Dissertations, Theses, and Masters Projects   Theses, Dissertations, & Master Projects

1995

Occurrence, fate and effects of polychlorinated terphenyls in an estuarine environment

Kathryn Gallagher

College of William and Mary - Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/etd

Part of the Environmental Sciences Commons

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

OCCURRENCE, FATE AND EFFECTS OF POLYCHLORINATED TERPHENYLS
IN AN ESTUARINE ENVIRONMENT

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

by

Kathryn Gallagher

1995

UMI Number: 9528260

UMI
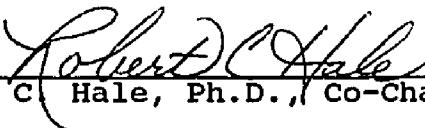
300 North Zeeb Road
Ann Arbor, MI 48103

APPROVAL SHEET
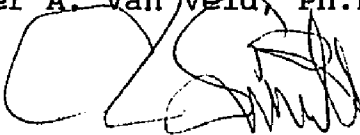
This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Kathryn Gallagher
Kathryn Gallagher

Approved, April 1995

Robert C. Hale
Robert C. Hale, Ph.D., Co-Chairman

Peter A. Van Veld
Peter A. Van Veld, Ph.D., Co-Chairman

Craig L. Smith, Ph.D.

Mark W. Luckenbach
Mark W. Luckenbach, Ph.D.

Robert J. Huggett
Robert J. Huggett, Ph.D.
U.S. Environmental Protection Agency

Jay W. Gooch
Jay W. Gooch, Ph.D.
The Procter and Gamble Company

ii

Dedication

This work is lovingly dedicated to my parents,
Johanna M. and Edward J. Gallagher,
and with gratitude for teaching me the value
of knowledge and persistence,
and in appreciation of their tremendous support and love.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Aroclor 5432, a mixture of polychlorinated terphenyls (PCT), was detected in sediment and several biological compartments including: saltmarsh cordgrass (*Spartina alterniflora*), American oysters (*Crassostrea virginica*), red-jointed fiddler crabs (*Uca minax*), wharf crabs (*Sesarma reticulatum*) and mummichogs (*Fundulus heteroclitus*) collected from Tabbs Creek. This tidal creek is located in the southern Chesapeake Bay region. Species from several phyla were selected to examine PCT accumulation in physiologically and ecologically different organisms. PCT concentrations in sediment, saltmarsh cordgrass, native oysters, and fiddler crabs decreased with distance downstream from the PCT outfall. Residues in transplanted oysters and mummichogs showed a more variable trend with distance downstream. The organism with the highest mean concentration (18,300 μg/kg dry weight) was the native oyster, a benthic filter feeder.

A preliminary study on the effects of Aroclors 5432 and 5460 showed induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity in mummichogs injected with Aroclor 5432. A second study examined the dose-response effects of PCT mixtures on levels of hepatic cytochrome P4501A (CYP1A) and associated EROD activity in the mummichog, relative to a the PCB mixture Aroclor 1254. Fish were injected intraperitoneally with PCT formulations Aroclor 5432, Aroclor 5460, or the PCB formulation Aroclor 1254, at doses of 0.32 to 100 mg/kg body weight. Elevated levels of CYP1A and EROD activity were detected in fish injected with Aroclor 5432 and 1254 doses of 32 and 100 mg/kg. Induction resulting from Aroclor 5432 was of the same order of magintude as caused by equivalent doses of Aroclor 1254. Treatment with Aroclor 5460 did not result in significant induction. This work represents the first report of hepatic CYP1A induction caused by Aroclor 5432 in teleosts and, similar to work in mammalian systems, suggests that the effects of this mixture may be mediated through Ah receptor binding.

Because commercial PCT mixtures contain small amounts of PCB, PCB may have contributed to the induction observed for Aroclor 5432. The planar PCB congener 3,3',4,4'-tetrachlorobiphenyl was identified in Aroclor 5432 by gas chromatography-mass spectrometry operating in negative chemical ionization mode.

Environmental induction was observed in mummichogs collected from Tabbs Creek. This study revealed CYP1A and EROD induction at the two most contaminated sites along the PCT gradient. Fish at the upper creek site also exhibited inhibition of enzyme activity. Definitive environmental induction by PCT could not be established due to the presence of other enzyme inducing pollutants in the creek. The results of this study contributed to the evaluation of remediation options for Tabbs Creek under the EPA Superfund.

OCCURRENCE, FATE AND EFFECTS OF POLYCHLORINATED TERPHENYLS

IN AN ESTUARINE SYSTEM

Chapter I

General Introduction

Polychlorinated terphenyls (PCT) are chlorinated aromatic compounds similar in structure and chemical properties to polychlorinated biphenyls (PCB) (Fig. 1). Compared to the well-studied PCB, little research has been conducted on the occurrence of PCT in the environment or their biological effects. This dissertation examines three aspects of environmental pollution by PCT: the distribution of PCT in biota and sediment collected from Tabbs Creek, a small tidal tributary of the Chesapeake Bay; the effects of laboratory exposure to PCT on the hepatic monooxygenase system of the mummichog (*Fundulus heteroclitus*); and the status of hepatic cytochrome P4501A in mummichogs collected from Tabbs Creek, as an indicator of potential environmental induction of fish by PCT.

PCT were produced in the United States by Monsanto from 1929 to 1972. During this time approximately 50,000 metric tons were manufactured (Jensen and Jørgensen, 1983), which was equal to 15% of PCB production by this company during this period (De Kok *et al.*, 1982). Several formulations of PCT were made, among these were: Aroclor 5432, a mixture of PCT containing 32% chlorine by weight, Aroclor 5460, 60% chlorine by weight, and Aroclor 5442, 42% chlorine by weight (Jensen and Jørgensen, 1983). PCT were also produced by European and

Figure 1. Structural diagrams of polychlorinated terphenyls
(top) and polychlorinated biphenyls.

Cl_{0-5} Cl_{0-4} Cl_{0-5}

Cl_{0-5} Cl_{0-5}
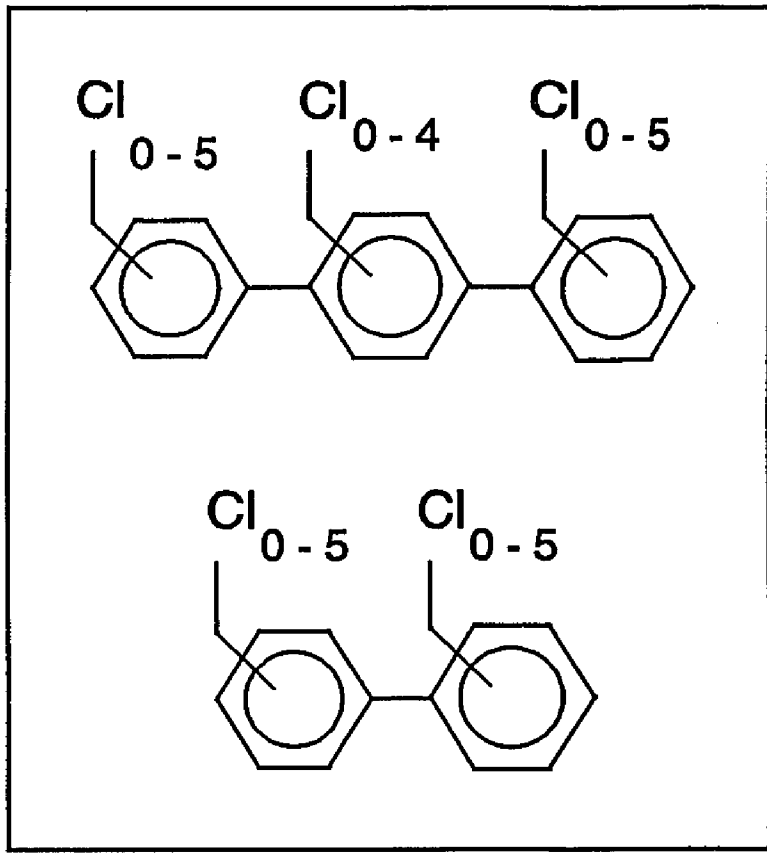
Japanese manufacturers (De Kok *et al.*, 1982).

PCT were used in hydraulic fluids, as plasticizers in investment castings for the production of high precision parts for aircraft (Jamieson, 1977), nuclear installations, and jewellery (Jensen and Jørgensen, 1983), in sealants for grain silos (Fries and Marrow, 1973), in paints, in adhesives for weatherstripping (Jamieson 1977), and as fire retardants (Jensen and Jørgensen, 1983). PCT were reported to be used as replacements for PCB, following the restrictive legislation of the latter (Jensen and Jørgensen, 1983).

PCT are directly regulated under section 8(a) of the Toxic Substances Control Act (TSCA); the Environmental Protection Agency must be notified of any manufacture or import. Small businesses are, however, exempt. PCT are also indirectly regulated under section 6(e) of TSCA due to the small amount of PCB contamination normally present in PCT mixtures (Code of Federal Regulations, 1984). Under this section of TSCA, PCB manufacture, processing or distribution for use in the U.S. is prohibited, with the exception of closed-system applications (Landfair, 1995). This PCB-related regulation, therefore, more severely restricts PCT manufacture and use.

Due to the structural similarities of PCT and PCB, and their documented effects on mammals, contamination of the environment by PCT is of concern. Highly chlorinated mixtures of terphenyls have been detected in human fat tissue

(Freudenthal and Greve, 1973; Watanabe et al., 1980) and blood (Doguchi and Fukano, 1975; Watanabe et al., 1980). These authors noted that PCT levels in blood exceeded those of PCB, despite the lower production and reported environmental levels of PCT. Highly chlorinated PCT have been observed in wild populations of eels and oysters in the Netherlands (Freudenthal and Greve, 1973), in the eggs and fat of herring gulls in Canada (Zitko et al., 1972), and in several species of birds in Britain (Hassell and Holmes, 1977). The less chlorinated mixture, Aroclor 5432, has been detected in white-tailed eagles and grey seals in Sweden (Renberg et al., 1978), and, more recently, in oysters from the Back River, Virginia (Hale et al., 1990, 1991a). During these studies, Hale et al. found the highest concentrations of Aroclor 5432 in samples from a small tributary, Tabbs Creek, which is situated on the property of a military-aerospace complex. Oysters from this creek contained concentrations of up to 35,000 μg/kg. This finding led to interest in the availability and potential for bioaccumulation of PCT by other organisms in the Tabbs Creek ecosystem. The purpose of the research described in the second chapter of this dissertation was to further investigate the spatial variation of Aroclor 5432 concentrations in Tabbs Creek sediment and to examine accumulation of PCT by several representative plant and animal species. Species collected were saltmarsh cordgrass (Spartina alterniflora), American oyster (Crassostrea virginica), red-jointed fiddler crab (Uca

minax), wharf crab (*Sesarma reticulatum*), and mummichog (*Fundulus heteroclitus*). The work described in Chapter II illustrates that Aroclor 5432 is bioavailable and can accumulate in species representing different ecological niches. Since minimal information was available on the effects of these compounds, further research was deemed necessary. Hepatic cytochrome P4501A was selected as the biological endpoint for studies on the effects of PCT on biota.

The Cytochrome P450 Monooxyenase System

The cytochrome P450 monooxygenase (MO) system in mammals consists of cytochrome(s) P450 (P450), nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome- P450 reductase, phosphatidylcholine, and in some cases cytochrome b_5 (Black and Coon, 1987). Recombined under optimum conditions, the MO system will catalyze the hydrolysis of specific substrates when the electron donor NADPH is added. P450 was first isolated by Omura and Sato (1964), and named for its carbon monoxide-reduced absorption peak at 450 nm. P450 contains the active site for the monooxygenation reaction. It consists of a protein group attached to a heme iron, with a mercaptide sulfur joined to the heme group. Specific reactions catalyzed by P450 include hydroxylation, epoxidation, N-,S- and O-dealkylation, N-,S-oxidation, dehalogenation, and C-C bond cleavage. P450 has been found in bacteria, and in almost all plant and animal tissues examined (Black and Coon, 1987). The

tissue with the highest concentration of P450 is the liver
(Dalh *et al.*, 1982; Black and Coon, 1987). In a cell, P450 is
most abundant in the endoplasmic reticulum (Stegeman and Hahn,
1994). Microsomes are remnants of the endoplasmic reticulum .
Liver P450s are referred to as hepatic microsomal
monooxygenases. Induction of the MO system is defined as an
increase in the rate of reactions catalyzed by the system
(Gruger *et al.*, 1977; Stegeman *et al.*, 1988).

The monooxygenase system (MO) in vertebrates catalyzes
reactions involving both endogenous and exogenous compounds.
Most substrates are hydrophobic and the affinity of P450 for
a substrate is partially determined by the lipophilicity of
the compound (Black and Coon, 1987). Endogenous substrate
reactions include synthesis and degradation of steroids,
prostaglandins, and fatty acids. Reactions involving non-polar
xenobiotic compounds include transformation to more water
soluble forms via oxidation, reduction or hydrolysis. These
reactions are referred to as Phase I reactions (Sivarajah *et
al.*, 1978). Phase II reactions generally involve conjugations
of Phase I products with sugars, amino acids, sulphates or
other groups (Sivarajah *et al.*, 1978; Bend and James, 1978),
and will not be examined in this dissertation. Transformation
of xenobiotics within the organism may result in an increase
in the carcinogenicity of some substrates (Stegeman, 1989).
Cytochrome P4501A (CYP1A) is a P450 which catalyzes reactions
involving exogenous compounds, such as planar halogenated

aromatic hydrocarbons (HAH).

Ah receptor

Induction of the hepatic microsomal monooxygenase CYP1A is a useful indicator of biological status following xenobiotic exposure. Most of the biological effects of PCB and other HAH in vertebrates are believed to be mediated through their binding to a cytosolic protein, the Ah receptor (Nebert, 1989; Poland and Knutson, 1982; Safe, 1986; Hahn et al., 1992; Heilmann et al., 1988). Induction of CYP1A and associated catalytic activities, such as ethoxyresorufin-*O*-deethylase activity (EROD), is one consequence of Ah receptor interaction (Okey et al., 1979). The binding affinity of a xenobiotic for the Ah receptor determines the magnitude of CYP1A induction (Poland and Knutson, 1982; Safe, 1986). Therefore, CYP1A induction is recognized as a sensitive indicator of HAH exposure and potential effects.

Common toxic responses in mammals produced by HAH include wasting, hyperplasia and/or altered differentiation (metaplasia), hypoplasia including thymus involution, and necrosis (Poland and Knutson, 1982). Toxic responses including thymus involution (Poland and Knutson, 1982), and teratogenesis (Poland and Glover, 1980) are more strongly exhibited in mammals that possess an Ah receptor with high affinity for HAH; toxicity segregates with the Ah locus.

The sequence of events involving the Ah receptor (AhR) in

mammals are believed to proceed as follows. The toxicant enters the cell, binds to the AhR complex which includes heat shock protein 90 (hsp90) and a nuclear translocator protein, Arnt. The AhR-ligand complex enters the nucleus, at which time hsp90 is released (Landers and Bunce, 1991). The AhR/Arnt-ligand complex binds to a regulatory sequence of DNA upstream of the CYP1A gene, the dioxin responsive enhancer (DRE) (Jones et al., 1985; Denison et al., 1989; Reyes et al., 1992). Binding of the complex to DRE may permit association of transcription promoting proteins with the promoter region downstream of DRE (Wu and Witlock, 1993), enabling the transcription of cyp1A mRNA (Israel et al., 1983). Expression of increased levels of CYP1A follows.

CYP1A is only one of the gene products regulated by AhR binding. Others include epidermal growth factor receptor (Madhukar et al., 1984) and protein kinase C (Bombick et al., 1988). The sequence of events leading from AhR binding to toxicity is not understood, but may involve the regulation of these and other genes. However, CYP1A induction has been directly correlated with toxicity in mammals (Poland and Knutson, 1982; Safe, 1990). Furthermore, Moorthy et al. (1993) reported a possible link between EROD induction and DNA adduct formation in rats. Unrepaired DNA adducts that are propagated during replication can result in mutation, alteration of gene product expression and, potentially, cancer. CYP1A effects can, therefore, be used to evaluate the potential Ah receptor-

mediated toxicity of a compound, and as such are valuable measurements to obtain.

Very little research has been conducted on monooxygenase induction by PCT. Because of the structural similarity of PCT to PCB, information on MO induction by PCB was researched as a reference point for this study.

Induction by PCB in Mammals and Birds

In general, when applied individually to mammals, planar (non-ortho-chlorinated) congeners produce CYP1A induction (Parkinson *et al.*, 1980); phenobarbital and non-planar PCBs induce a different P450 protein, CYP2B (Stegeman and Hahn, 1994). Planar PCB also induce CYP1A activity in birds. In addition to planarity, PCB molecules must have adjacent halogen atoms in at least two lateral positions on each ring (eg. 3,3',4,4') to increase CYP1A levels in birds. When there is an unsubstituted position between two halogen substitutions the molecule does not induce CYP1A. (Poland and Glover, 1977). EROD activity seems to be the most sensitive indicator of induction by PCB (Ankley et al., 1986).

