of benzene (δ= 7.27 ppm), the introduction of a chlorine atom into the aromatic ring causes the ortho protons to be shifted approximately 0.02 ppm downfield, and the meta and para protons approximately 0.06 and 0.04 ppm, respectively, upfield. Chlorination of the 2, 2', 6 or 6' positions hinders the rotation of the aromatic rings about the bridging bond. The chemical shift of the proton at the 2 position is shifted upfield approximately 0.15 ppm for each chlorine at the 2' or 6' positions. The upfield shift is only 0.05 ppm if a chlorine atom is substituted in the 6 position.

c. Methods of Determination and Quantitation

As stated earlier, there occur differing commercial preparations of PCBs, under a variety of tradenames. The following discussion will be limited to the Aroclor type mixtures because these have been the most extensively used in North America.

The analysis of environmental samples for PCBs requires certain considerations. There are three major problem areas in this type of analysis[9]. These are summarized as follows:

i) Composition: In almost all cases, PCBs occur as complex mixtures of different isomers. Not all of the 209 possible congeners are present in these mixtures (see Chapter II, B.6.a.)

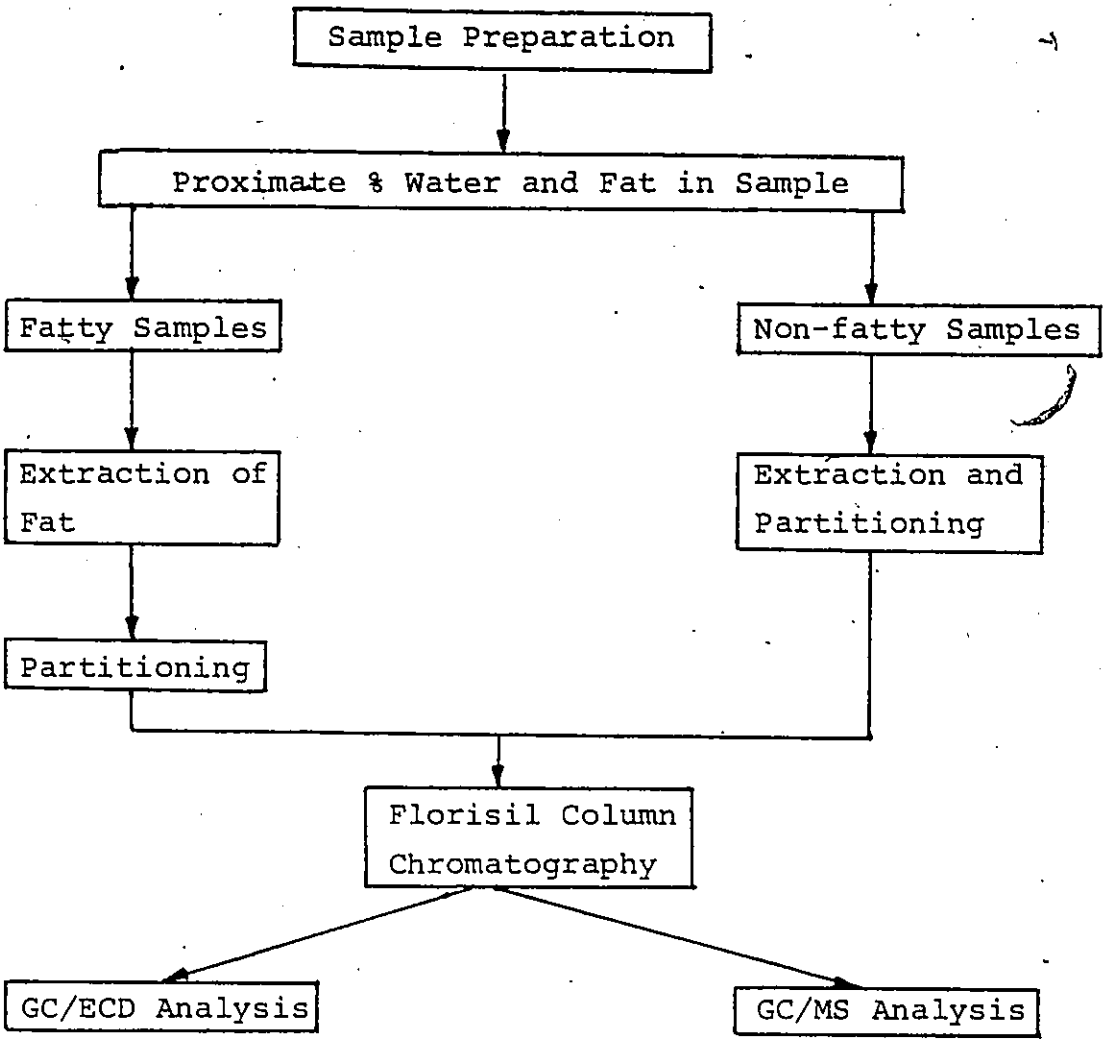
ii) Interfering organochlorine pesticides: Most environmental samples require some sort of prior separation or "clean-up" of the extracts. Many of these procedures are flawed by variable recoveries.

iii) Metabolism: PCB elution patterns from most biological samples do not resemble those of the commercial preparations. This is most likely due to the presence of metabolites or degradation products (see Chapter II, B.6.e.).

The preferred method for the characterization of commercial PCB mixtures is gas chromatography[34]. Usually, the organochlorine contaminants are initially extracted from the biological matrix using suitable organic solvents. These include acetonitrile[84], acetone[85], methylene chloride[85] and petroleum ether[84]. The extract is concentrated and "cleaned-up" using column chromatography. The purpose of the "clean-up" is to separate from the compounds of interest, any pollutants or organic substances which may interfere with the analysis. Florisil[84], silica gel[86] and cesium silicate[87] have all been suggested as suitable stationary phases for this step of the procedure. The "cleaned-up" extract is subsequently injected in a GC for qualitative and quantitative analysis. A typical scheme for the analysis of a sample is illustrated in Figure 3a.

Analyses for PCBs are carried out for either scientific or practical (environmental control) purposes. Thus, various methods for their quantitation have been developed. These include determination as total PCBs, determination as

Fig. 3a: Schematic Representation of Sample Preparation for PCB Analysis[36]


Sample Preparation
Proximate % Water and Fat in Sample
Fatty Samples
Non-fatty Samples
Extraction of Fat
Extraction and Partitioning
Partitioning
Florisil Column Chromatography
GC/ECD Analysis
GC/MS Analysis

a specific Aroclor or the quantitation of specific isomers. All of these methods involve the use of GC techniques which have been related to recent advances in this field.

Total PCB concentration is generally estimated by relating the PCB concentration to that of a single species. This may be accomplished by the following means:

i) Determination as the carbon skeleton; that is, the PCBs are reduced on a column (5% Pt at 180°C with H_2 as the carrier gas) to biphenyl[88].

ii) Perchlorination with $SbCl_5$, then quantitation as decachlorobiphenyl[89].

iii) Microcoulometric determination of the total chlorine content as HCl[90].

Until recently, most PCB analyses were carried out using packed column chromatographic/electron capture detection techniques[91-96]. With packed columns, the qualitative and quantitative characterizations of commercial Aroclor mixtures has been empirical[97], due to inadequate resolution of the chromatographic peaks. Initial studies by Zitko, Hutzinger and Safe[98] showed that retention times and ECD responses for some individual chlorobiphenyls are dependent upon both the structure and the degree of chlorination of the specific isomer.