Induction by PCB in Teleosts

In general, levels hepatic microsomal monooxygenases appear to be increased in fish by HAH which induce mammalian CYP1A, but not by HAH which induce CYP2B in mammals (Bend and James, 1978). CYP1A is the primary monooxygenase form induced

in fish by planar PCB (Gooch *et al.*, 1989). To date, non-planar PCB (ortho-substituted) have not been shown to induce teleost enzyme systems (Melancon *et al.*, 1981; Lech *et al.*, 1982; Melancon and Lech, 1983; Gooch *et al.* 1989), with the exception of one report of EROD induction in rainbow trout (*Oncorhynchus mykiss*) following exposure to the mono-*ortho* chlorinated 2,3'4,4',5- pentachlorobiphenyl (Skaare *et al.*, 1991). However, this mono-*ortho* congener did not induce scup CYP1A (Gooch *et al.*, 1989), suggesting potential species differences.

Numerous authors have noted induction of hepatic monooxygenase activity by PCB mixtures in teleosts in laboratory studies (Hill *et al.*, 1976; Gruger *et al.*, 1977; Addison *et al.*, 1978; Sivarajah *et al.*, 1978; Narbonne and Gallis, 1979; Melancon *et al.*, 1981; Melancon and Lech, 1983; Ankley *et al.*, 1986). Few experiments on enzyme induction by PCB have been conducted by applying doses via the diet or water. However, Gruger *et al.* (1977) observed that dietary doses of 1 ppm Aroclor 1254 increased enzyme activity in young coho salmon. Hill *et al.* (1976) noted that exposure of fish to Aroclor 1254 via water also results in enzyme induction.

The majority of experiments on enzyme induction by PCB were conducted by injection into the intraperitoneal space of the fish. In general, intraperitoneal (i.p.) injections of 0.1 to 100 mg Aroclor 1254/ kg body weight (bw) increase enzyme activity (Sivarajah *et al.*, 1978, Melancon and Lech, 1983,

Ankley *et al.*, 1986). Planar PCB congeners can induce MO
activity at lower doses than commercial formulations such as
Aroclor 1254. Induction of teleost MO systems at 0.01 to 0.1
mg of planar PCB/kg bw has been demonstrated (Melancon and
Lech, 1983, Gooch *et al.*, 1989). It is thought that planar PCB
are responsible for the majority of induction caused by PCB
mixtures in fish. At high doses, however, some planar PCB may
act as competitive inhibitors of enzyme induction. Gooch *et
al.* (1989) injected ortho and non-ortho substituted PCB into
scup (*Stenotomus chrysops*). EROD activity increased following
a single i.p. injection of 1 mg/kg bw 3,3',4,4'-
tetrachlorobiphenyl (BZ 77). However, at higher doses (5 and
10 mg/kg bw) EROD activity decreased. BZ 77 was found to be a
competitive inhibitor of EROD activity for scup microsomes *in-
vitro*.

Induction and Additional Toxic Effects in Mammals of PCT

Most research on enzyme induction caused by PCT has
involved the use of the highly chlorinated mixture, Aroclor
5460; little work has addressed the effects of Aroclor 5432.
Dietary exposure of rats to the highly chlorinated PCT
formulation, Aroclor 5460, causes an increase in liver weight
and in total cytochrome P450 (Sosa-Lucero *et al.*, 1973).
Intraperitoneal injection of rats with the same mixture caused
an increase in aryl hydrocarbon hydroxylase activity and total
P450 (Nilsen and Totgard, 1981). An increase in liver weight

and liver tumor formation was reported in mice exposed to Kanechlor C (Shirai *et al.*, 1978). This PCT formulation, produced in Japan, possesses a degree of chlorination similar to Aroclor 5460 (De Kok *et al.*, 1982). Rhesus monkeys fed Aroclor 5460 exhibited a decrease in body weight, hair loss, subcutaneous edema, chloracne, liver hypertrophy attributed to proliferation of smooth endoplasmic reticulum, and hyperplasia and dysplasia of gastric mucosa suggestive of eventual development of neoplastic transformations (Allen and Norback, 1973). Toftgård *et al.* (1980) noted an increase in proliferation of hepatic endoplasmic reticulum, total cytochrome P450 levels and EROD activity in rats injected (i.p.) with the less chlorinated PCT formulation Aroclor 5432; these authors described Aroclor 5432 as a potent inducer of rat liver microsomal P450. Aroclor 5460 induced EROD activity to a lesser degree than 5432. Monooxygenase induction in teleosts by PCT mixtures has not been previously reported.

In Chapter III, a pilot study on the effects of Aroclors 1254, 5432 and 5460 on EROD activity in the mummichog is described. This work indicates MO induction by the less chlorinated PCT formulation, Aroclor 5432. Chapter IV describes a full dose-response study on the relative effects of 5432 and 5460 on CYP1A and associated EROD activity. These effects are compared with those caused by exposure to 1254, a PCB mixture known to cause monooxygenase induction in

teleosts. The magnitude of CYP1A and EROD induction resulting from Aroclor 5432 injection was approximately equal to that caused by the known CYP1A inducer Aroclor 1254. Aroclor 5460 did not cause significant enzyme induction.

Because commercial PCT mixtures contain small amounts of PCB (Cooke et al., 1978), and because planar PCB congeners are known CYP1A inducers (Melancon et al., 1981; Lech et al. 1982; Melancon and Lech, 1983; Gooch et al. 1989), it is important to consider the potential effects of PCB contaminants in induction studies using these mixtures. Nilsen and Toftgård (1981) reported that Aroclor 5460 contained 0.75% PCB. These authors estimated that the PCB contamination of PCT caused 20% of the observed increase in P450 concentration, and concluded that the PCT components of the mixture caused the remainder of the induction. Induction studies based on exposure to PCT mixtures must consider the potential effects of PCB components in the mixture. Therefore, the Aroclor formulations 5432 and 5460 were analyzed using gas chromatography-mass spectrometry (GC-MS) for the planar PCB congeners 3,3',4,4'-tetrachlorobiphenyl (BZ 77), 3,3',4,4',5-pentachlorobiphenyl (BZ 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (BZ 169). The presence of the non-ortho-chlorinated PCB congener, and known CYP1A inducer in fish, 3,3',4,4'-tetrachlorobiphenyl in Aroclor 5432 was confirmed.

There have been many reports in the literature of induction of CYP1A and resultant EROD activity in fish

collected from a variety of environments (Stegeman *et al.*, 1990) and in different organs including liver and intestine (Van Veld *et al.*, 1990). Several authors have noted a gradient of induction in fish along a contamination gradient. Van Veld *et al.* (1990) found liver MO induction in spot (*Leiostomus xanthurus*) to be positively correlated with an environmental gradient of sediment polycyclic aromatic hydrocarbon (PAH) contamination. Addison *et al.* (1994) reported increasing hepatic CYP1A and EROD levels in winter flounder (*Pseudopleuronectes americanus*) along a gradient of increasing PAH contamination. Elskus and Stegeman (1989) found elevated CYP1A and EROD levels, relative to those at a control site, in mummichogs environmentally exposed to PCB and PAH. These authors found a three-fold induction in EROD activity and a smaller increase in CYP1A concentrations. There have been several reports on hepatic enzyme induction in fish environmentally exposed primarily to PCB. Addison and Edwards (1988) found EROD activity to be significantly positively correlated with total PCB in sediment, as indicated by hepatic PCB concentrations in flounder (*Platichthys flesus*) collected from a contaminated site in Norway. In a companion study, Stegeman *et al.* (1988) found increased CYP1A and EROD levels in the same species to be positively correlated with a field gradient of PCB, as indicated by PCB levels in mussels collected at the four sites. PAH were also present at these sites. There have been no previous reports of environmental

induction in PCT contaminated environments.

In Chapter V, the status of CYP1A in mummichogs collected from Tabbs Creek is examined. CYP1A induction in Tabbs Creek mummichogs was observed, with the magnitude of induction increasing with increasing PCT sediment concentrations. Because other CYP1A inducing compounds are present in Tabbs Creek (Hale and Smith, 1988), the induction observed in Tabbs Creek mummichogs cannot be solely attributed to PCT.

Chapter II

**Accumulation of Polychlorinated Terphenyls in Aquatic Biota
of an Estuarine Creek**

by K. Gallagher, R.C. Hale,

J. Greaves, E.O. Bush and D.A. Stilwell‡

‡ U.S. Fish and Wildlife Service, Cortland, NY 13045

ABSTRACT

Aroclor 5432, a mixture of polychlorinated terphenyls (PCT), was detected in several biological compartments including: saltmarsh cordgrass (*Spartina alterniflora*), American oysters (*Crassostrea virginica*), red-jointed fiddler crabs (*Uca minax*), wharf crabs (*Sesarma reticulatum*) and mummichogs (*Fundulus heteroclitus*) collected from Tabbs Creek. This tidal creek is located in the southern Chesapeake Bay region and contains sediments with high concentrations of PCT. Samples were collected at four sites, ranging from a suspected outfall near the head of the creek, to its mouth, approximately 2.5 river kilometers downstream. Species from several phyla were selected in order to examine PCT accumulation in physiologically and ecologically diverse organisms. PCT concentrations in sediment, saltmarsh cordgrass, native oysters, and fiddler crabs decreased with distance downstream. Residues in transplanted oysters and mummichogs showed a more variable trend with distance downstream. The organism with the highest mean concentration (18,300 μg/kg dry weight) was the native oyster, a benthic filter feeder.

Introduction

Polychlorinated terphenyls (PCT) are chlorinated aromatic compounds similar in structure and chemical properties to polychlorinated biphenyls (PCB). Compared to the well-studied PCB, little research has been conducted on the occurrence of PCT in the environment or their biological effects. This chapter describes the distribution of PCT in biota and sediment from Tabbs Creek, a small tidal tributary of Back River, which in turn enters the southern Chesapeake Bay (Fig. 2).

PCT were produced in the United States by Monsanto from 1929 to 1972. During this time approximately 50,000 metric tons were manufactured (Jensen and Jørgensen, 1983), which was equal to 15% of PCB production by this company during this time (De Kok et al., 1982). Several formulations of PCT were made, among these were: Aroclor 5432, a mixture of PCT containing 32% chlorine by weight, Aroclor 5460, 60% chlorine by weight, and Aroclor 5442, 42% chlorine by weight (Jensen and Jørgensen, 1983). PCT were also produced by European and Japanese manufacturers (De Kok et al., 1982).

The effects on mammals of dietary exposure to the highly chlorinated PCT (Aroclor 5460) include hair loss, chloracne, subcutaneous edema, hyperplasia and dysplasia of gastric mucosa suggestive of eventual development of neoplastic transformations, increase in liver weight due to proliferation

Figure 2. Sampling locations for the monitoring study of PCT
levels in Tabbs Creek, a tributary of the Chesapeake
Bay. The bars indicate general sampling areas.
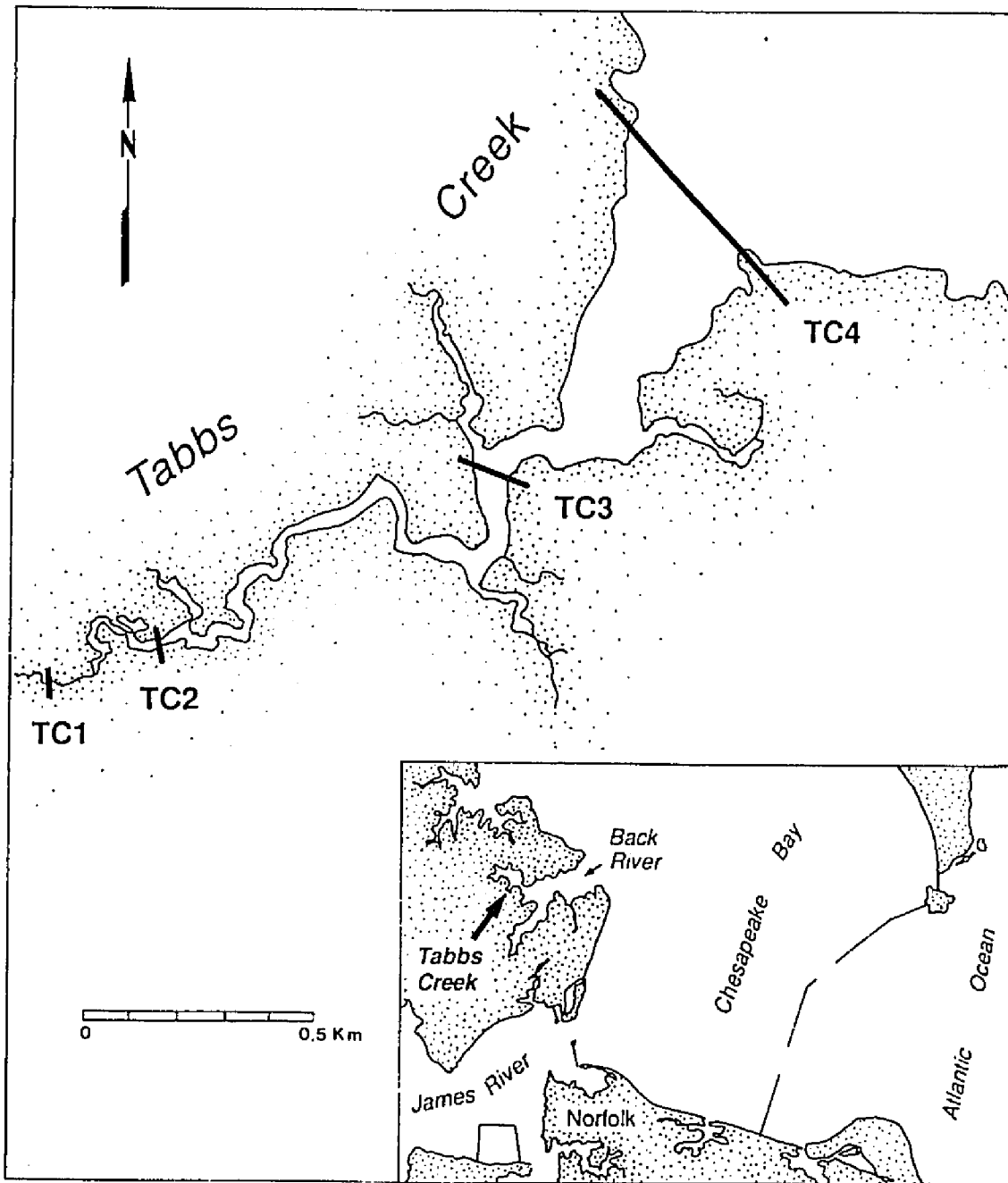
FIG. 2. Sampling locations in Tabbs Creek.

of the endoplasmic reticulum, an increase in cytochrome P450 levels, and induction of hepatic microsomal enzymes (Allen and Norback, 1973; Sosa-Lucero et al., 1973; Nilsen and Toftgård, 1981; Toftgård et al., 1980). Shirai et al. (1978) found an increase in liver weight and liver tumor formation in mice exposed to Kanechlor C, a PCT formulation produced in Japan. Kanechlor C has a similar degree of chlorination as Aroclor 5460 (De Kok et al., 1982). Toftgård et al. (1980) noted an increased proliferation of hepatic endoplasmic reticulum, and an increase in cytochrome P450 levels and EROD activity in rats injected with the less chlorinated mixture, Aroclor 5432; these authors described Aroclor 5432 as a potent inducer of rat liver microsomal P450.

Due to the structural similarities of PCT to PCB and their documented effects on mammals, contamination of the environment by PCT is of concern. Highly chlorinated mixtures of terphenyls have been detected in human fat tissue (Freudenthal and Greve, 1973; Watanabe et al., 1980) and blood (Doguchi and Fukano, 1975; Watanabe et al., 1980). These authors noted that PCT levels in blood exceeded those of PCB, despite the lower production and reported environmental levels of PCT. Highly chlorinated PCT have been observed in wild populations of eels and oysters in the Netherlands (Freudenthal and Greve, 1973), in the eggs and fat of herring gulls in Canada (Zitko et al., 1972), and in several species of British birds (Hassell and Holmes, 1977). The less

chlorinated mixture, Aroclor 5432, has been detected in white-tailed eagles and grey seals in Sweden (Renberg *et al.*, 1978), and recently in oysters from the Back River, Virginia (Hale *et al.*, 1990, 1991a). During these studies, Hale *et al.* (1990, 1991a) found the highest concentrations of Aroclor 5432 in samples from a small tributary, Tabbs Creek, which is situated on the property of a military-aerospace complex. Oysters from this creek contained concentrations of up to 35,000 μg/kg. This finding led to interest in the availability and potential for bioaccumulation of PCT by other organisms in the Tabbs Creek ecosystem. The purpose of this chapter was to further investigate the spatial variation of Aroclor 5432 concentrations in Tabbs Creek sediment and to examine accumulation of PCT by several representative plant and animal species. Initial pilot studies at Tabbs Creek, a tributary of the Chesapeake Bay, revealed PCT contamination in sediment and oysters (Hale *et al.*, 1990, 1991a).

To assess the extent of contamination of the biota of Tabbs Creek, organisms of several different phyla were considered for analysis for PCT content. Individual species were selected based on their feeding strategy, mobility and abundance. Saltmarsh cordgrass (*Spartina alterniflora*) was selected as a representative plant species. It is present in great abundance in tidal areas such as Tabbs Creek, and is a major primary producer in east and Gulf coast saltmarshes of the U.S. (Reidenbaugh, 1983). A mollusk, the American oyster

(*Crassostrea virginica*), was also sampled. Consumption of contaminated oysters by man is a source of concern, since oysters are part of a large commercial fishery. The red-jointed fiddler crab (*Uca minax*) was also selected for sampling. At some stations, fiddler crabs were not available in sufficient numbers for analysis. At these stations, wharf crabs (*Sesarma reticulatum*) were used as an alternative semi-terrestrial crustacean. The diet of the wharf crab is similar to that of fiddlers and includes marsh vascular plants and, occasionally, animal matter (Bursey, 1982). In this study, wharf crabs occupied the same spatial location as the red-jointed fiddler. The similarity of diet and zonation suggested that PCT burdens in wharf crabs may be similar to those in fiddler crabs. The mummichog (*Fundulus heteroclitus*) was collected to represent teleosts in the creek. It is known to be a resident species of tidal creek ecosystems, particularly during summer (Lotrich, 1975), and, therefore, is a potentially useful indicator of PCT contamination of biota within a localized area.

Materials and Methods

A flowchart for the analytical methodology used in this chapter is shown in Figure 3. Sampling was conducted from September 1989 through January 1990 at four sites within Tabbs

Figure 3. Flowchart of analytical methods used for analysis
of PCT content in sediment and biota.

```
        ┌─────────────────────────────────────┐
        │     Collect/Resect Samples          │
        └─────────────────────────────────────┘
                        ↓
        ┌─────────────────────────────────────┐
        │        Lyophilize (48 hr)           │
        └─────────────────────────────────────┘
                        ↓
    ┌─────────────────────────────────────────────────┐
    │   Add Surrogate Standard Decachlorobiphenyl      │
    │  Soxhlet Extract with Dichloromethane (48 hr)    │
    └─────────────────────────────────────────────────┘
                        ↓
    ┌─────────────────────────────────────────────────┐
    │       Gel Permeation Chromatography              │
    │   (Remove High Molecular Weight Biogenics)       │
    └─────────────────────────────────────────────────┘
                        ↓
       ┌──────────────────────────────────────────┐
       │      Florisil Column Chromatography       │
       │   (Remove Remaining Polar Compounds)      │
       └──────────────────────────────────────────┘
                        ↓
    ┌─────────────────────────────────────────────────┐
    │        Gas Chromatography with ELCD              │
    │  Quantification Relative to Decachlorobiphenyl   │
    └─────────────────────────────────────────────────┘
                        ↓
       ┌──────────────────────────────────────────┐
       │        GC-NCI Mass Spectrometry           │
       │           for Selected Samples            │
       └──────────────────────────────────────────┘
```

Creek. It was noted that the furthest site upstream, TC1, went nearly dry at low tide. In order to obtain an estimate of the variation in PCT concentration at each location, three composite samples of sediment and biota were collected within an area of approximately 30 meters. All sampling equipment was thoroughly cleaned and solvent rinsed prior to use. Sediments were collected in shallow water with a metal scoop and the upper 2 cm retained for analysis.

Saltmarsh cordgrass was obtained from the banks of the creek and held in a cold storage room (10°C). At the time of analysis, sediment was removed from the plant by rinsing with tap water. Five grams (lyophilized weight) of root and 5 g of shoot were combined for each composite.

Indigenous oysters were collected by hand. Additional specimens were collected from the Rappahannock River, since this river was believed to be free of PCT. The Rappahannock River oysters were transplanted to Tabbs Creek to evaluate bioaccumulation of PCT. The oysters were placed in mesh bags and allowed to clear gut contents overnight. Three composites of Rappahannock River oysters were analyzed to confirm the absence of detectable levels of PCT, prior to placement of samples in Tabbs Creek. The remaining oysters were transplanted to Tabbs Creek for approximately 30 days, with 10 individuals in each bag. Oysters were allowed to clear gut contents for 24 hours following final collection. The entire soft tissue was used for analysis. Individual sizes ranged

from 60 to 125 mm for indigenous oysters, and 40 to 135 mm for transplants; five to eleven individuals were homogenized for each composite. The number of oysters used in each composite was determined by the number available, for the indigenous oysters, and the number remaining alive at the end of 30 days, for the transplants.

Fiddler and wharf crabs were collected by hand, and their legs removed prior to analysis, to minimize contamination by sediment adhering to fine leg hairs. Crabs were washed with tap water and crushed prior to lyophilization. Fiddler crabs were 24 to 36 mm in width. At site TC3 male wharf crabs were collected; widths ranged from 21 to 28 mm. At site TC4 only female wharf crabs were available; widths ranged from 14 to 18 mm. Fifteen crabs were combined and analyzed for each composite sample.

Mummichogs were collected in baited minnow traps, and the entire body used for analysis. Sizes ranged from 35 to 105 mm. Nine to eleven individuals were analyzed per composite.

After collection, all samples except saltmarsh cordgrass were transported to the lab on ice, and retained frozen until analysis. Due to the size of the saltmarsh cordgrass samples, they were transported to the lab at room temperature, and then placed in a cold storage room. Analytical glassware was thoroughly cleaned and solvent rinsed prior to use. Volumetric glassware was dried at 120°C, non-volumetric glassware was baked at 400°C. High purity solvents (Burdick and Jackson)

were used. Composites that were subsampled for analysis were homogenized prior to lyophilization. All samples were lyophilized for 48 to 72 hours. A surrogate standard, decachlorobiphenyl, was added to the samples prior to extraction. Samples were then soxhlet extracted for 48 hours with dichloromethane. Solvent volumes were subsequently reduced on a rotary evaporator and the solvent exchanged to a 1:1 (v:v) mixture of dichloromethane/cyclohexane.

High molecular weight biogenic compounds, such as lipids, were removed via gel permeation chromatography on a 60 g bed of Biobeads SX-3 resin (Bio-Rad), using a 1:1 solvent mixture of dichloromethane and cyclohexane (Hale *et al.*, 1991b). Volumes were reduced to 1.0 ml cyclohexane and polar biogenic compounds were removed via open column chromatography on a bed of 10 g of activated Florisil, using dichloromethane as the eluent.

The solvent was exchanged to hexane, and the purified extracts injected onto a gas chromatograph (Varian 3300) equipped with a capillary column (J & W Scientific, DB-5 fused silica column, 30 m x 0.25 mm i.d., 25 μm film thickness). PCT were detected with an electrolytic conductivity detector (OIC 4420) operating in the halogen-specific mode. The electrolyte was n-propanol. The GC column was held at 90°C for 1 min, programmed at 4°C/min to 310°C, and held at 310°C for 10 min. The response factors of the Aroclor 5432 standards, relative to decachlorobiphenyl, were found to be linear over two orders

of magnitude. Concentrations in tissues were determined via computer integration (Hewlett-Packard 3350A Laboratory Automation System) of summed peak areas of PCT formulations, using decachlorobiphenyl as an internal standard.

Quantitation limits were 50 μg/kg for sediment and 100 μg/kg for biota. Accuracy and precision of the method were calculated by adding 276 μg/kg Aroclor 5432, prior to extraction, to oyster tissue free of detectable levels of PCT. Determined values (n=3) were found to be 81.8% (s=12.8) of nominal concentration. Blanks were analyzed with each batch of samples as a check on laboratory contamination; PCT were not detected in any of these. For selected samples, identification of compounds present was confirmed using a GC (DB-5 column) coupled with an Extrel ELQ 400-2 quadrupole mass spectrometer operating in the negative chemical ionization mode. Methane was used as the moderator gas (Hale *et al.*, 1990)

Results and Discussion

Reported values are expressed on a dry weight basis. Chromatograms of an Aroclor 5432 standard and representative samples are shown in Figures 3 through 9. Figures 10 trough 15 are mass spectra of Aroclor 5432 and representative samples.

Figure 4. GC-ELCD chromatogram of Aroclor 5432 standard.

AROCLOR 5432 STANDARD
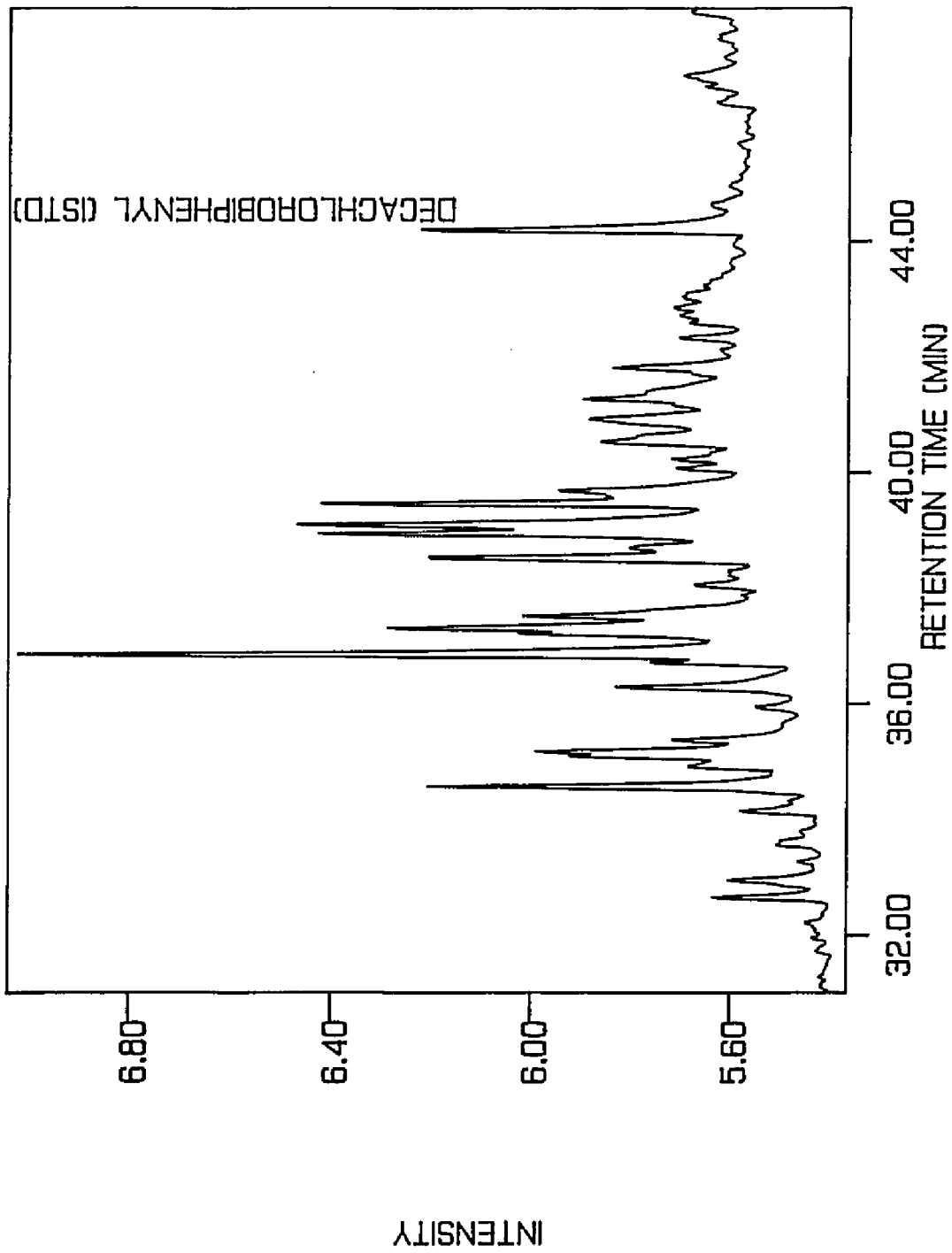
Figure 5. GC-ELCD chromatogram of Tabbs Creek sediment extract.

SEDIMENT TC1B

DECACHLOROBIPHENYL (ISTD)

RETENTION TIME (MIN)

INTENSITY
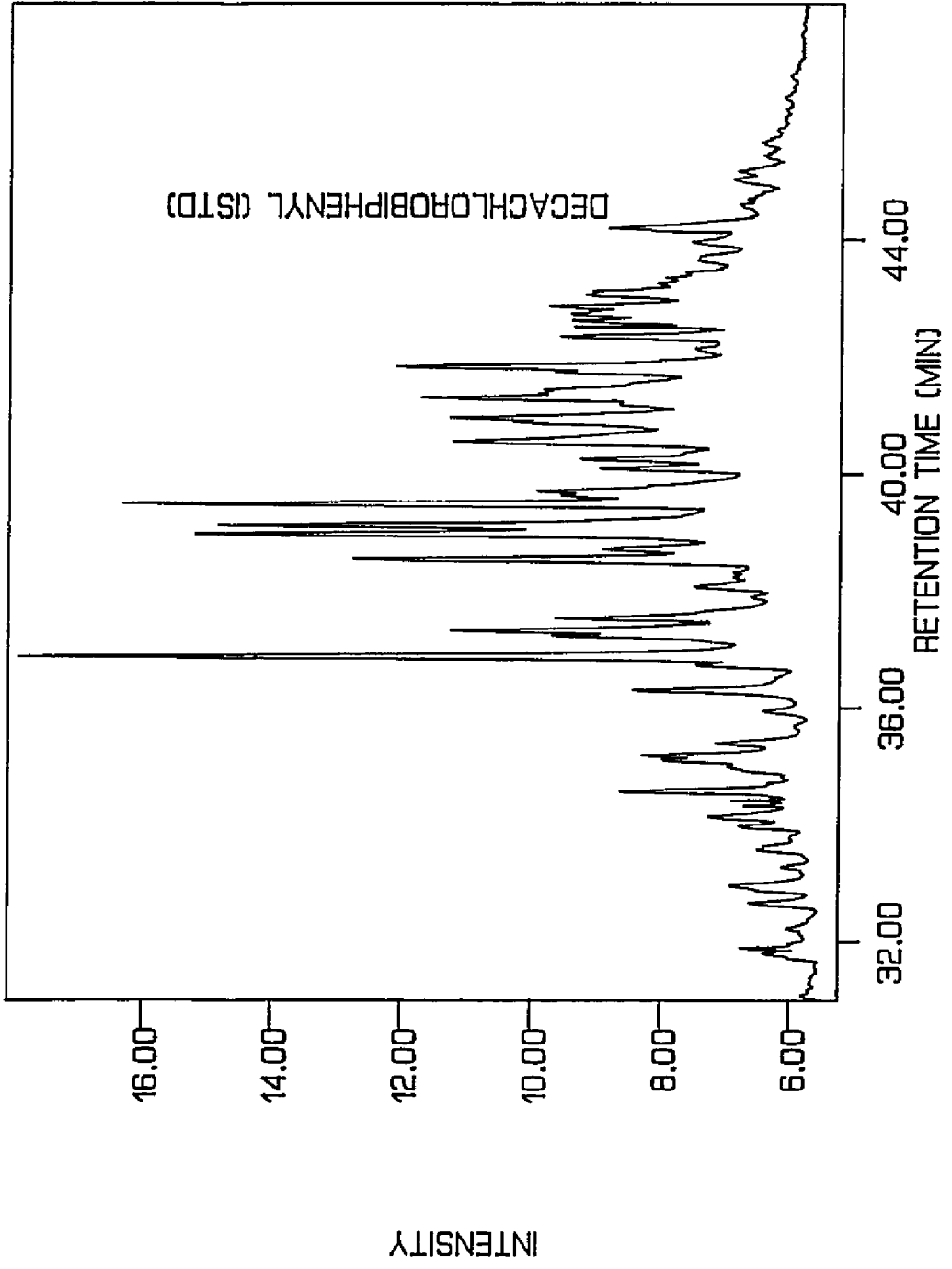
Figure 6. GC-ELCD chromatogram of extract of saltmarsh

cordgrass collected at Tabbs Creek.