Rote and Murphy[99] attempted to quantitate the chlorinated components of the various commercial PCB preparations. The authors used the semilogarithmic relationship between the detector response and the average chlorine content of

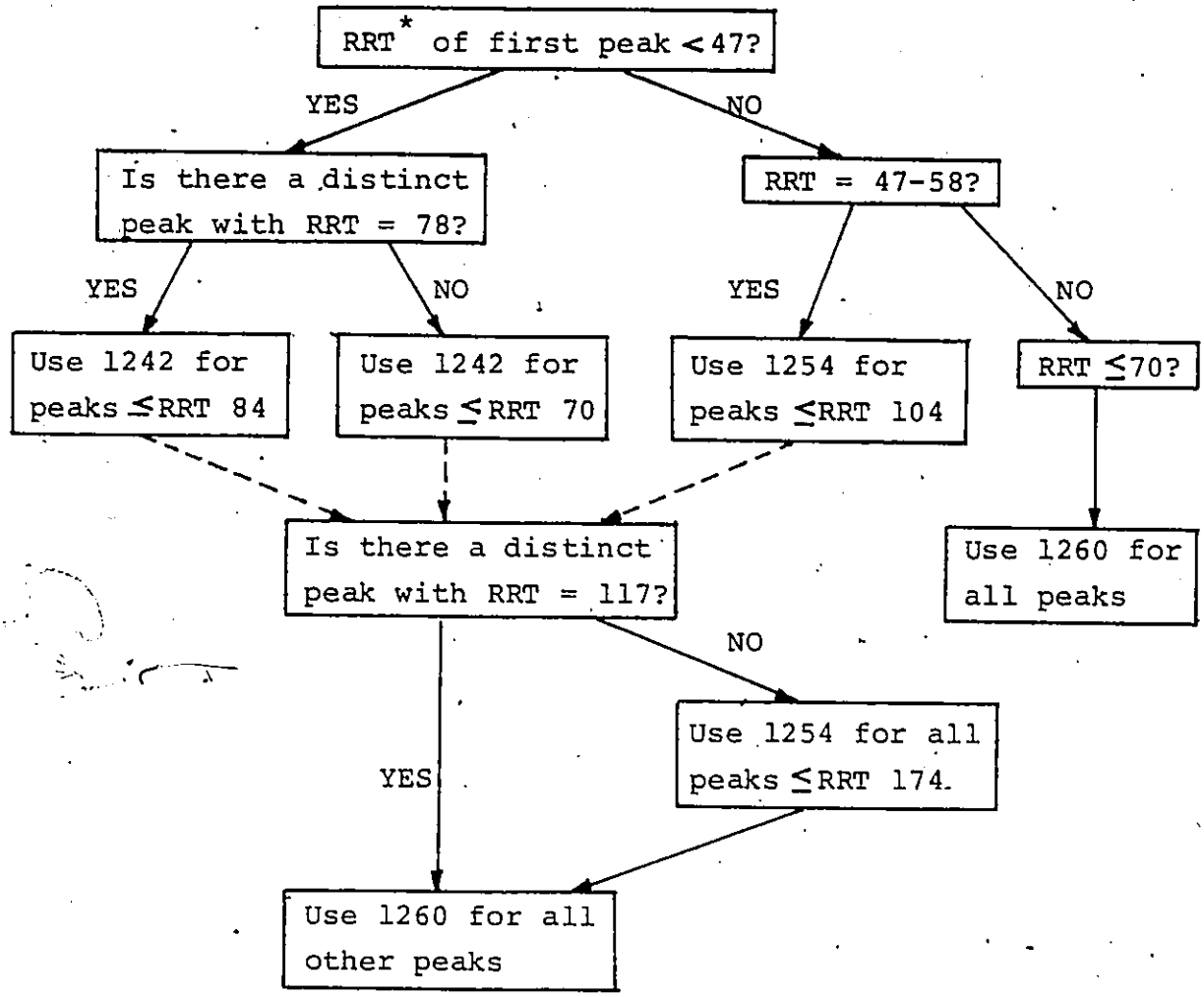
each Aroclor to determine the theoretical response of the detector to each of the chlorinated components (i.e. mono- through deca-). Using these theoretical detector responses, the authors could approximate the amount of chlorinated component represented by each peak in the chromatogram.

The most widely accepted method for the quantitation of PCBs using packed column GC is that of Webb and McCall[100]. Using GC/MS and chlorine specific detection techniques (electrolytic conductivity detector), the authors determined the isomeric distributions and weight percent of PCB represented by each peak of the electron capture gas chromatogram of a particular Aroclor mixture and thus determined the ECD responses for each identified chlorobiphenyl. Using appropriate standards, the authors carried out a "peak by peak" quantitation and reported their results as total PCBs

Chau and Sampson[101], who sought a uniform quantitation scheme for PCBs, recommended that the procedure by Webb and McCall was the most applicable. In summary, the authors concluded, that along with its applicability to samples from a wide variety of sources, this method eliminates the need for mixed standards and yields more realistic results. Its simplicity, reportedly, facilitates its applicability to computerized data techniques (see Figure 4).

The advent of capillary columns has greatly improved the resolving capabilities of the GC/ECD, thus improving the qualitative and quantitative aspects of PCB analysis. The increased separation allowed by glass capillary columns has

Fig. 4: Chromatogram Partitioning Flow Diagram for the Quantitation of Aroclors 1242, 1254 and 1260[100]



* RRT p,p'-DDE = 100

allowed for a compound by compound analysis of PCB mixtures[102, 117]. Because glass capillary columns are manufactured simply and inexpensively, their use has become the accepted method for PCB analysis[103], rendering the use of packed columns almost obsolete. This, along with the increased availability of specific PCB isomer standards, has given the analyst the opportunity to quantitate specific PCB congeners.

d. Toxicity

The severity of PCB toxicity was first realized in 1968 with the outbreak of "Yusho" in Japan[9]. This poisoning was due to the presence of PCBs in rice oil. The symptoms included chloracne, discoloration of the gums and nailbeds, swelling of joints, waxy secretions from the glands surrounding the eyes, and general lethargy and pain. This outbreak led to increased attention to the levels of PCBs in foods.

It is difficult to determine the toxicity of PCBs because of the heterogeneity of the mixtures and analytical difficulties including separation, identification and quantitation.

Since the late sixties, an abundance of information regarding the acute and chronic toxicity of PCBs in many animals has been obtained[58]. However, most of the studies were carried out using commercial PCB preparations, and little is known about the toxicities of specific PCB isomers.

Because of their lipophilic nature, PCBs tend to accumulate in the liver and fatty tissues. It appears that

this accumulation is higher in the case of tetra- and pentachlorobiphenyls. The Aroclors show moderate acute toxicity. More disturbing are their chronic toxicological effects, due to the cumulative action of PCBs[104]. The acute oral LD_{50} in mammals ranges from 2000-10000 ppm, whereas chronic ingestion of small amounts (10 ppm) for 50 days leads to chloracne in humans[9]. In general, it appears that PCB toxicity increases with decreased chlorine content[9]. It also seems, that PCB toxicity parallels its ability to compete *in vitro* for specific binding sites in cytosol carrier proteins[9].

e. Metabolism

It has been reported[105], that the GC elution patterns for PCBs extracted from biological matrices do not correspond with those obtained for PCB standards. This, as well as the environmental impact of PCBs, caused extensive investigation[106,107] of their metabolic fates. The results of these studies indicated that the major metabolites are hydroxylated PCBs. It was also shown[108], that as the degree of chlorination increases, the rate of metabolism decreases. Similarly, it appears[107,109] that PCB isomers with unsubstituted para positions tend to be more readily metabolized.

Safe *et al*[110] have shown that the major metabolic pathways for PCBs are hydroxylation, chlorination and arene oxide formation. The results of the study indicated that hydroxylation occurs predominantly at the para positions of the biphenyl, and at positions ortho and para to the chlorine substituents.

It has been suggested[9], that dechlorination of the higher chlorinated biphenyls may yield a GC profile which is indicative of a less chlorinated Aroclor, thus causing discrepancies in the choice of a suitable standard. Similarly, the removal of certain congeners from the mixture by metabolic hydroxylation creates difficulties in the determination of the Aroclor type, as the chlorobiphenylols are generally not eluted from the chromatographic column in the "clean-up" step of the extraction procedure.

CHAPTER III

DISCUSSION AND OBJECTIVES OF THE STUDY

Because of the relatively unknown implications of OCS contamination, and the well established hazards of PCBs, the levels of these compounds in biological matrices, for either scientific purposes or environmental control, require further investigation. The purpose of the following study is to determine the levels of OCS and PCBs in clams obtained from the Lake St. Clair area. The report will emphasize the levels of OCS and relate them to the areas from which the clams were obtained and other characteristics of the clams, if possible. PCBs are studied, but to a lesser extent, due to the difficulties and discrepancies encountered in their quantitation.

Because of the increased demand for improved analytical methods, the report will also deal with the development of improved methodologies for the extraction and quantitation of OCS and PCBs from biological matrices.

The synthesis and methods of characterization of OCS are examined in this study. This was required, since OCS is not available commercially as a standard.

CHAPTER IV

EXPERIMENTAL TECHNIQUE AND PREPARATION OF MATERIALS

A. Synthesis of OCS

The synthesis of OCS was carried out according to the procedure outlined by Korte and Bieniek[21]. Modifications to this procedure included the use of a Florisil column to further purify the synthetic product[111]. OCS standards of greater than 99% purity were obtained as a gift from Dr. I. Chu, Environmental Health Directorate, Health Protection Branch, Ottawa, Canada.

To 400 ml of carbon tetrachloride in a 1000 ml round bottom flask, was added 1.0 ml of 2,6-dichlorostyrene. Cl_2 gas was bubbled into the system for 220 minutes and the mixture was stirred at room temperature for 20 hours. The solvent was removed by distillation and 4.3 ml of antimony pentachloride was added to the remaining residue. The mixture was heated in an oil bath at 190 $\pm$ 2°C for 4 hours, with a calcium chloride drying tube attached. The mixture was allowed to cool to room temperature, and 40 ml of 20% (v/v) hydrochloric acid was added. The solution was extracted twice with 50 ml portions of hexane. The combined extracts were washed with 100 ml of distilled water and dried over anhydrous sodium sulfate. This mixture was filtered and the filtrate evaporated at reduced pressure to dryness. The remaining oil was crystallized in methanol, and recrystallized twice, once with 100 ml of met-

hanol and finally with 30 ml of methanol.

B. Characterization of Synthetic Products

Apart from the observation of physical properties, the products obtained from the OCS syntheses were characterized by ^{13}C-NMR spectroscopy, GC, GC/MS, UV spectrometry and IR spectrometry. GC/MS and ^{13}C-NMR spectral data were compared to that found in the literature[21]. UV and IR spectral data appear to have not yet been published.

^{13}C-NMR chemical shift data for OCS were obtained using a Bruker CXP Pulsed NMR Spectrometer containing a 90 MHz magnet. An almost saturated solution of OCS, in a chloroform solvent, was used as a sample.

GC/MS data were obtained on a Finnegan 4000 quadrupole MS interfaced via a glass jet separator to a Finnegan 9610 packed column GC. Operating conditions for the analyses were as follows:

Column: 6' x 4 mm (i.d.) glass column;
3% OV-1 on Chromosorb W (HP)
Injector Temp.: 220°C
Column Temp.: 2.0 min. at 100°C;
100-260°C at 8°C/min.;
10.0 min. at 260°C
Separator Temp.: 260°C
Carrier Gas: He at 20 ml/min.
Ionizer Temp.: 250°C
Emission Current: 0.50 mA
Electron Multiplier: 1200 V
Electron Energy: 70 eV

GC data were obtained, initially, using a Beckman GC45 packed column GC equipped with a flame ionization detector. Latter results were obtained using a Hewlett-Packard 5790A capillary column GC equipped with a pulsed ^{63}Ni ECD (see Chapter IV, D.3.a. for operating conditions).

IR and UV spectral data were obtained using a Beckman IR-20A Spectrophotometer and a Shimadzu UV-240 UV-visible Spectrophotometer, respectively.

C. PCB Standards

All PCB standards used in this study were obtained from Ultra Scientific Ltd., Hope, Rhode Island. The commercial mixtures studied were Aroclors 1242, 1254 and 1260. The specific isomers used in the semi-quantitative analysis of PCBs in clam tissues were 2,5,2'-trichlorobiphenyl, 2,5,2',5'- and 2,4,3',4'-tetrachlorobiphenyls, 2,4,5,2',5'- and 2,4,6,3',4'-pentachlorobiphenyls, 2,3,4,2',4',5'- and 2,4,5,2',4',5'-hexachlorobiphenyls and 2,3,4,5,6,2',5'-heptachlorobiphenyl.

D. Clam Analysis

1. Sampling Technique

The clams studied were all of the species *Lampsilis radiata siliquoidea*. Samples were obtained by divers at various stations along the St. Clair River, Lake St. Clair and the Detroit River (Figures 5-7)*. The clam meat was immediately removed from the shell, and both the shell and meat were

* The sampling program was coordinated by Dr. Chris Pugsley of the Great Lakes Institute.