SALTMARSH CORDGRASS TC1B

DECACHLOROBIPHENYL (ISTD)

INTENSITY

RETENTION TIME (MIN)
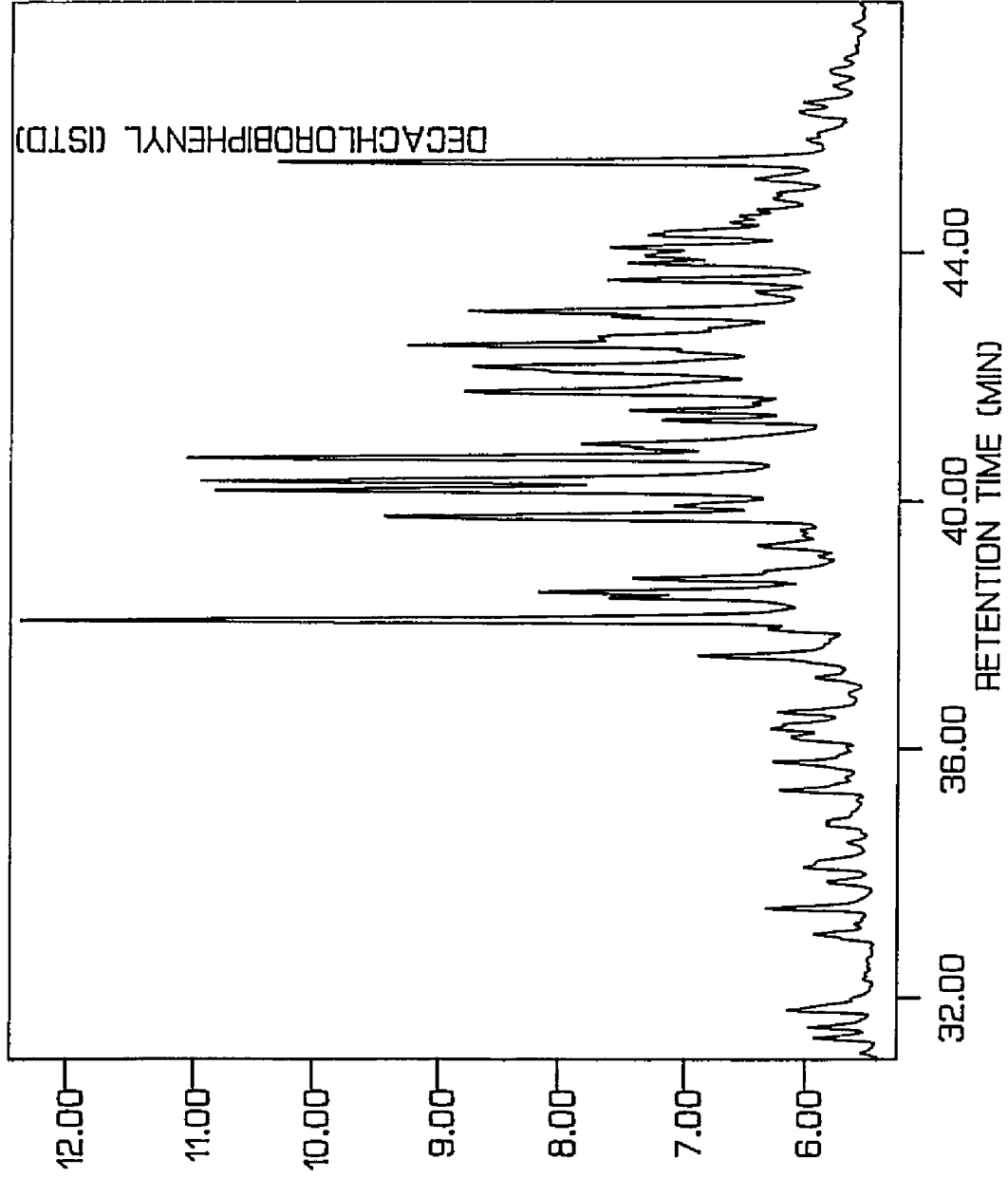
Figure 7. GC-ELCD chromatogram of extract of indigenous

oysters collected from Tabbs Creek.

INDIGENOUS OYSTER TC3B

DECACHLOROBIPHENYL (ISTD)

INTENSITY

RETENTION TIME (MIN)
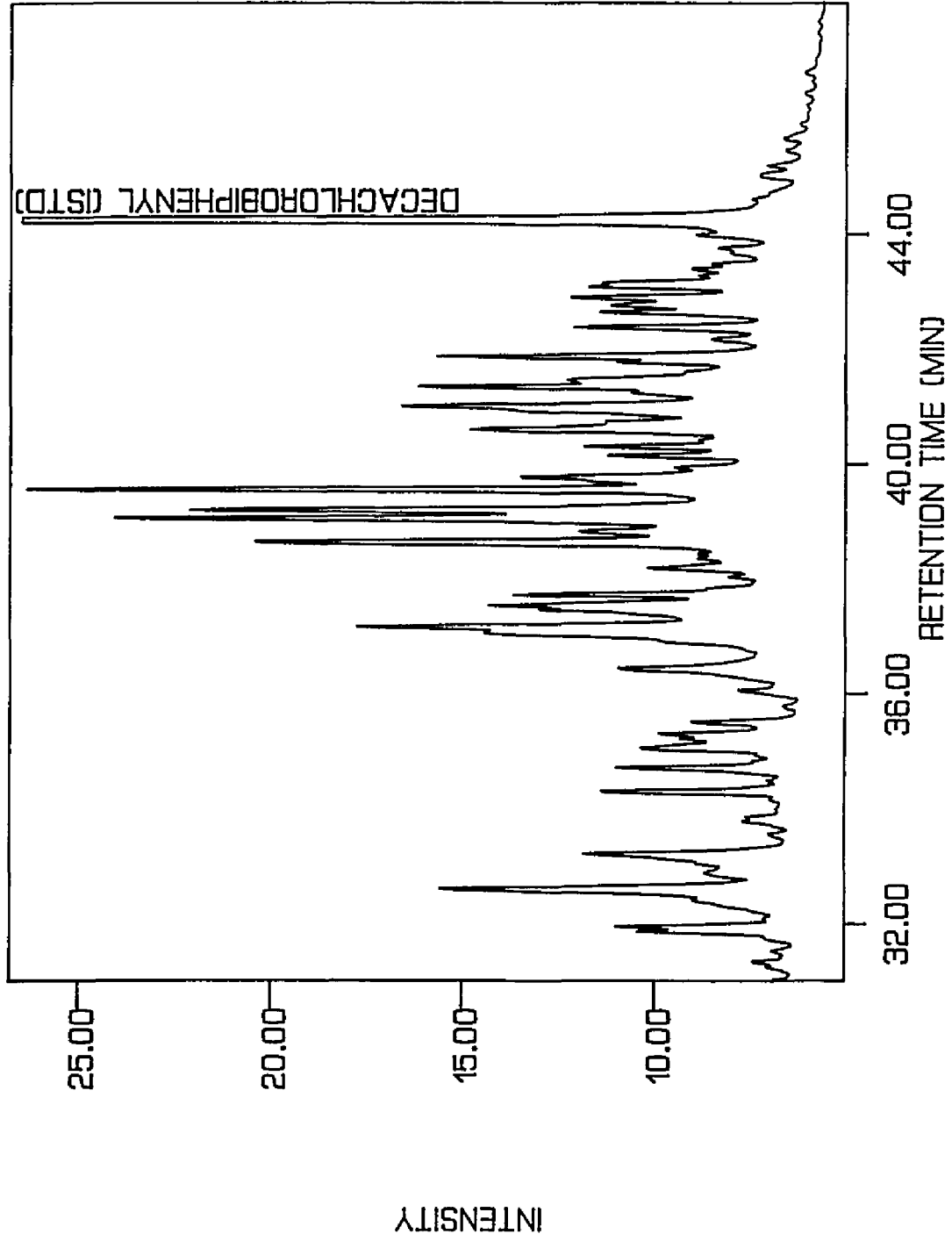
Figure 8. GC-ELCD chromatogram of extract of oysters
transplanted to Tabbs Creek for one month.

TRANSPLANTED OYSTER TC2B

DECACHLOROBIPHENYL (ISTD)

INTENSITY

RETENTION TIME (MIN)
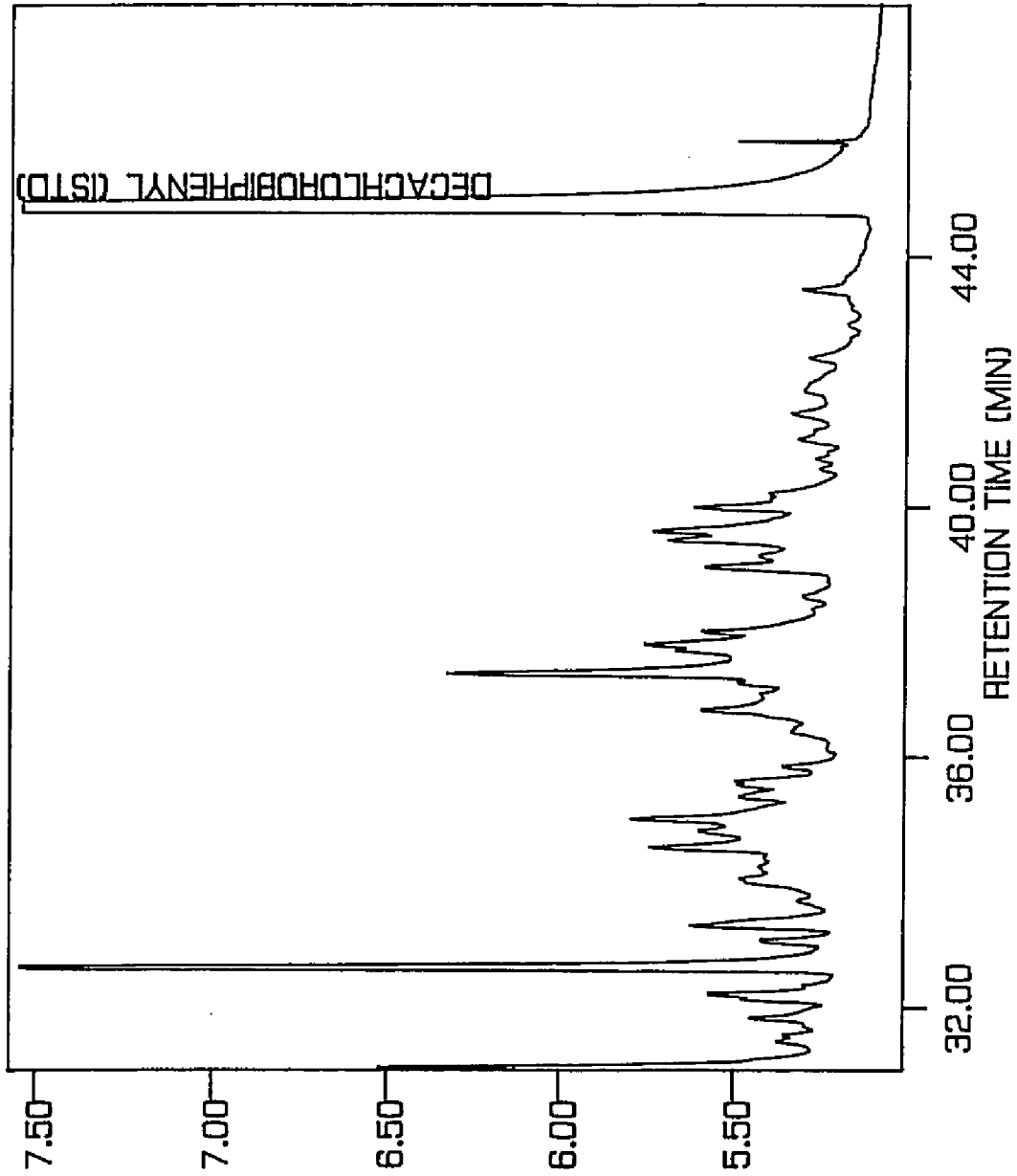
Figure 9. GC-ELCD chromatogram of extract of fiddler crabs collected at Tabbs Creek.

FIDDLER CRAB TC1B

DECACHLOROBIPHENYL (ISTD)

INTENSITY

RETENTION TIME (MIN)
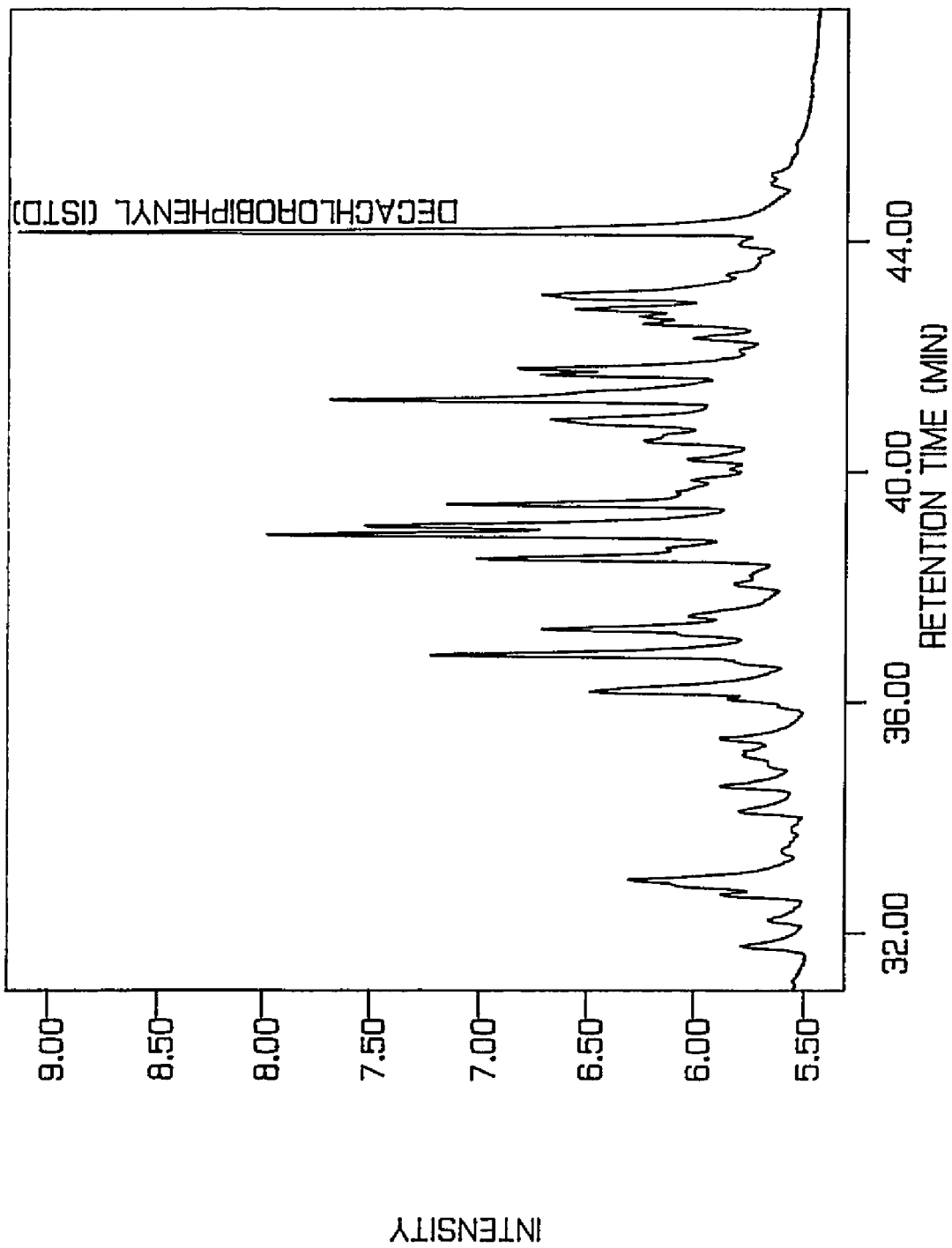
Figure 10. GC-ELCD chromatogram of extract of mummichogs

collected from Tabbs Creek.