Fig. 5: Sampling Stations in Lake St. Clair

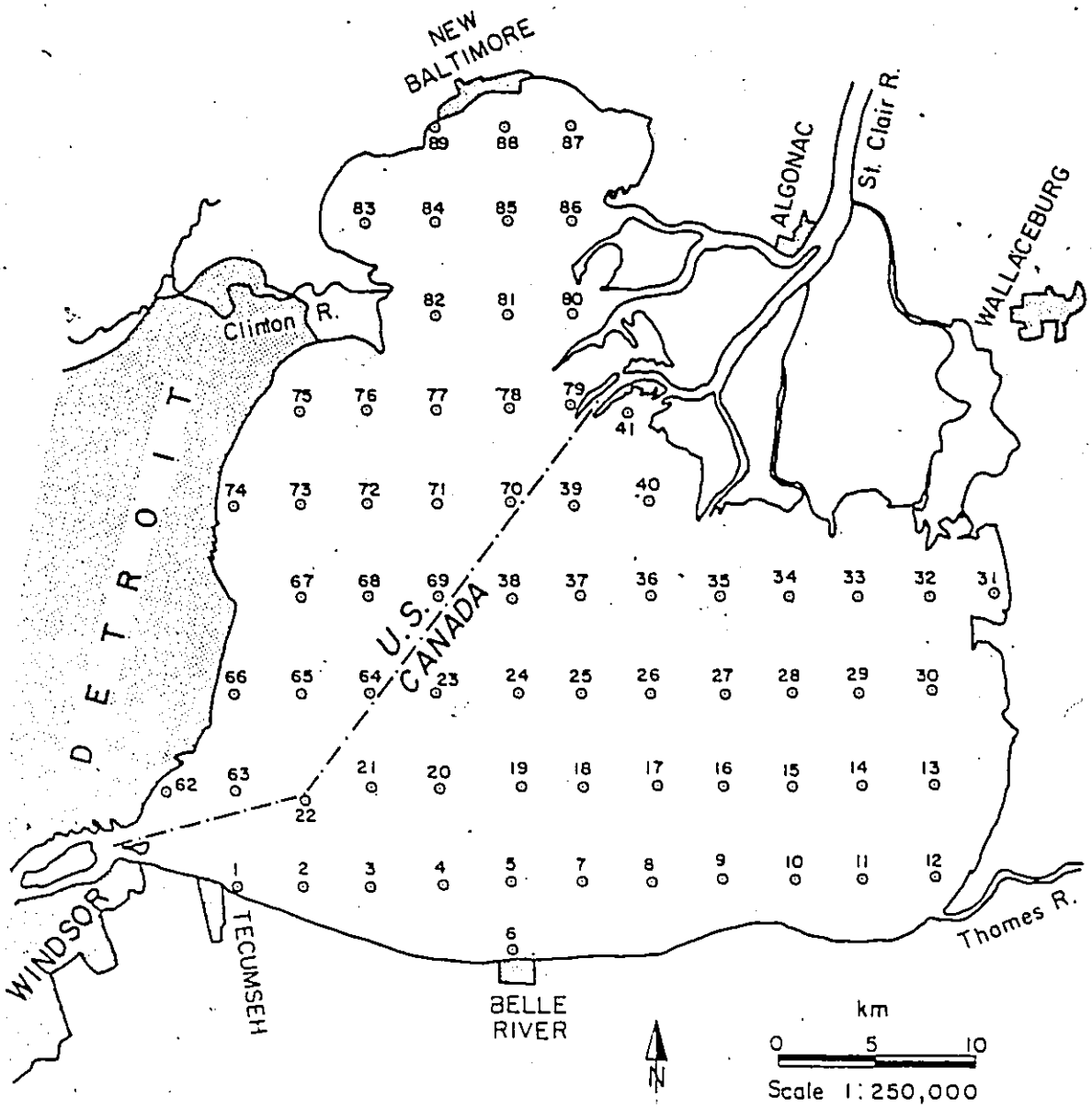

NEW BALTIMORE
ALGONAC
St. Clair R.
WALLACEBURG
Clinton R.
DETROIT
U.S.
CANADA
WINDSOR
TECUMSEH
BELLE RIVER
Thomes R.
km
0 5 10
Scale 1:250,000

Fig. 6: Sampling Stations along the Detroit River

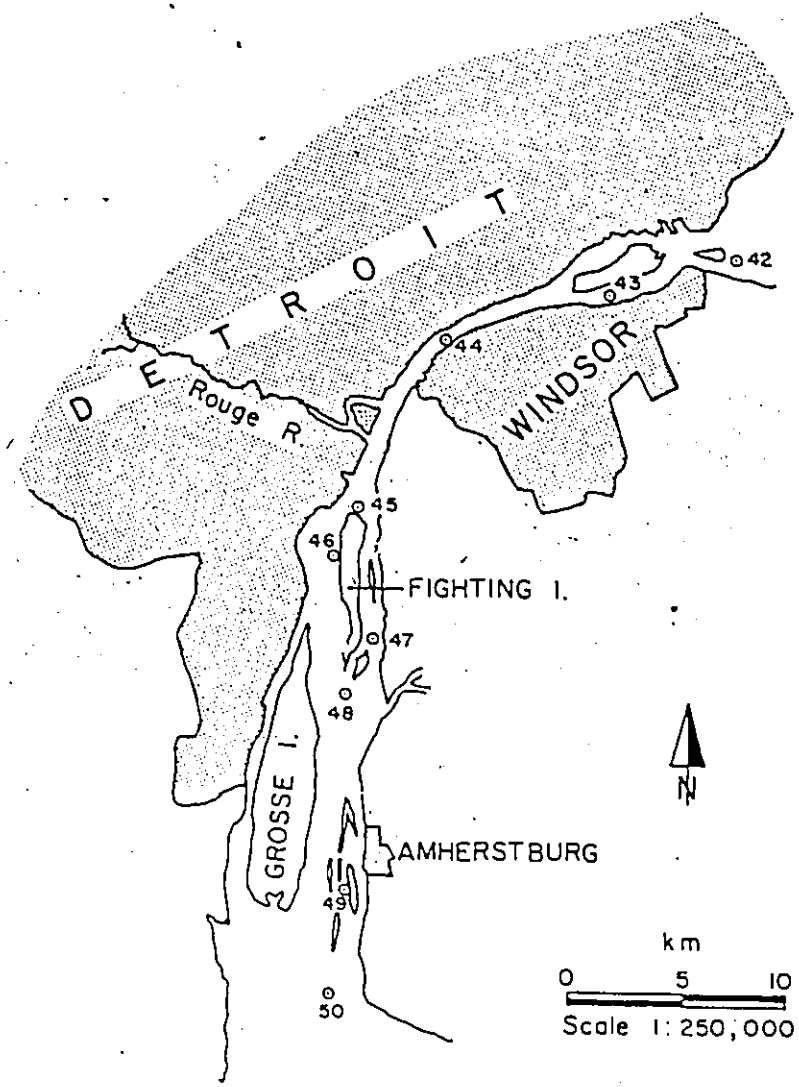
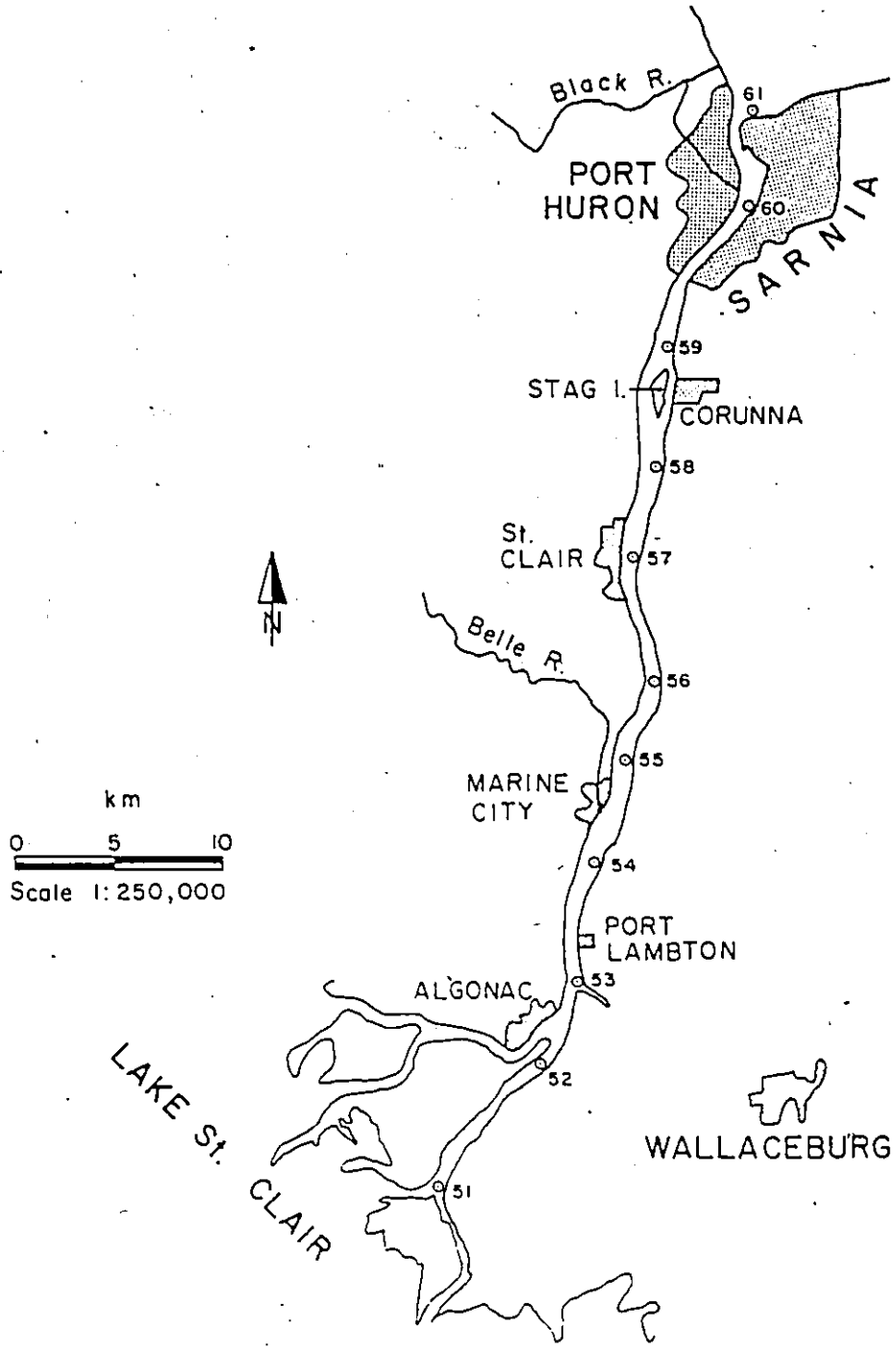

DETROIT
Rouge R.
WINDSOR
42
43
44
45
46
FIGHTING I.
47
48
GROSSE I.
AMHERSTBURG
49
50
N
km
0 5 10
Scale 1:250,000

Fig. 7: Sampling Stations along the St. Clair River


Black R.
61
PORT
HURON
60
SARNIA
59
STAG I.
CORUNNA
58
St.
CLAIR
57
N
Belle R.
56
55
MARINE
CITY
km
0 5 10
Scale 1:250,000
54
PORT
LAMBTON
ALGONAC
53
52
LAKE St. CLAIR
WALLACEBURG
51

labelled and stored in ice. The samples were transported to the laboratory and frozen at -21°C. All samples were wrapped in heavy duty aluminum foil, prerinsed in acetone, petroleum ether and pesticide grade hexane (Caledon Laboratories Ltd., Georgetown, Canada). The shells were used for speciation, age determinations, shell measurements and determination of sex.

The clam samples were speciated by T. Freitag (U.S. Army Corps of Engineers, Detroit, Michigan), based on clam shape, beak size and structure, and the width of the green bands radiating out from the beak. *Lampsilis radiata siliquoidea* have elliptical shells exhibiting concentric wrinkles and lines of growth. Their beaks are low, and project slightly above the hinge line. Other characteristics of the beak are shallow cavities, a coarse sculpture and numerous concentric bars having shallow central situation. The green bands of this species are narrow, compared to that of *Lampsilis ovata ventricosa*, which is the most similar species to *Lampsilis radiata siliquoidea* in this area.

The sex of the clam was determined by observing the shape of the shell. A female has a rectangular shell shape, while a male exhibits a rounder shell. The female also has a characteristic invagination on the ventral margin of the shell.

The ages of the clams were estimated by counting the number of growth rings on the shell. Each growth ring corresponds to approximately one year.

Measurements of the clam shells were made on the left valve of each clam to determine the length, height and shell thickness of each sample. The length of the shell is defined by the distance from the anterior to the posterior end of the valve. Shell height is determined by measuring the distance from the umbo to the ventral margin of the shell. These measurements were obtained using a pair of Vernier calipers. Shell thickness was measured at the anterior muscle attachment location using a Mitutoga point micrometer.

2. Sample Preparation

a. Preparation of Glassware

All glassware used in this study was cleaned according to a procedure suggested[112] by the Canadian Centre for Inland Waters (CCIW), with certain modifications. The following is an outline of the procedure used to clean glassware in order to obtain adequate, or clear blank analyses.

The glassware was initially washed with hot, soapy water. After rinsing well with hot water, the glassware was rinsed three times using technical grade (wash) acetone. The glassware was further rinsed three times with petroleum ether (b.p.= 30-60°C), and finally, once with pesticide grade hexane. The solvent rinse volumes were generally 2-3% of the volume of the particular piece of glassware.

This procedure omits the suggested use of an initial chromic acid rinse. It is known that chromic acid cleaning

solution tends to activate the surface of the glassware; that is, it tends to increase the adsorptive capability of the glass. Chromic acid was used, however, when cleaning the sintered glass funnels, as small amounts of biological matter could not be removed otherwise. This tissue tended to block the sintered glass and thus hinder the filtration of the extract.

b. Handling of Materials

Because of the toxic or potentially toxic nature of the standards used in this study (see Chapter II, A.3.d. and B.6.d.), all materials were handled in fumehoods and transferred using sterile, disposable pipettes. The importance of working in an adequately ventilated area cannot be overemphasized, as many of the organic materials used show high volatilities and are potentially hazardous.

c. Sample Extraction and Clean-up

All samples were prepared, extracted and cleaned-up according to a procedure similar to that recommended by the CCIW[113]. Various modifications were required, since OCS and PCBs were the only contaminants of interest, and because of the types of instrumentation used.

Frozen samples were weighed whole, then placed in 600 ml beakers. 120 ml of pesticide grade acetonitrile (Caledon Laboratories Ltd., Georgetown, Ontario) was added, and the mixture homogenized, using a Polytron (Brinkmann Instruments,

New York), for 1.0-1.5 minutes. This mixture was filtered with suction through a sintered glass funnel. This step was repeated twice, once with 120 ml of acetonitrile combined with 40 ml of water, and once with 50 ml of acetonitrile. After the final extraction, the beaker was rinsed twice with 20 ml portions of acetonitrile. The aqueous content of the combined extracts was made up to 20% with deionized water.

Back-extraction of the combined filtrates was carried out with 150 ml, then twice with 75 ml of pesticide grade petroleum ether (Caledon Laboratories Ltd., Georgetown, Ontario). The petroleum ether extracts were combined, and the aqueous phase discarded. The combined extracts were washed with 200 ml of distilled water and dried by passage through columns containing approximately 15 g of anhydrous sodium sulfate, previously heated to 600°C overnight and stored at 130°C. The dried extracts were concentrated to approximately 5 ml using a Kuderna-Danish evaporator, in preparation for the clean-up.