MUMMICHOG TC2A

DECACHLOROBIPHENYL (ISTD)

RETENTION TIME (MIN)

INTENSITY
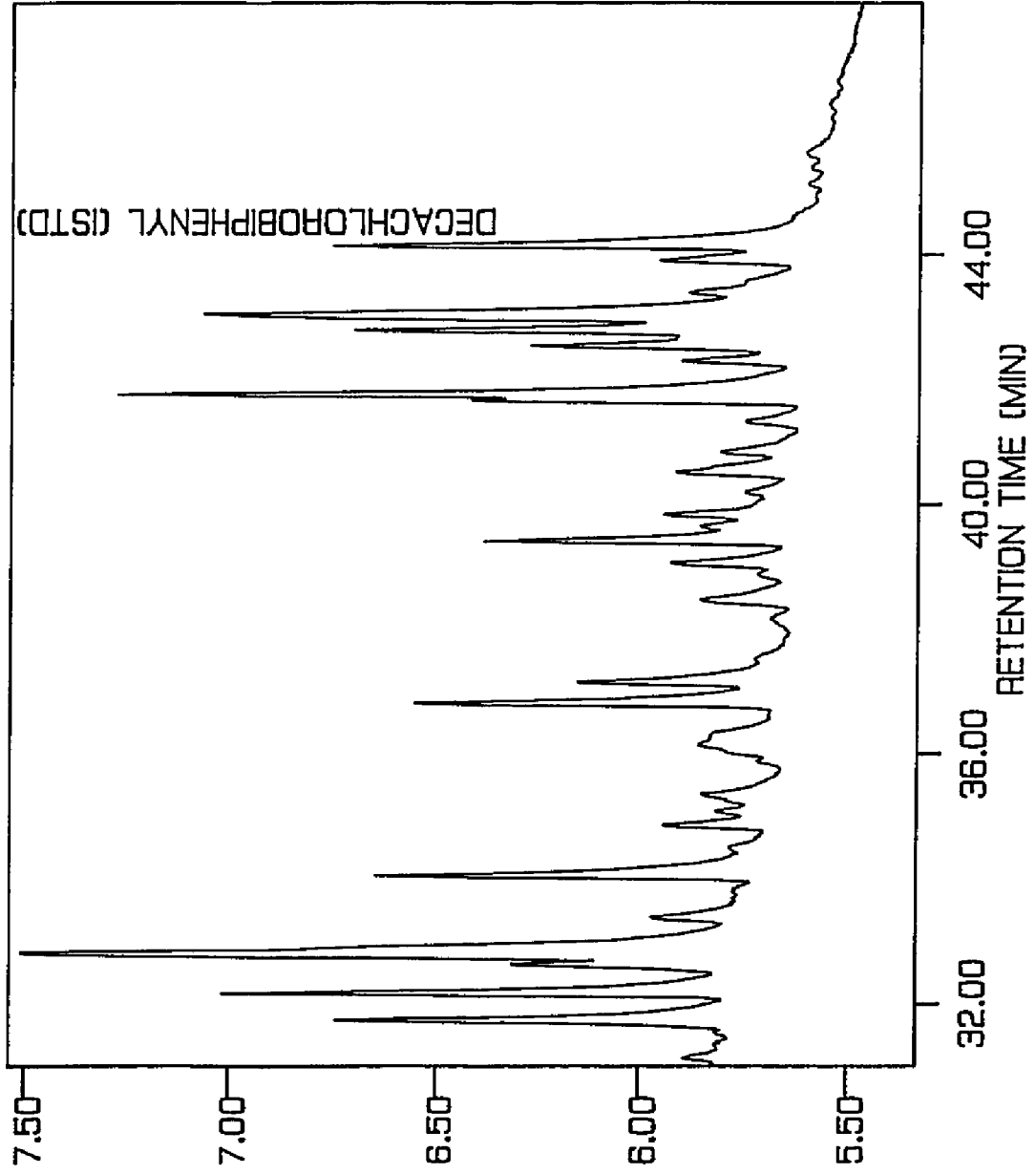
Figure 11. NCI mass spectrum of a trichloroterphenyl from an
Aroclor 5432 standard.

EXAR5432;10 5432 3655UG/ML 1UL -CI CH4 90/1/4/M/310
10-FEB-95 SCAN 2702 TIME 51.48 MIN.
100 % = 1806

TOTAL SCALE
8329 1*

297

332

595
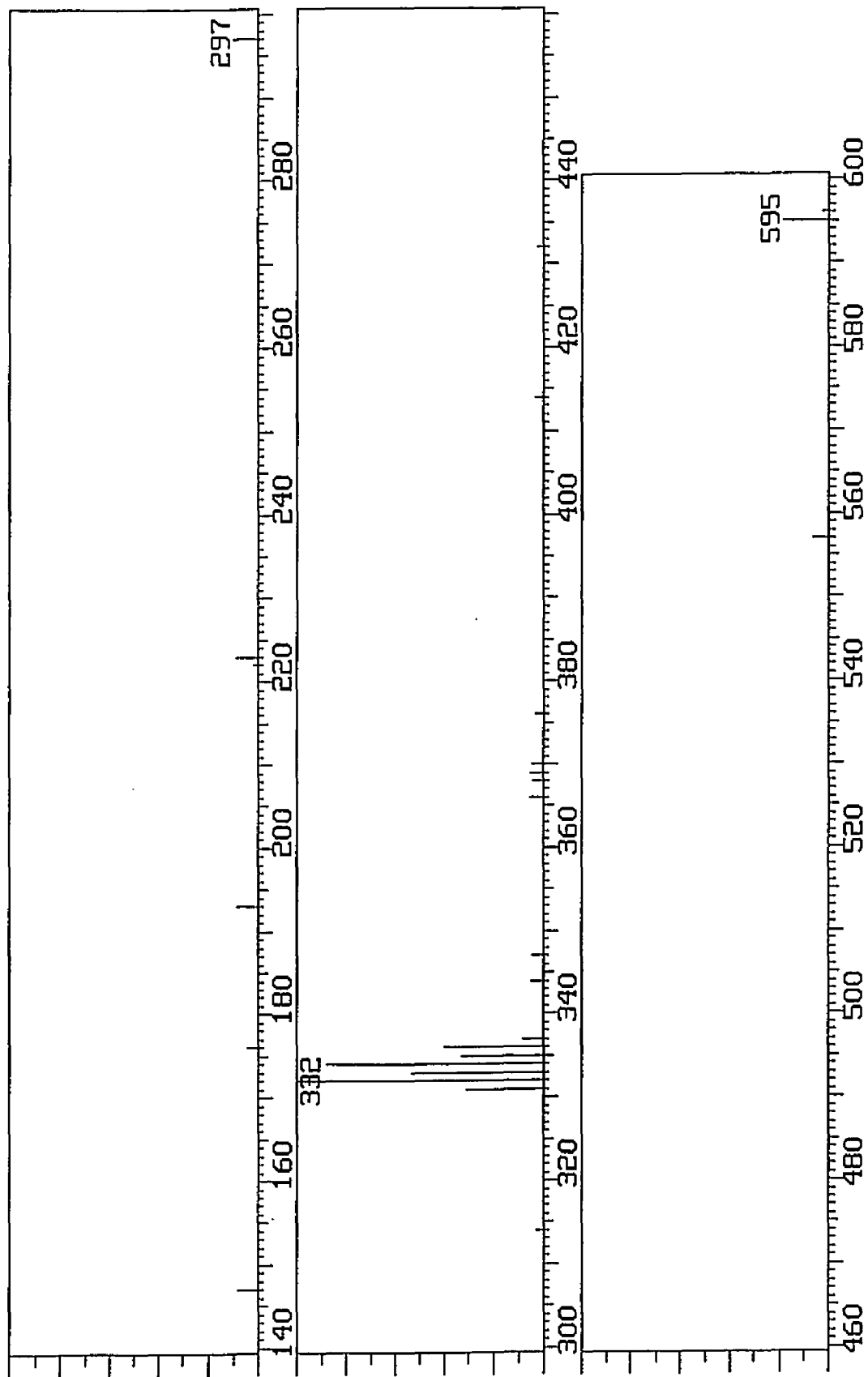
Figure 12. NCI mass spectrum of a trichloroterphenyl from a
Tabbs Creek sample of saltmarsh cordgrass.

EXTC2BSA TC2B SPARTINA FV0.2ML 2.0UL
12-FEB-95 SCAN 2698 TIME 51.37 MIN.
100 % = 416

TOTAL SCALE
2545 1*

121

147

223

332

595
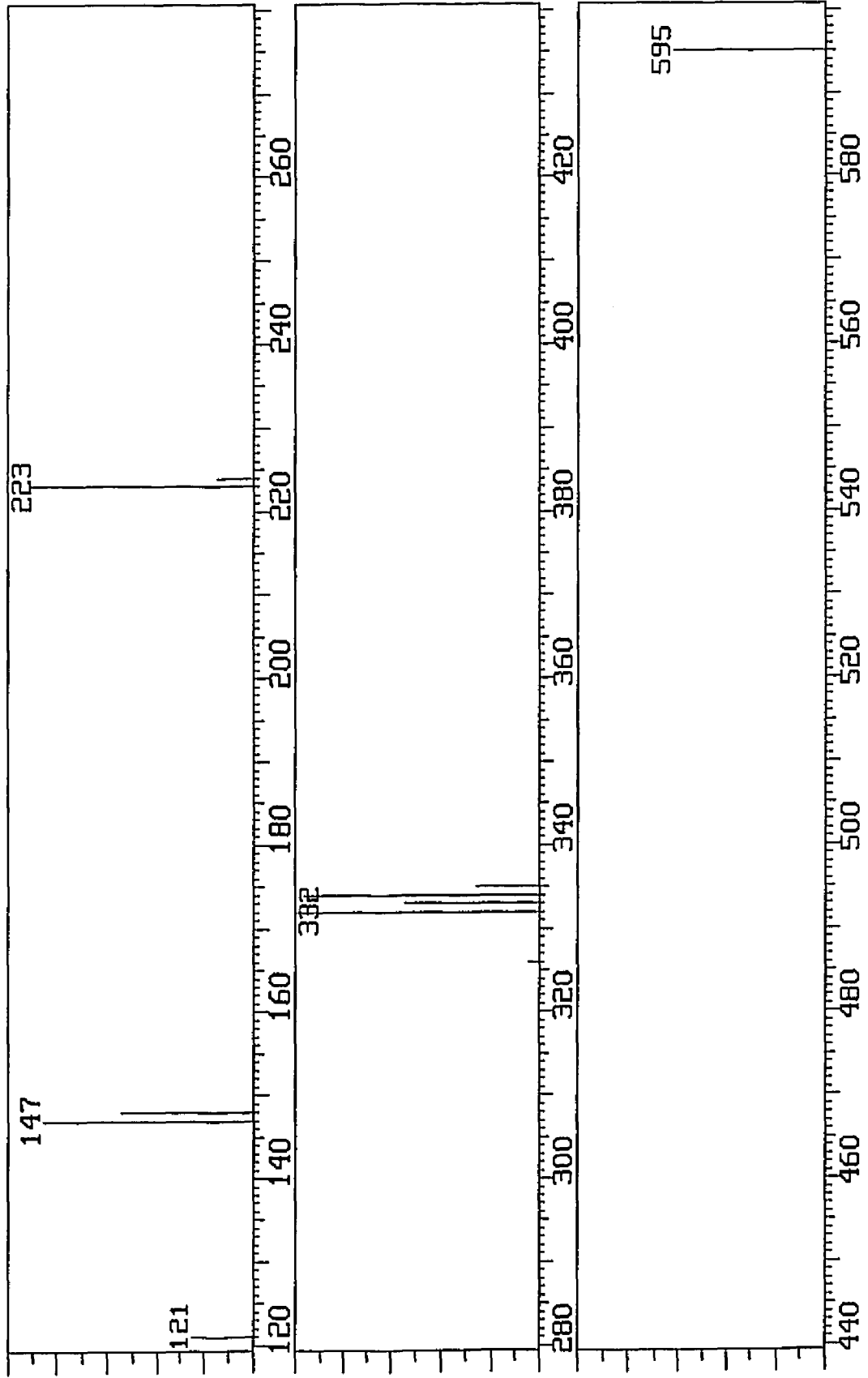
Figure 13. NCI mass spectrum of a tetrachloroterphenyl from an
Aroclor 5432 standard.

EXAR5432;10 5432 3655UG/ML 1UL -CI CH4 90/1/4/M/310
10-FEB-95 SCAN 2776 TIME 52.73 MIN.
100 % = 2031

TOTAL SCALE
9474 1*
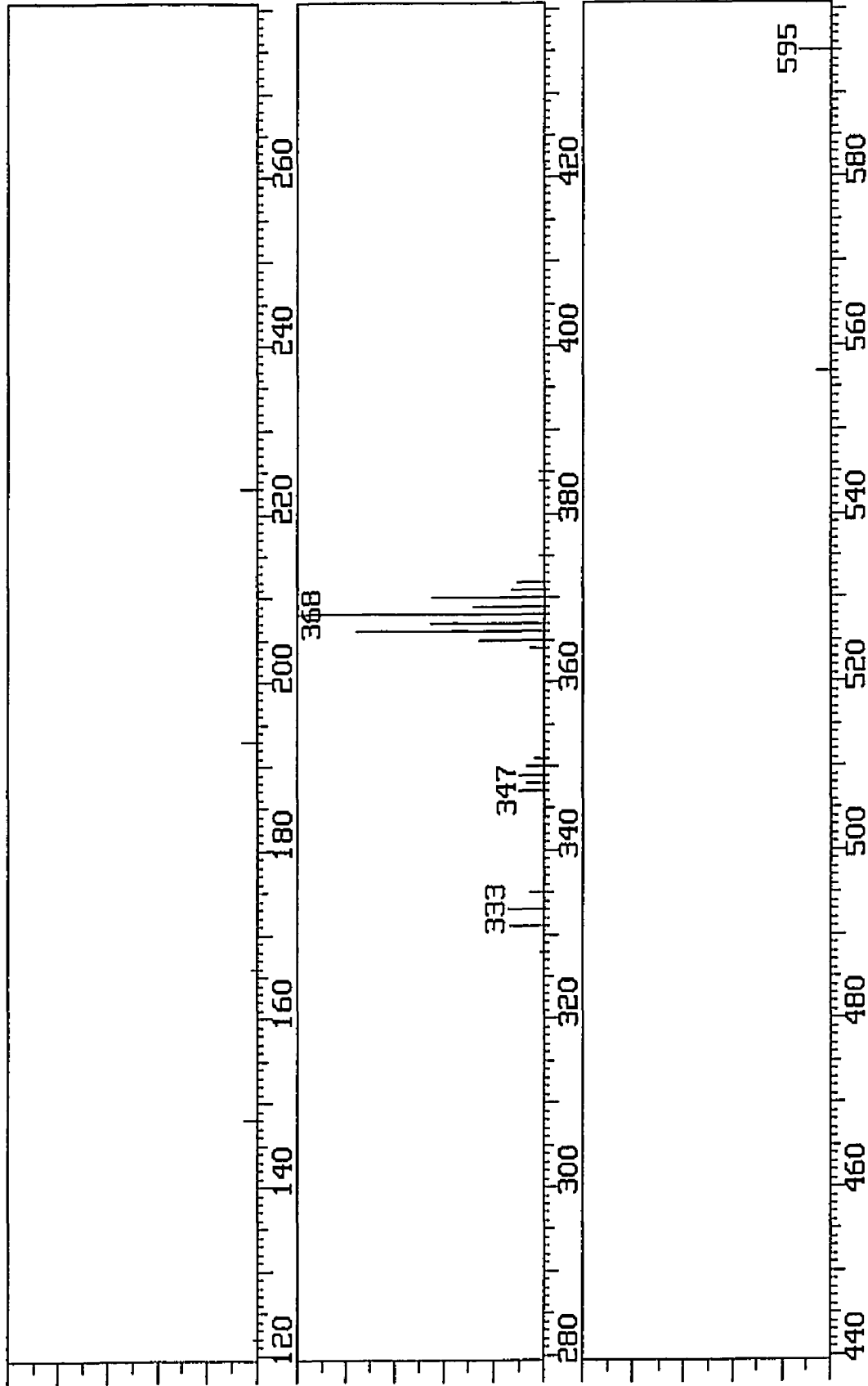
Figure 14. NCI mass spectrum of a tetrachloroterphenyl from a
sample collected at Tabbs Creek, fiddler crab.