Clean-up of the extracts was done using 20 mm x 40 cm glass columns containing approximately 30 g of Florisil. The Florisil was also heated to 600°C overnight and stored at 130°C before use. The Florisil columns contained a top layer (1-2 cm) of anhydrous sodium sulfate. The concentrated extracts were pipetted onto the Florisil columns and eluted with 200 ml of petroleum ether. The eluant was collected in a 500 ml round bottom flask and concentrated in a Kuderna-Danish evaporator to 4-5 ml. The concentrate was diluted to

10 ml with pesticide grade hexane (Caledon Laboratories Ltd., Georgetown, Ontario), and a 1.0 ul aliquot was injected into the GC/ECD. A summary of this procedure is illustrated in Figure 8.

3. Data Analysis

a. Quantitative Analysis of OCS

OCS was quantitated in the clam samples by the external standard method. 1.0 ul aliquots were injected into a Hewlett-Packard 5790A capillary column GC/ECD. The column used was 15 m x 0.25 mm fused silica, containing a cross-linked DB-1 stationary phase, supplied by J & W Scientific, Rancho Cordova, California. The following operating conditions were used for the analyses:

Injector Temp.: 250°C
Column Temp.: 0.5 min. at 50°C
50-250°C at 7.0°C/min.
10.0 min. at 250°C
Detector Temp.: 300°C
Carrier Gas: He at 1.5 ml/min.
Detector Make-up Gas: 5% methane/95% argon
at 60 ml/min.
Injection Mode: splitless

Peak areas were determined using a Hewlett-Packard 3390A integrator. The external standards, obtained from Dr. I. Chu (see Chapter IV, A.), were diluted in hexane to 24 ppb.

The quantitation of OCS in clam tissues is illustrated

Fig. 8: Procedure Used for the Analysis of OCS and PCBs in Clam Tissues

Sample weighed and homogenized with acetonitrile

↓

Filter → Discard homogenate or store for further analysis

↓

Adjust aqueous content to 20%

↓

Extract three times with petroleum ether → Discard aqueous portion

↓

Wash with 200 ml distilled water → Discard aqueous portion

↓

Dry extract on sodium sulfate column

↓

Concentrate dried extract to 4-5 ml

↓

Clean-up extract on Florisil column

↓

Elute with 200 ml petroleum ether

↓

Concentrate eluant to 4-5 ml

↓

Dilute concentrate to 10 ml

↓

Inject aliquot for GC analysis

by the following equation,

$$\text{OCS (mg/kg)} = \frac{A_s}{A_{std}} \times W_{std} \times \frac{1}{V_{inj}} \times 10^{-2} \times \frac{1}{Y}$$

A_s = peak area (sample)
A_{std} = peak area (standard)
W_{std} = weight standard (pg) to give A_{std}
V_{inj} = volume injected (ul)
Y = weight sample (g)

Samples were analyzed in batches of five, that is each run consisted of four clam samples and one blank. Samples were injected into the GC in duplicates and standards were run with each batch of samples analyzed to compensate for any variance in detector response.

Blank samples were prepared identically to the clam samples. Volumes of acetonitrile equal to those used for the clam samples were homogenized with the Polytron and filtered. The filtrates were combined, extracted, cleaned-up and analyzed in the same manner as outlined in Chapter IV, D.3.c.

b. Qualitative Analysis of OCS

Qualitative (confirmational) analyses for OCS were carried out on a Hewlett-Packard 5790A GC/ECD and a Finnegan 4000 GC/MS (see Chapter IV, D.3.a. and Chapter IV, B., respectively, for operating conditions). The GC/ECD results were used mainly to determine the retention time of the major component in the standard. An aliquot of this standard was then injected into the GC/MS, and the structure of the major

component determined. After determining the structure of the major component to be OCS, the retention time of this compound, initially determined by GC/ECD, may be used to confirm the identity of the unknown peak in the GC/ECD chromatogram obtained for a sample.

c. Semi-quantitative Analysis of PCBs

Aroclor 1254 and 1260 levels, in clam tissues, were estimated based on the levels of two specific isomers found in the commercial preparations. Studies[102,114] have determined the % (w/w) of specific isomers in the Aroclor mixtures. Using these values, one may roughly estimate the amount of Aroclor present, based on the detector response toward that specific isomer. Aroclor 1254 was quantitated using 2,4,5,2',4',5'-hexachlorobiphenyl (7.4% w/w) and 2,3,4,5,6,2',5'-heptachlorobiphenyl (1.3% w/w). Aroclor 1260 was quantitated based on the levels of 2,3,4,2',4',5'-hexachlorobiphenyl (9.8% w/w) and 2,3,4,5,6,2',5'-heptachlorobiphenyl (6.14% w/w). The general scheme used to semi-quantitatively determine PCB levels in clams is illustrated in Figure 9.

Procedure 1: Aroclors 1254 and 1260 are both present.

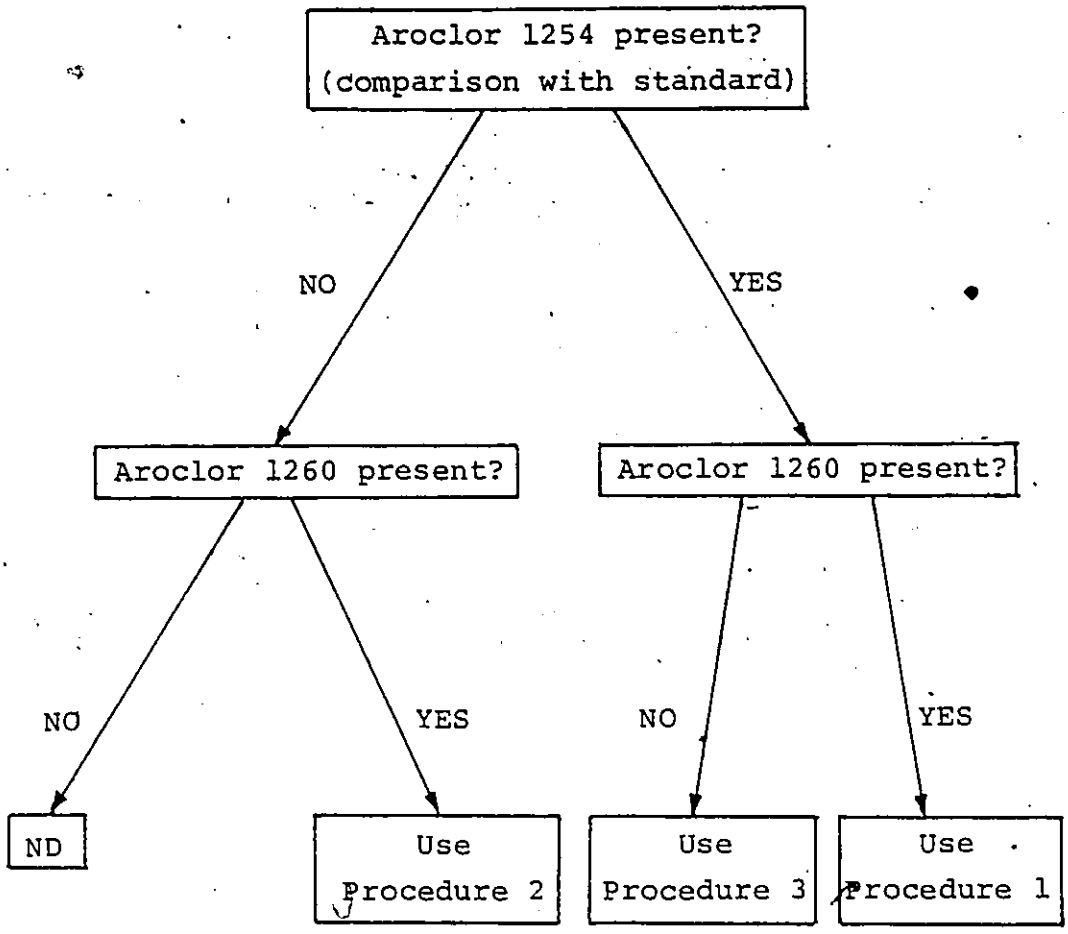
Quantitation of Aroclor 1254

Peak corresponding to:

2,4,5,2',4',5'- $B = (0.457)\frac{(A/RF \times 0.4)}{7.4\%}$

2,3,4,5,6,2',5'- $C = (0.175)\frac{(A/RF \times 0.7)}{1.3\%}$

Fig. 9: Scheme for the Semi-quantitation of Aroclors 1254 and 1260


Aroclor 1254 present?
(comparison with standard)
NO
YES
Aroclor 1260 present?
Aroclor 1260 present?
NO
YES
NO
YES
ND
Use
Procedure 2
Use
Procedure 3
Use
Procedure 1

$$\text{Aroclor 1254 (mg/kg)} = \frac{B + C}{2} \times \frac{10}{Y}$$

Quantitation of Aroclor 1260

Peak corresponding to:

2,3,4,2',4',5'- $\quad D = (0.620)\dfrac{(A/RF \times 0.9)}{9.8\%}$

2,3,4,5,6,2',5'- $\quad E = (0.825)\dfrac{(A/RF \times 0.2)}{6.14\%}$

$$\text{Aroclor 1260 (mg/kg)} = \frac{D + E}{2} \times \frac{10}{Y}$$

Procedure 2: Aroclor 1254 absent

Peak corresponding to:

2,3,4,2',4',5'- $\quad G = \dfrac{(A/RF \times 0.9)}{9.8\%}$

2,3,4,5,6,2',5'- $\quad H = \dfrac{(A/RF \times 0.2)}{6.14\%}$

$$\text{Aroclor 1260 (mg/kg)} = \frac{G + H}{2} \times \frac{10}{Y}$$

Procedure 3: Aroclor 1260 absent

Peak corresponding to:

2,4,5,2',4',5'- $\quad I = \dfrac{(A/RF \times 0.4)}{7.4\%}$

2,3,4,5,6,2',5'- $\quad J = \dfrac{(A/RF \times 0.7)}{1.3\%}$

$$\text{Aroclor 1254 (mg/kg)} = \frac{I + J}{2} \times \frac{10}{Y}$$

A = peak area (sample)
RF = response factor of the ECD toward the particular PCB isomer
Y = weight sample (g)

CHAPTER V

EXPERIMENTAL RESULTS AND DISCUSSION

A. Synthesis of OCS

The synthesis of OCS yielded off-white to white crystals, depending on the purity of the product. It appears that a decrease in OCS purity is indicated by a change in colour from white to off-white. For three syntheses, the yields obtained ranged from 2.3-29.1%, as compared to the cited 60%[21]. The products showed melting points between 94-97°C. Recrystallization using a methanol solvent, rather than ethanol appears to produce higher yields and better formed crystals.

The purities of the synthetic products varied from 92.6% to 98.8% as shown by GC/ECD. It has been suggested[111], that the purity may be improved by Florisil column chromatography. The use of 60/100 mesh Florisil did not effect complete separation of OCS from the impurities present. It was realized, however, that the mesh size of the adsorbent is an important factor in separating OCS from other impurities. The suggested mesh size is 100/120 mesh, and further studies regarding this purification technique are required.

An interesting physical property of OCS, determined in this laboratory, is its volatility. Many organochlorine contaminants show low volatilities. OCS appears to have a higher volatility than the others, such that upon rapid heating it tends to sublime. This indicates that a simple distil-

lation apparatus or rotary evaporator may not be adequate for the concentration of sample extracts, as some OCS may be lost. A Kuderna-Danish evaporator shows little or no loss of OCS as the extract is concentrated. Similarly, OCS sublimation poses an interesting question in terms of a possible purification technique rather than Florisil column chromatography.

The synthetic products were characterized by an assortment of analytical techniques. ^{13}C-NMR chemical shift data obtained for the synthesized OCS are listed in Table 3. This data corresponds quite well with that cited from the literature[21]. The sensitivity of the instrument appeared to be quite low, even with a highly concentrated sample and after 58825 scans. The low natural abundance of ^{13}C and the effect of the attached chlorine atoms may account for this.

The most useful technique for confirming the structure of the synthetic products was GC/MS. The data obtained showed the chlorine clusters characteristic of the peaks at $[M]^{+\cdot}$ (m/z = 376,378,...,392), $[M-Cl]^{+}$ (m/z = 341,343,...,355) and $[M-2Cl]^{+\cdot}$ (m/z = 306,308,...,318). The mass spectrum obtained for OCS is illustrated in Figure 10.

On two separate occasions, the product obtained from the OCS synthesis was a mixture of the characteristic OCS crystals and a brownish oil. GC/MS analysis showed this product to be a mixture of OCS and HCS (approximately 1:1). The GC/MS data for these products are illustrated in Figures 11-12. The mass spectrum for HCS shows characteristic peaks

Table 3

Experimental ^{13}C-NMR Chemical Shift Data for OCS

Carbon Number	$\delta_{lit.}$[21] (ppm)	$\delta_{obs.}$ (ppm)
C-1, 4	135.74, 134.13	135.8, 134.2
C-2, 6; C-3, 5	132.60, 132.66	132.7
C-7, 8	124.41, 125.29	124.5, 125.3

Fig. 10: The Mass Spectrum of OCS

MASS SPECTRUM
06/16/83 12:38:00 + 14:33
SAMPLE: OCTA-,HEPTACHLOROSTYRENES
CONDS.: EI

DATA: OCSMIX #291
CALI: CALI8 #2

BASE M/E: 308
RIC: 765952.