EXTC1BUM TC1B UCA DBSCOL,DEARCH FULL, 2.0UL,-CI CH4 90/1/4/M/
08-FEB-95 SCAN 2777 TIME 52.73 MIN.
100 % = 1558

TOTAL SCALE
5667 1*

148

368

349

595
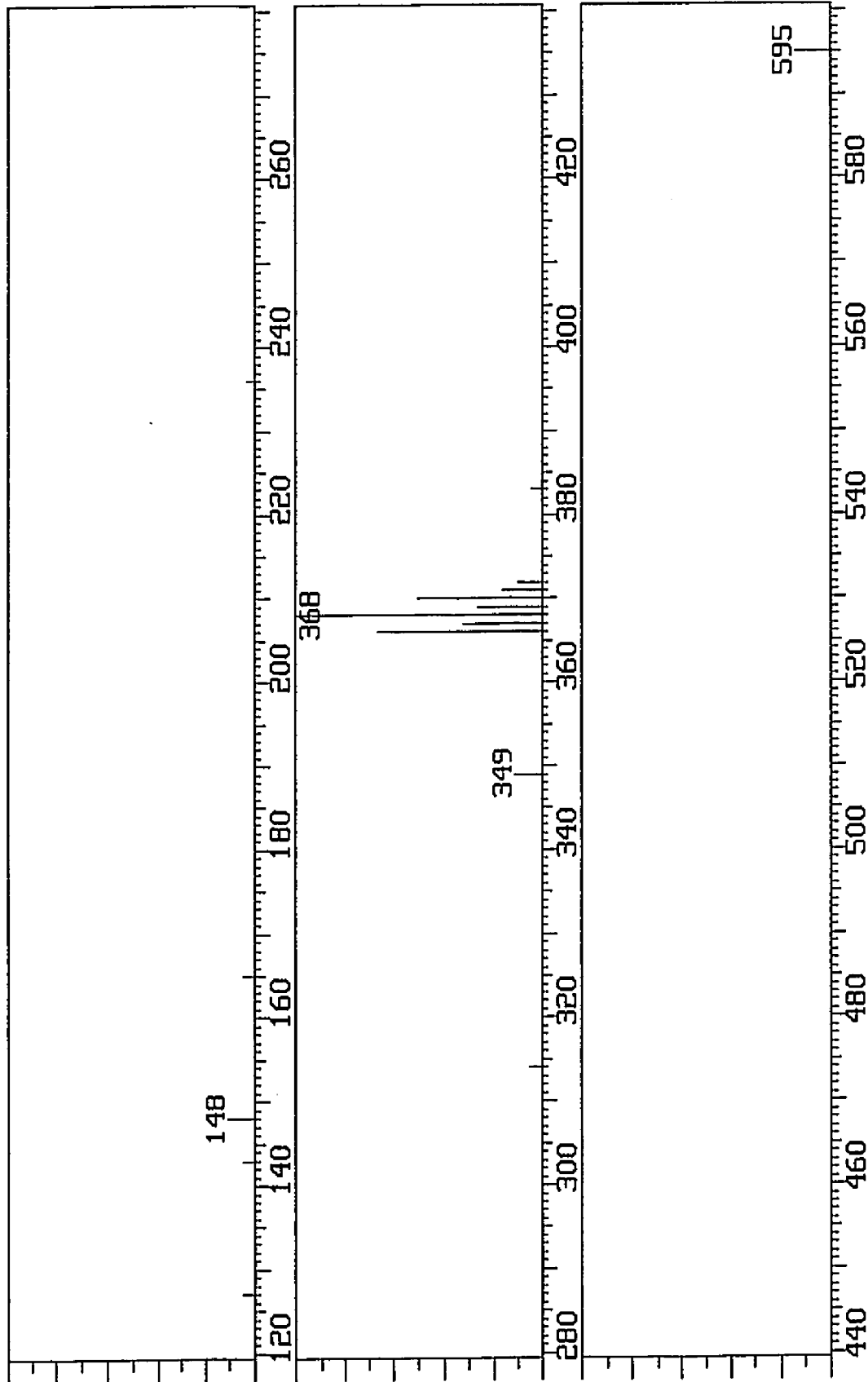
Figure 15. NCI mass spectrum of a pentachloroterphenyl from an
Aroclor 5432 standard.

EXAR5432;10 5432 3655UG/ML 1UL -CI CH4 90/1/4/M/310
10-FEB-95 SCAN 2999 TIME 56.48 MIN.
100 % = 7280

TOTAL SCALE
23849 1*

402
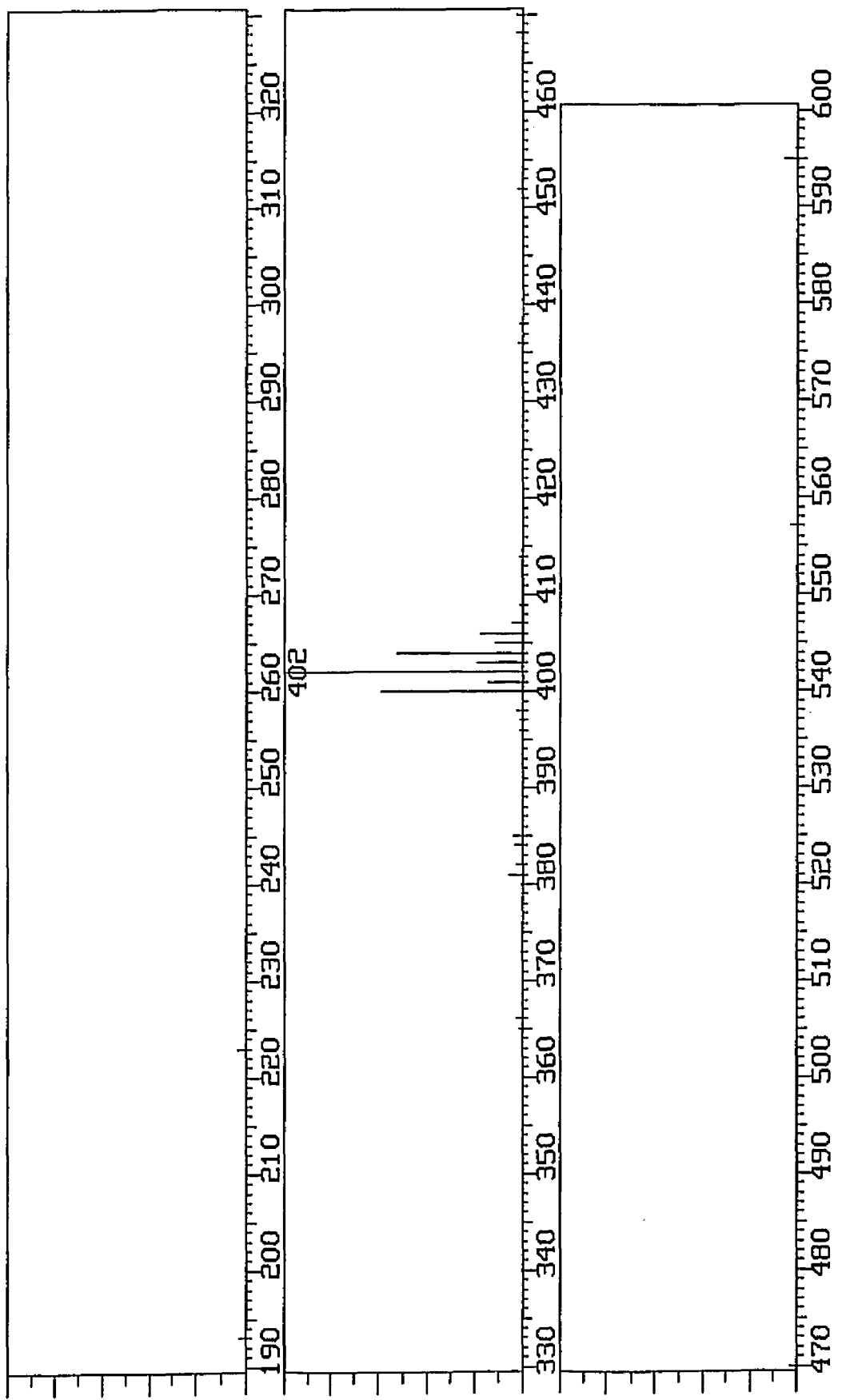
Figure 16. NCI mass spectrum of a pentachloroterphenyl from a

Tabbs Creek sample, mummichog.

EXTC2AF2 TC2AFISH DEARCH,FV 0.2-0.5ML, 2UL,CI CH4
09-FEB-95 SCAN 2994 TIME 56.38 MIN.
100 % = 3552

TOTAL SCALE
12428   1*

402
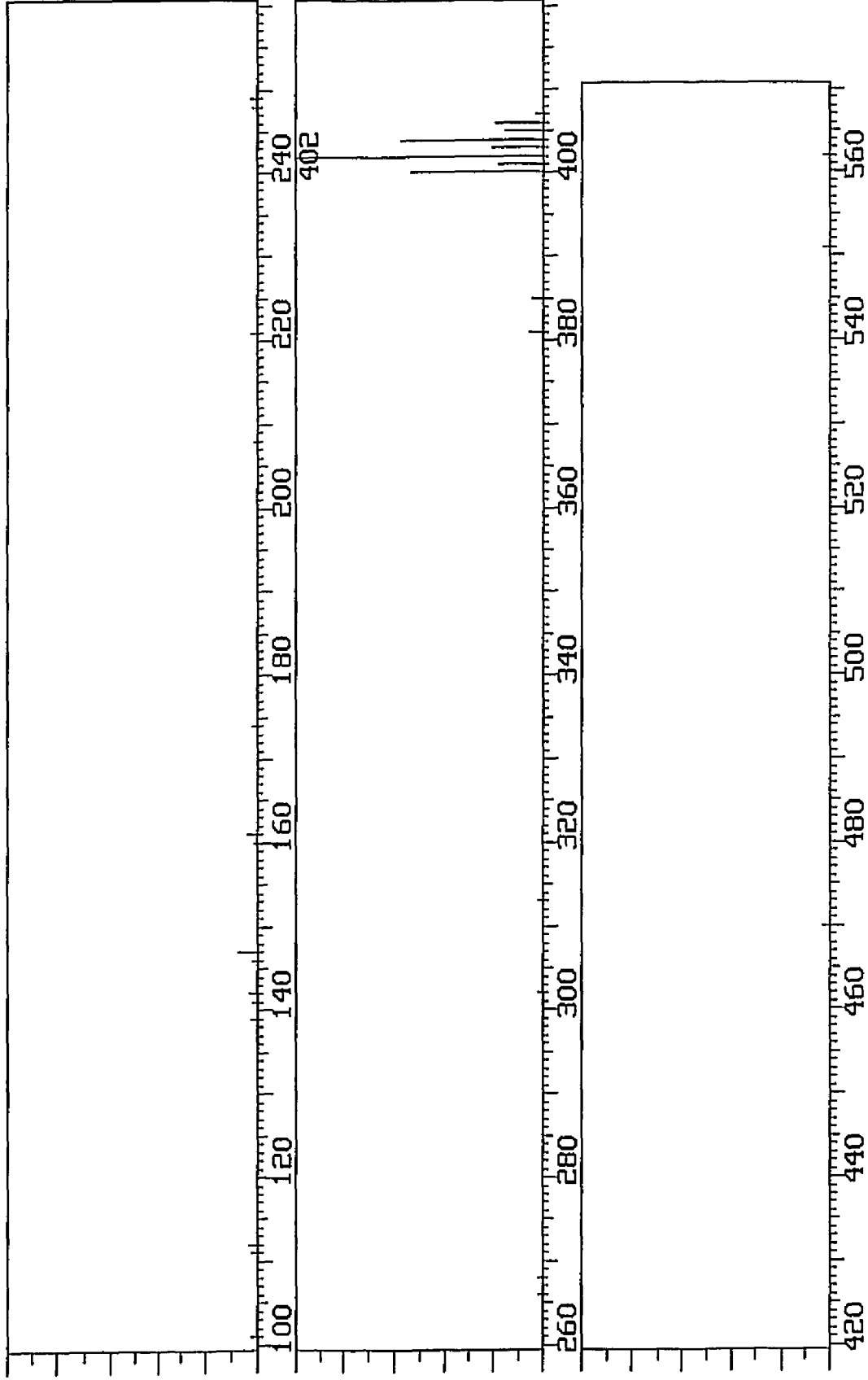
Sediment

Mean concentrations of Aroclor 5432 in sediment decreased with distance downstream (Table 1). The upstream site, TC1, contained the highest mean concentration (n=3) of Aroclor 5432 in the sediment (43,300 μg/kg). This site is nearest the suspected outfall, as determined in a previous study (Hale et al., 1990). The lowest mean concentration in the sediment was found at the mouth of the creek (<171 μg/kg). PCT concentrations varied greatly at site TC1. When sediment concentrations were normalized to total organic carbon content (TOC), variations in PCT levels within a site were reduced (Table 2). For example, the coefficient of variation for PCT concentration in sediment was 34.8% at TC1. Following normalization to TOC, the coefficient of variation was reduced to 14.6%. Thus, some of the variations in PCT sediment concentrations within a given site may be attributed to variation in TOC. Other causes of within-site variation of PCT concentration may include multiple inputs, and variations in creek flow rates and sediment deposition/redistribution.

Saltmarsh Cordgrass

The trend in concentrations of PCT in saltmarsh cordgrass matched those found in the sediment, with the mean concentrations decreasing with distance downstream (Table 1). The highest mean concentration of Aroclor 5432 in saltmarsh cordgrass (5080 μg/kg) was found at the upstream site TC1, and

Table 1. Concentrations of Aroclor 5432 in Sediment and Biota in µg/kg dry weight.

Site	Sediment	Saltmarsh Cordgrass	Indigenous Oyster	Transplanted Oyster[1]	Fiddler/Wharf Crab	Mummichog
TC1	46,900	3860	---	527	9600	6420
	56,300	7560	---	567	5740	5550
	26,800	3810	---	870	5760	5790
TC1 Mean (SD)	43,300 (15,100)	5080 (2150)	---	655 (188)	7030 (2220)	5920 (449)
TC2	33,900	3230	---	2620	1260	6390
	32,600	2680	---	2060	2250	6650
	30,600	1830	---	2290	1830	7820
TC2 Mean (SD)	32,400 (1660)	2580 (705)	---	2320 (281)	1780 (497)	6950 (762)

Table 1 (continued). Concentrations of Aroclor 5432 in Sediment and Biota in µg/kg dry weight.

Site	Sediment	Saltmarsh Cordgrass	Indigenous Oyster	Transplanted Oyster[a]	Fiddler/Wharf Crab	Mummichog
TC3	2790	131	12,700	1530	BQL	BQL
	2020	BQL	21,900	2540	398	BQL
	2550	BQL	20,400	4380	562	680
TC3 Mean (SD)	2450 (394)	<110	18,300 (4940)	2820 (1450)	<353	<293
TC4	410	BQL	2140	312	103	BQL
	BQL	BQL	2120	630	BQL	BQL
	52.7	BQL	1450	367	BQL	BQL
TC4 Mean (SD)	<171	<100	1900 (393)	436 (170)	<101	<100

Note. Quantitation limits were 50 µg/kg and 100 µg/kg for sediment and biota, respectively. Concentrations expressed with less than signs include one or more replicates with determined values below the quantitation limit (BQL). Dashes indicate samples were not available at these stations.
[a] Background Aroclor 5432 levels were BQL.

Table 2. Normalization of PCT Concentration in Sediment to Total Organic Carbon (TOC) Content.

Site	PCT Concentration in Sediment (µg/kg)	Mean PCT Concentration in Sediment (s) (µg/kg)	TOC (% of dry weight)	PCT/TOC (µg/kg TOC)	Mean PCT/TOC (s) (µg/kg TOC)
TC1A	46,900	43,300	6.795	690,200	678,200
TC1B	56,300	(15,100)	7.302	771,000	(99,300)
TC1C	26,800	$s/\bar{X}$= 34.8%	4.674	573,400	$s/\bar{X}$= 14.6%
TC2A	33,900	32,400	7.943	426,800	430,000
TC2B	32,600	(1660)	7.281	447,700	(16,400)
TC2C	30,600	$s/\bar{X}$= 5.1%	7.367	415,400	$s/\bar{X}$= 3.8%
TC3A	2790	2450	5.777	48,300	42,900
TC3B	2020	(394)	5.608	36,000	(6270)
TC3C	2550	$s/\bar{X}$= 16.1%	5.753	44,300	$s/\bar{X}$= 14.6%
TC4A	410	<171	2.661	15,400	<12,200
TC4B	BQL[a]	(NA)[b]	0.407	NA[b]	(NA)[b]
TC4C	52.7	$s/\bar{X}$= NA[b]	0.592	8900	$s/\bar{X}$= NA[b]

Note. s is standard deviation. $s/\bar{X}$ is the coefficient of variation.
[a] BQL= below quantitation limit of 50 µg/kg.
[b] NA = not applicable to this site because value was BQL at TC4B.

the lowest near the mouth of the creek, at site TC4 (<100 µg/kg). Mean plant PCT burdens were lower than sediment levels at all sites. Concentrations of PCT in saltmarsh cordgrass are of interest because the *Spartina* food chain is an important food source for the other species examined. This plant may be a route for contamination of oysters, which consume small amounts of plant detritus (Truitt, 1931). *Spartina* may also be an important source of PCT for fiddler crabs. Montague (1980) noted that where this plant is abundant, fiddler crab fecal pellets may be packed with vascular plant detritus. Additionally, Hughes and Scherr (1983) determined that 44% of a mummichog's body carbon is derived from the *Spartina* food chain. Thus, all species in this study were linked to the *Spartina* food chain. Whether the PCT was internally absorbed by *Spartina* or sorbed to the surface was not determined. In either case, PCT may still be introduced into the food chain by ingestion of this plant or its breakdown products.

Indigenous Oysters

Indigenous oyster populations were only present at sites TC3 and TC4. The mean concentration of Aroclor 5432 in indigenous oysters at TC3 was 18,300 µg/kg, which was over seven times greater than that in the sediment at this location, and was the highest found for any species, at any site in this study. This concentration of 18,300 µg/kg (dry weight) is equivalent to 2550 µg/kg (s= 1020) on a wet weight

basis. Thus, the mean wet weight concentration for indigenous oysters at TC3 is slightly higher than the federal standard of 2000 μg/kg established for PCB in seafood destined for human consumption (Code of Federal Regulations, 1988). No corresponding limit has been established for PCT.