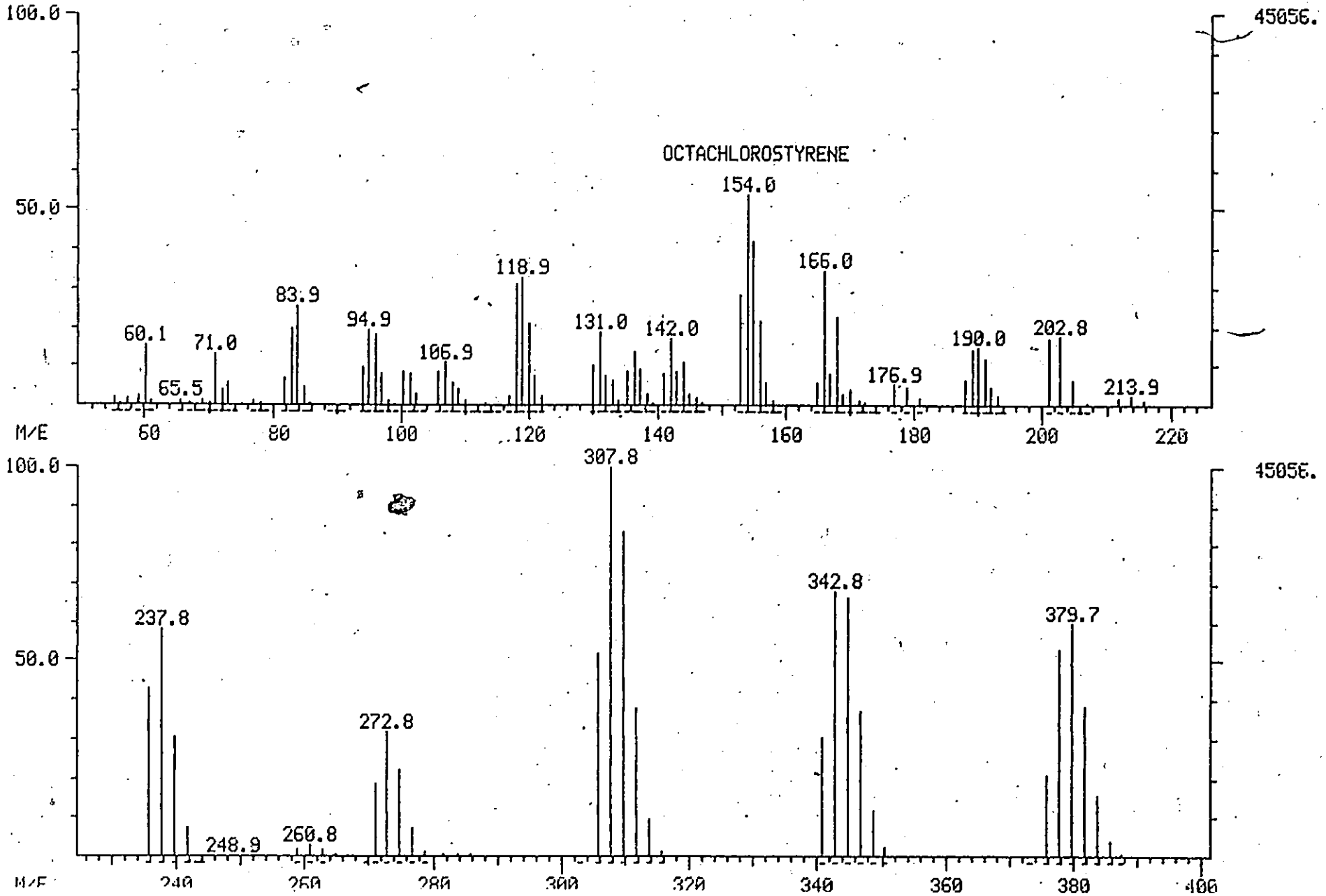


Fig. 11: Mass Chromatogram Obtained for an OCS/HCS Mixture

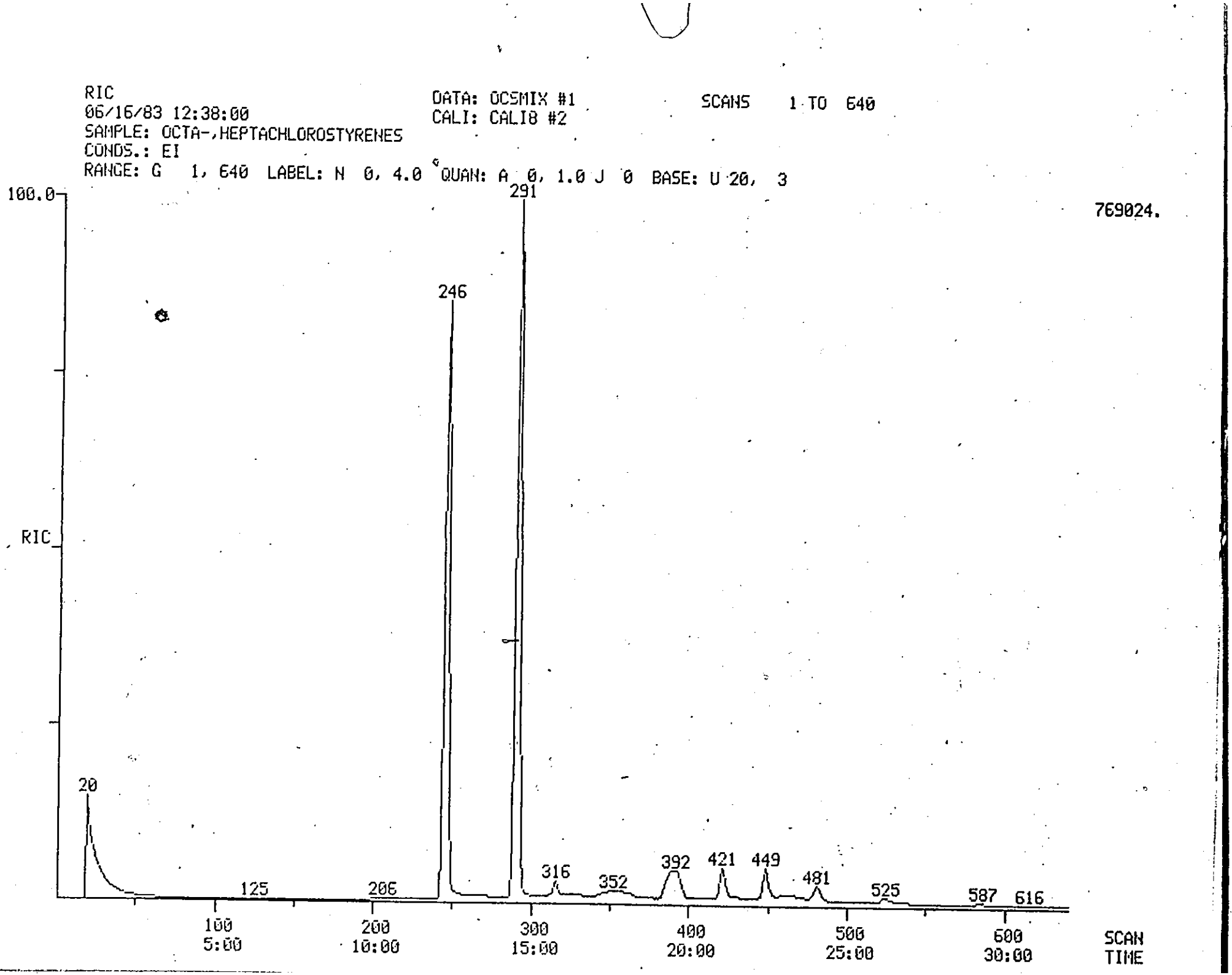

RIC
06/16/83 12:38:00
SAMPLE: OCTA-,HEPTACHLOROSTYRENES
CONDS.: EI
RANGE: G 1, 640 LABEL: N 0, 4.0
DATA: OCSMIX #1
CALI: CALI8 #2
SCANS 1 TO 640
QUAN: A 0, 1.0 J 0 BASE: U 20, 3
100.0
769024.
RIC
291
246
20
125
206
316
352
392
421
449
481
525
587
616
100
5:00
200
10:00
300
15:00
400
20:00
500
25:00
600
30:00
SCAN
TIME

Fig. 12: The Mass Spectrum of HCS

MASS SPECTRUM
06/16/83 12:38:00 + 12:18
SAMPLE: OCTA-,HEPTACHLOROSTYRENES
CONDS.: EI

DATA: OCSMIX #246
CALI: CALI8 #2

BASE M/E: 274
RIC: 657408.