Transplanted Oysters

Oysters were also transplanted to all four of the sites, to further examine PCT accumulation patterns. Transplants were found to have PCT levels below the quantitation limit (BQL) prior to placement in Tabbs Creek. The highest mean PCT concentration in transplanted oysters, 2820 μg/kg, was again found at TC3. However, the mean PCT concentration in native oysters was over six times that of the transplants at this site. A similar pattern was observed at site TC4, where the mean PCT burden found in native oysters was approximately four times that of the transplanted oysters at this site. These data suggest that the transplanted oysters may not have achieved equilibrium with environmental concentrations of PCT after 30 days of residence at the site. However, the demonstrated PCT accumulation by transplanted oysters within a relatively short period, 30 days, illustrates the bioavailability PCT and potential for rapid bioaccumulation of these compounds to high levels.

It is interesting to note that the highest mean concentrations in transplanted oysters were not observed at

the two upstream sites (TC1 and TC2), where sediment levels were highest, but at the third site downstream, TC3. The lack of indigenous oysters at TC1 and TC2 indicates that conditions there may not have been optimal for oyster growth and survival. Low mean PCT concentrations in transplanted oysters at TC1 suggests that the oysters were not open and feeding as actively at this site as they were at TC3, causing lower levels of PCT accumulation. Low salinity may have affected the accumulation of PCT at the upstream locations. The salinity at TC1 was 10 ppt and at site TC3 was 16 ppt; these measurements were made near the time of high tide. Maurer and Watling (1973) found that, while oysters in Delaware Bay can tolerate a salinity range of 5 to 35 ppt, the optimum salinity range for adult oysters was 14 to 28 ppt. Oysters exposed to low salinity water (< 5 ppt) for prolonged periods have been reported to close their valves and die of anoxia (Andrews, 1982). The salinity measured at TC1 was near the low end of the toleration limit for oysters, suggesting conditions were not optimal for oysters placed at the head of the creek; this may have contributed to the lower PCT levels detected in transplanted oysters at TC1 and TC2.

Food availability may also have limited PCT accumulation in the transplanted oysters at the upper creek sites. The water at site TC1 was observed to have freshwater runoff and to be relatively clear, suggesting that less algae may have been present for the oysters to harvest. Higgins (1980) noted

that the activity of juvenile oysters is quantitatively affected by the presence of food; when food is absent, oysters generally remain closed. Lack of food may have thus caused the transplanted oysters to close their valves at the sites furthest upstream, limiting the exposure of oysters to PCT at the sites furthest upstream. Viewed from another standpoint, the observed lack of turbidity at TC1 indicates lower levels of suspended sediment. Since suspended sediment may be a source of PCT to biota, lower levels of suspended sediment may have contributed to lower PCT burdens in oysters transplanted at the upper sites (TC1 and TC2).

Crabs

Similar to sediment and saltmarsh cordgrass, mean PCT concentrations in fiddler/wharf crabs decreased with distance downstream. Fiddler crabs from site TC1 had a mean tissue concentration of 7030 μg/kg; values decreased downstream to 1780 μg/kg at TC2. Fiddler crabs were not available at TC3 or TC4C. At TC4A and TC4B, fiddler crab concentrations were at or near the quantitation limit. The mean concentration of Aroclor 5432 in wharf crabs at TC3 was less than 353 μg/kg, while a combination of wharf and fiddler crabs at site TC4 contained less than 101 μg/kg. Fiddler crab PCT burdens were consistently below sediment levels, but were within the same order of magnitude as those of saltmarsh cordgrass and mummichogs at all sites. Foods of the red-jointed fiddler crab

include algae, organic debris, and dead fish (Gray, 1942). Consumption of mummichog tissue may have, therefore, been a route by which PCT entered fiddler crabs. Fiddlers are also one of the primary foods of mummichogs (Kneib et al., 1980). Thus, the food chain is not unidirectional from fiddler crabs to mummichogs and each species may be contributing to the PCT burden in the other. The diet of wharf crabs is similar to that of fiddlers, as noted above. Predators of fiddlers include blue crabs (Callinectes sapidus) and a large number of bird species (Montague 1980); contamination of fiddler crab tissues may be another source of PCT for organisms in this ecosystem including blue crabs, and a means for transport of PCT out of this ecosystem via avian predators. Montague (1980) noted racoon (Procyon lotor) feces contained Uca parts; this was also observed at Tabbs Creek, suggesting a pathway for PCT movement into terrestrial food chains.

Mummichogs

PCT concentrations in mummichogs were higher at the two upstream sites (TC1 and 2) than at the two downstream sites (TC3 and 4). The highest mean concentration, 6950 μg/kg, was observed at the second site downstream, TC2, while the mean level at the extreme upstream site was 5920 μg/kg. Considering the standard deviations in concentration at the sites, these levels are similar. The lack of a definitive decreasing trend downstream, as observed in the sediment may be due, in part,

to the fact that mummichogs are mobile. Although these fish maintain a summer range of 36 to 38 m, they may move as much as 375 m at times (Lotrich, 1975). Furthermore, as noted earlier, the furthest site upstream, TC1, went nearly dry at low tide. Thus, the fish at site TC1 would be expected to move downstream, towards site TC2, at low tide, and thereby be exposed to varying PCT concentrations via the sediment during different parts of the tidal cycle. Heterogeneity of PCT contamination of the sediment within a site may also have contributed to anomalous concentrations in mummichogs. Major foods of mummichogs include fiddler crabs, wharf crabs, other small crustaceans, and polychaetes (Kneib et al., 1980). Thus, PCT in fiddler crab tissue may contribute to mummichog PCT burdens, as noted above. Prinslow et al., (1974) found that mummichogs ingest large quantities of detritus, but do not assimilate it. PCT sorbed to sediment may be absorbed by the mummichog as it passes through the mummichog's digestive tract, thus contaminated sediment or detritus may contribute to PCT levels in these organisms. As noted above, the Spartina food chain is a food source for these fish (Hughes and Scherr, 1983); PCT associated with Spartina may also contribute to PCT levels in mummichogs. Predators of these fish include blue crabs (Butner and Brattstrom, 1960), illustrating another means for PCT to be spread within the Tabbs Creek food web. Avian predators of these fish may be a means of transport of PCT out of the creek. Valiela et al. (1977) noted that very

high population and production rates of mummichogs make them an important species in the functioning of the saltmarsh ecosystem (Valiela *et al.* 1977). An alteration in population levels brought on by deleterious effects of contaminants may thus affect the overall production of the marsh.

From the previous discussion concerning the interrelationships of the species examined, it is clear that the pathway for PCT transfer between organisms is complex. Research on other chlorinated hydrocarbons reveals a difference in opinion over the major pathways for bioaccumulation of these types of contaminants in biota. Biomagnification along a food chain has been described as the most significant source of input of chlorinated hydrocarbons to biota at higher levels of the food chain by some authors (Macek and Korn 1970; Oliver and Niimi 1988; Rasmussen *et al.*, 1990). Others argue that equilibrium partitioning of chlorinated organics between the lipid phase of the organism and water is critical (Hamelink *et al.*, 1971; Shaw and Connell, 1982). The mechanism for PCT accumulation in the organisms of this study is thought to be a combination of biomagnification and bioconcentration. Initial sources of PCT to biota may include sediment, suspended sediment, and PCT dissolved in the water. Through the complex food web described above, the organisms in this study may also be contributing to PCT burdens in each other.

Conclusions

Aroclor 5432 was accumulated by all species collected from Tabbs Creek: saltmarsh cordgrass, American oysters, fiddler crabs, wharf crabs and mummichogs. The highest concentrations (18,000 μg/kg) were found in the native oyster. In general, PCT concentrations decreased with distance downstream from a suspected outfall near the head of the creek.

Chapter III

A Pilot Study of EROD Induction

in the Mummichog (<u>Fundulus heteroclitus</u>)

by Polychlorinated Terphenyl Formulations

Aroclor 5432 and 5460,

and Polychlorinated Biphenyl Formulation Aroclor 1254.

ABSTRACT

A pilot study on the effects of the PCT mixtures Aroclor 5432 and 5460 on activities of CYP1A associated hepatic ethoxyresorufin O-deethylase (EROD) in the mummichog (*Fundulus heteroclitus*) was completed. EROD levels were compared to those following injection of the known EROD inducer Aroclor 1254. Fish were injected intraperitoneally with PCT formulations Aroclor 5432, Aroclor 5460, or the PCB formulation Aroclor 1254, at doses of approximately 100 mg/kg body weight. A 20 mg/kg Aroclor 5432 dose was also applied. Statistically significant elevation of EROD activity was only detected in fish injected with Aroclor 5432 at a dose of approximately 100 mg/kg. Mean EROD activity resulting from Aroclor 5432 injection was greater than that caused by an approximately equal dose of Aroclor 1254. Treatment with Aroclor 5460 resulted in the lowest mean EROD of the three mixtures.

Introduction

The work of Chapter II illustrates that Aroclor 5432 can accumulate in species representing different ecological niches. Since minimal information was available on the effects of these compounds, further research was deemed necessary. Hepatic monooxygenase induction was selected as an indicator of the biological status of the mummichog following PCT exposure, because most of the biological effects of PCB and other halogenated aromatic hydrocarbons (HAH) in vertebrates are believed to be mediated through their binding to a cytosolic protein, the Ah receptor (Nebert, 1989; Poland and Knutson, 1982; Safe, 1986; Hahn et al., 1992; Heilmann et al., 1988). Induction of the hepatic monooxygenase cytochrome P4501A (CYP1A) and associated catalytic activities, such as ethoxyresorufin-O-deethylase activity (EROD), is one consequence of Ah receptor interaction (Okey et al., 1979) and is recognized as a sensitive indicator of HAH exposure and effects.

Numerous authors have reported induction of hepatic monooxygenase activity in teleosts following laboratory exposure to PCB mixtures, primarily Aroclor 1254 (Hill et al., 1974; Gruger et al., 1977; Addison et al., 1978; Sivarajah et al., 1978; Melancon et al., 1981; Melancon and Lech, 1983; Ankley et al., 1986). Monooxygenase induction in teleosts by PCT mixtures has not been previously reported.

Materials and Methods

Figure 17 illustrates the methods followed in this chapter. Male mummichogs were collected from Kings Creek, Virginia, on May 5, 1990. After anesthetization in a 200 mg/l solution of tricaine methanesulfonate, fish were given intraperitoneal injections (22G1.5 needle) with one of five treatments: corn oil, 100 mg/kg body weight (bw) Aroclor 1254, 32 or 100 mg/kg bw Aroclor 5432, or 100 mg/kg bw Aroclor 5460. Aroclor standards were purchased from Chem Service (West Chester, PA). The Aroclor mixtures were corn oil based. Ten μl were injected per g of fish. Six fish were injected per treatment. Injected fish were maintained in tanks containing filtered York River water; a 50% water change was made daily during a post-injection holding period. There was no mortality due to injection, nor during the holding period. Fish were sacrificed three days after injection, by stunning in York River water/ice slurry followed by cervical transection. Livers were immediately removed, rinsed in an ice-cold 1.15% KCl solution, frozen in liquid nitrogen, and stored at -70°C until analysis (within six weeks).

Microsome preparation essentially followed Van Veld *et al.* (1990). Samples were centrifuged twice at 10,000 xg for 10 min in a Sorvall RC28S centrifuge and the resulting supernatants centrifuged for 1 h at 100,000 xg. Samples were then homogenized with a glass rod and forced through a 22

Figure 17. Flowchart of methods used in pilot study of
EROD induction in fish injected with PCT.

Inject Samples
with Aroclor/Corn Oil

↓

Sacrifice 3 Days Post-Injection
Remove Liver, Freeze in Liquid Nitrogen

↓

Homogenize Liver,
Create Microsomal Fraction
through Centrifugation

↓

Bradford Total Protein Analysis

↓

Catalytic Assay
for CYP1A Activity: EROD

gauge needle. Microsomal pellets were frozen in liquid nitrogen and transferred to a -70°C freezer prior to analysis.

Total microsomal protein concentrations were determined by the Bradford Total Protein Assay (Bradford, 1976). Twenty μl of sample was diluted with 380 μl of deionized water. Five ml of Bradford Protein Dye Reagent (Bio-Rad, Richmond, CA) was added to 100 μl of diluted sample or standard, samples were incubated at room temperature for ten min, and absorbance at 595 nm was read on a Gilford Response spectrophotometer (Ciba Corning). Due to the small volume of each sample, single analyses for total protein content were conducted per sample. Protein concentrations were determined relative to a standard curve created with dilutions of bovine serum albumin. Samples that were not within the range of the standard curve were reanalyzed with a appropriate deionized water dilutions.

EROD reactions were measured spectrophotometrically (Klotz et al., 1984). EROD reaction mixtures contained 100 Mm Tris-HCl (pH 7.90), 100 mM NaCl, 2 μM of 7-ethoxyresorufin (Sigma Chemical Corp, St. Louis, MO), and 0.40 mM NADPH (Sigma) and microsomal protein (25-50 μg/ml). Absorbance at 572 nm was measured for 2 minutes. Blanks contained reaction mixture without NADPH. The small size of the mummichog liver precluded completion of duplicate analyses.

Multi-variable analysis of variance (p=0.05) followed by an a posteriori Tukey multiple comparison test (p=0.05) was performed to identify significant differences between doses of

a compound, using the *Toxstat®* statistical package (Gulley *et al.*). For statistical analysis, data were log transformed due to heteroscedasticity of variance.

Results

This preliminary study of induction resulting from the intraperitoneal injection of the PCB formulation Aroclor 1254, and the PCT formulations Aroclor 5432 and 5460 into mummichog indicated EROD induction for the approximately 100 mg/kg doses of Aroclor 5432 and 1254, although the level of induction was highly variable among fish of the same treatment (Table 3). The mean level of EROD activity in controls was 210 pmol/min/mg microsomal protein. The 22 mg/kg dose of Aroclor 5432 resulted in a mean EROD activity of 510 pmol/min/mg, and the 118 mg/kg dose of this mixture resulted in a mean EROD level of 1920 pmol/min/mg. The same 118 mg/kg dose of the PCB formulation Aroclor 1254 resulted in a lower mean EROD of 680 pmol/min/mg. The higher chlorinated PCT mixture, Aroclor 5460, gave a mean EROD of 420 pmol/min/mg. Only the highest dose of Aroclor 5432 caused a significant increase in EROD activity (Figure 18) (Analysis of Variance, p = 0.05; subsequent Tukey Multiple Comparison Test, p= 0.05).

Table 3. Pilot Study Results on EROD Activity in Mummichogs I.P. Injected with PCB and PCT.

Replicate	Corn Oil	Aroclor 1254 118 mg/kg dose	Aroclor 5432 22.5 mg/kg dose	Aroclor 5432 118 mg/kg dose	Aroclor 5460 126 mg/kg dose
1	140	750	380	1420	270
2	250	1240	1490	6250	200
3	140	580	420	1440	470
4	460	920	300	1820	1300
5	160	380	80	290	90
6	100	240	380	300	170
Mean	210	680	510	1920	420
(SD)	(130)	(370)	(500)	(2210)	(450)

Note. SD denotes standard deviation.

Figure 18. Results of pilot study of EROD induction in fish
injected with the PCB formulation Aroclor 1254, and
the PCT formulations Aroclor 5432 and 5460.

Error bars indicate standard error. * Denotes
statistical significance in ANOVA and subsequent
Tukey multiple comparison test ($p= 0.05$),
in which data were log transformed.

Pilot Study on EROD Induction
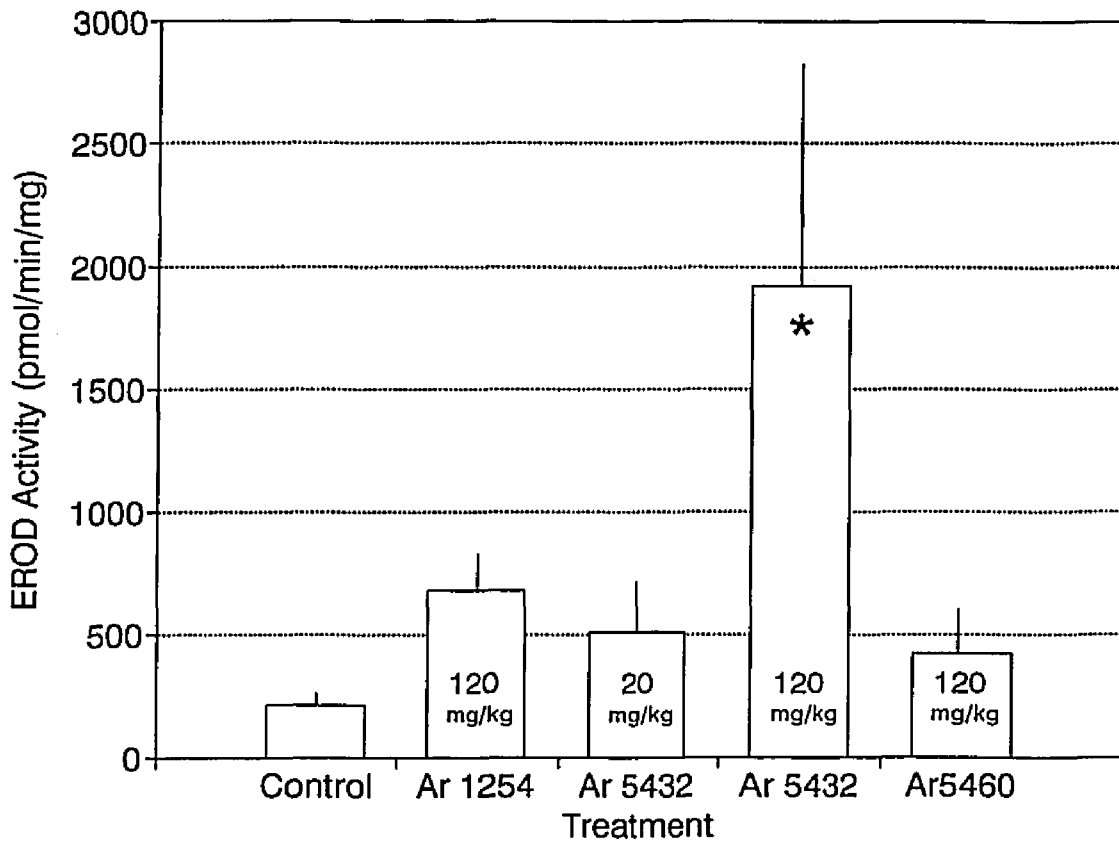
Discussion

EROD induction was observed only in fish injected with the high Aroclor 5432 dose. Examination of the data, however, indicated a trend toward increased EROD levels for the 118 mg/kg dose of Aroclor 1254, and possibly for the remaining two Aroclor treatments.

Heterogeneity of the microsomal mixture may have affected the consistency of values within treatments; the liver homogenate was observed to be clumpy. Introduction of air during the procedure may also have affected results. Additional experience with the procedure, in addition to modifications in the method to produce a more homogenous microsomal fraction, was expected to reduce variability within treatments.

These results demonstrated that further study on CYP1A induction in the mummichogs by PCT was warranted. Furthermore, the results of this chapter indicated that a dose-response study would be useful in the effects of variations in induction with changing doses of potential inducer.

Chapter IV

Induction of Cytochrome P4501A

in the Mummichog (<u>Fundulus heteroclitus</u>)

by the Polychlorinated Terphenyl Formulation Aroclor 5432.

by Kathryn Gallagher, Peter A. Van Veld,

Robert C. Hale and John J. Stegeman‡

‡Biology Department, Woods Hole Oceanographic Institution,

Woods Hole, Massachusetts 02543

ABSTRACT

Accumulation of the polychlorinated terphenyl formulation Aroclor 5432 in aquatic species has previously been reported. Polychlorinated terphenyls (PCT) are structurally similar to polychlorinated biphenyls (PCB), and were used in related applications. However, their biological effects have not been thoroughly studied. Effects of PCT mixtures on levels of hepatic cytochrome P4501A (CYP1A) and associated ethoxyresorufin O-deethylase (EROD) activity in the mummichog (*Fundulus heteroclitus*), a common estuarine fish, were assessed. Fish were injected intraperitoneally with PCT formulations Aroclor 5432, Aroclor 5460, or the PCB formulation Aroclor 1254, at doses of 0.32 to 100 mg/kg body weight. Elevated levels of CYP1A and EROD activity were detected in fish injected with Aroclor 5432 and 1254 doses of 32 and 100 mg/kg. Induction resulting from Aroclor 5432 was of the same order of magnitude as that caused by equivalent doses of Aroclor 1254. Treatment with Aroclor 5460 did not result in significant induction. Because commercial PCT mixtures contain small amounts of PCB, the PCB components may have contributed to the induction observed for Aroclor 5432. This work represents the first report of hepatic cytochrome CYP1A induction caused by Aroclor 5432 in teleosts and, similar to work in mammalian systems, suggests that the effects of this mixture may be at least partially mediated through Ah receptor binding.

Introduction

Polychlorinated terphenyls (PCT) are halogenated aromatic compounds similar in structure and chemical properties to polychlorinated biphenyls (PCB). Approximately 50,000 metric tons of PCT were produced in the United States by Monsanto (St. Louis, MO) from 1929 to 1972. Several PCT formulations were produced including Aroclor 5432 and 5460, which contain 32 and 60 percent chlorine by weight, respectively (Jensen and Jørgensen, 1983). Little information is available on either the distribution of PCT in the environment or their biological effects.

Dietary exposure of rats to the highly chlorinated PCT formulation, Aroclor 5460, has been reported to cause an increase in liver weight and in total cytochrome P450 (Sosa-Lucero et al., 1973). Intraperitoneal (i.p.) injection of rats with the same mixture caused an increase in aryl hydrocarbon hydroxylase activity and total P450 (Nilsen and Toftgård, 1981). An increase in liver weight and liver tumor formation was reported in mice exposed to Kanechlor C (Shirai et al., 1978). This PCT formulation, produced in Japan, possesses a degree of chlorination similar to Aroclor 5460 (De Kok et al., 1982). Rhesus monkeys fed Aroclor 5460 exhibited a decrease in body weight, hair loss, subcutaneous edema, chloracne, liver hypertrophy attributed to proliferation of smooth endoplasmic reticulum, and hyperplasia and dysplasia of gastric mucosa

suggestive of eventual development of neoplastic transformations (Allen and Norback, 1973). An increase in proliferation of hepatic endoplasmic reticulum, total cytochrome P450 levels and ethoxyresorufin *O*-deethylase (EROD) activity was noted in rats injected (i.p.) with the less chlorinated PCT formulation Aroclor 5432. Aroclor 5460 induced EROD activity to a lesser degree than 5432 (Toftgård *et al.*, 1980).

Because commercial PCT mixtures contain small amounts of PCB (Cooke *et al.*, 1978), it is important to consider the potential effects of PCB contaminants in induction studies using these mixtures. Nilsen and Toftgård (1981) found that Aroclor 5460 contained 0.75% PCB. These authors estimated that the PCB contamination of PCT caused 20% of the observed increase in P450 concentration, and concluded that the PCT components of the mixture caused the remainder of the induction.

Numerous authors have reported induction of hepatic monooxygenase activity in teleosts following laboratory exposure to PCB mixtures, primarily Aroclor 1254 (Hill *et al.*, 1974; Gruger *et al.*, 1977; Addison *et al.*, 1978; Sivarajah *et al.*, 1978; Melancon *et al.*, 1981; Melancon and Lech, 1983; Ankley *et al.*, 1986). Cytochrome P4501A (CYP1A) is the primary monooxygenase form induced in fish by planar PCB (Gooch *et al.*, 1989). Most of the biological effects of PCB and other halogenated aromatic hydrocarbons (HAH) in vertebrates are

believed to be at least partially mediated through their binding to the Ah receptor (Nebert, 1989; Poland and Knutson, 1982; Safe, 1986; Hahn et al., 1992; Heilmann et al., 1988). Induction of CYP1A and associated catalytic activities, such as EROD, is one consequence of Ah receptor interaction (Okey et al., 1979) and is recognized as a sensitive indicator of HAH exposure and effects. Monooxygenase induction in teleosts by PCT mixtures has not been previously reported.

We recently reported accumulation of PCT in biota of Tabbs Creek, a small tidal creek draining into the Chesapeake Bay, Virginia (Gallagher et al., 1993). Aroclor 5432, was detected in saltmarsh cordgrass (*Spartina alterniflora*), American oysters (*Crassostrea virginica*), red-jointed fiddler crabs (*Uca minax*), wharf crabs (*Sesarma reticulatum*), and mummichogs (*Fundulus heteroclitus*). This work illustrated that Aroclor 5432 can accumulate in species representing different ecological niches. Since minimal information was available on the effects of these compounds, further research was deemed necessary. In the present study, we examined the effects of Aroclors 5432 and 5460 on hepatic CYP1A and associated EROD activity in the mummichog, and compared these effects with those caused by exposure to the PCB formulation Aroclor 1254, a mixture known to cause induction of CYP1A in teleosts.

The results of the previous chapter indicated EROD induction by the PCT mixture Aroclor 5432. In this chapter,

a more extensive analysis of CYP1A induction was undertaken, using a range of doses of the three Aroclor mixtures, Aroclor 1254, 5432 and 5460. In addition, a second assay for enzyme induction, immunochemical analysis of CYP1A levels, was conducted. Immunochemical analysis of CYP1A measures the level of CYP1A protein, the EROD catalyst, in the microsomal fraction using an antibody specific to the protein. The addition of this assay was necessary and useful as a confirmation of EROD results, and to assess potential inhibition of catalytic activity of the protein resulting from exposure.

Materials and Methods

Figure 19 outlines methods used in this chapter. Male mummichogs *(Fundulus heteroclitus)* (6.6 g ± 1.0) were collected from Kings Creek, Virginia, a relatively clean reference site [Van Veld, personal communication]. The fish were transported to laboratory aquaria, where they were maintained in clean water and fed commercial aquarium food (TetraWerke, Germany) for approximately one month prior to treatment.

At the time of injection, fish were removed from aquaria and anesthetized by placement in a solution of 200 mg/l tricaine methanesulfonate in water for several minutes. The fish received intraperitoneal injections of Aroclor 5432

Figure 19. Flowchart of methods used in the CYP1A and EROD

induction dose-response study.

```
┌─────────────────────────────────────────┐
│              Inject Samples             │
│          with Aroclor/Corn Oil          │
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│      Sacrifice 3 Days Post-Injection,   │
│  Remove Liver, Freeze in Liquid Nitrogen│
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│             Homogenize Liver,           │
│         Create Microsomal Fraction      │
│             via Centrifugation          │
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│    Micro-Bradford Total Protein Analysis│
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│    Immunochemical Analysis for CYP1A    │
│        Using Mab 1-12-3 to Scup         │
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│            Catalytic Assay for          │
│          CYP1A Activity: EROD           │
└─────────────────────────────────────────┘
```

(PCT), Aroclor 5460 (PCT), or Aroclor 1254 (PCB) at doses of 0.32, 1.0, 3.2, 10, 32, and 100 mg/kg body weight in a corn oil carrier. Carrier oil dose was constant between treatments, with 10 μl of carrier oil injected per g of fish body weight for each treatment. Aroclor standards were purchased from Chem Service (West Chester, PA). Corn oil controls and no-injection controls were also analyzed. Each treatment consisted of three or four fish. Following injection, fish were maintained for 72 hours in randomly selected, separate, aerated aquaria at 20 to 22°C containing York River water. Tank water was replaced daily. There was no mortality following injection, nor during the holding period. The fish were killed by immersion in a York River water-ice slurry, and subsequent cervical transection. Livers were immediately removed, rinsed with ice-cold 1.15% KCl, frozen in liquid nitrogen, and stored at -70°C until analysis.

Microsome preparation essentially followed Van Veld *et al.*(1990). Samples were centrifuged twice at 10,000 xg for 10 min in a Sorvall RC28S centrifuge and the resulting supernatants centrifuged for 1 h at 100,000 xg. Replicate analyses demonstrated that the heterogeneity of microsomal fractions indicated in the pilot study could be reduced by repeatedly forcing the sample through a smaller gauge needle (25G). Following this protocol, samples were found to be homogenous by protein assay. Microsomal pellets were frozen in liquid nitrogen and transferred to a -70°C freezer prior to

analysis.

In order to maximize sample volume for replicate enzyme analyses, total microsomal protein concentrations were determined by a modified Bradford Total Protein Assay (Bradford, 1976), which required only 25% of the sample volume per replicate used in the pilot study. Five μl of each sample was diluted with 55 μl deionized water, placed in a microplate, and 200 μl of Protein Assay Dye Reagent (Bio-Rad, Richmond, CA) were added to each well. The mixed solutions were incubated at room temperature for 10 minutes, and absorptions at 570 nm were measured with a Dynatech MR5000 microplate reader. Samples were analyzed in triplicate. Protein concentrations were determined relative to a standard curve created with dilutions of bovine serum albumin. Samples that were not within the range of the standard curve were reanalyzed after appropriate deionized water dilutions.

Immunochemical analyses were based on the methodology of Kloepper-Sams et al. (1987), with the following modifications. Microsomal proteins were electrophoretically separated on polyacrylamide (12%) gels and transferred to 0.20 μm nitrocellulose papers, using a Bio-Rad Mini-Protean II apparatus. The blots were placed in a solution of monoclonal antibody (Mab 1-12-3) to scup CYP1A (Park et al., 1986) for one h. The secondary antibody was alkaline phosphatase (AP) conjugated goat anti-mouse IgG (Bio-Rad). The blots were then placed in a color development solution of Bio-Rad AP Color

Reagent A and B. CYP1A contents were quantitated on a Shimadzu CS-930 scanner coupled to a Shimadzu DR-2 data recorder. Spot (*Leiostomus xanthurus*) microsomal CYP1A, previously calibrated against purified scup CYP1A (Klotz *et al.*, 1983), was diluted for standard curve preparation. CYP1A concentrations are expressed as CYP1A equivalents due to potential species differences in antibody reactivity. CYP1A measurements were not completed for the 0.32 and 1 mg/kg Aroclor 5460 doses because the EROD showed no significant induction, and there was a limited quantity of antibody available at the time.

EROD reactions were measured spectrophotometrically (Klotz *et al.*, 1984). EROD reaction mixtures contained 100 Mm Tris-HCl (pH 7.90), 100 mM NaCl, 2 μM of 7-ethoxyresorufin (Sigma Chemical Corp, St. Louis, MO), and 0.40 mM NADPH (Sigma) and microsomal protein (25-50 μg/ml). Absorbance at 572 nm was measured for 2 minutes. Blanks contained reaction mixture without NADPH. When sufficient sample was available, duplicate analyses for EROD activity were completed on the samples. In these cases, the reported EROD activity was the mean of the two analyses. The small size of the mummichog liver precluded completion of duplicate analyses for all fish.

Multi-variable analysis of variance (p=0.05) and subsequent *a posteriori* Tukey multiple comparison tests (p=0.05), where appropriate, were performed to identify significant differences between doses of a compound. The *Toxstat*® statistical package (Gulley *et al.*) was used for

these tests. Corn oil controls were treated as the 0 mg/kg dose of each compound; there was no significant difference between corn oil and uninjected controls. For statistical analysis, data were log transformed due to heteroscedasticity of variance.

To attempt to quantify potential effects of planar PCB in the Aroclor mixtures, standards of Aroclors 1254, 5432, and 5460, 3,3',4,4'- tetrachlorobiphenyl (BZ 77), 3,3',4,4',5-pentachlorobiphenyl (BZ 126), 3,3',4,4',5,5'-hexachlorobiphenyl (BZ 169), and 2,3,3',4',6-pentachlorobiphenyl (BZ 110) were analyzed using GC-MS. The Aroclor mixtures were purchased from Chem Service (West Chester, PA), and the PCB congeners from Ultra Scientific (North Kingston, RI). A Hitachi gas chromatograph equipped with a capillary column (J & W Scientific, DB-5 fused silica column, 60 m x 0.25 mm i.d., 25 μm film thickness) coupled to an Extrel ELQ 400-2 quadropole mass spectrometer was operated in negative chemical ionization (NCI) mode, with methane as the moderator gas. The temperature program for the GC was 90°C for 1 min, 4°C per min to 310°C, with a 30 min hold. Helium was the carrier gas. Source temperature was 100°C and source pressure was 1000 mTorr. The mass spectrometer was operated in the full scan mode to obtain ion spectra. It was also used in selected ion monitoring (SIM) mode to minimize interference by co-eluting compounds, in an attempt to quantitate any coplanar

PCB identified.

Due to the complexity of the formulations, a second capillary column (Chrompack, CPSIL CB5/C18, 100m x 0.32 mm i.d., 0.10 μm film thickness), coupled to the mass spectrometer operating in NCI mode, was evaluated for these separations under similar mass spectrometer conditions. The temperature program for the GC was 75°C for 1 min, 4°C per min to 270°C, with a hold at 270°C for 60 min. The mass spectrometer was operated in full scan mode to obtain ion spectra.

Results

Statistically elevated levels of CYP1A were detected in fish exposed to Aroclor 5432 (PCT) and 1254 (PCB) doses of 32 and 100 mg/kg (Figs. 20a and 21a)(p=0.05), relative to corn oil controls. Elevated EROD activities were also detected in fish exposed to Aroclor 1254 and 5432 doses of 32 and 100 mg/kg (p=0.05)(Figs. 20b and 21b). Treatment of fish with Aroclor 1254 at the 10 mg/kg dose also resulted in a statistically significant increase in EROD activity. There was a trend toward higher EROD activity, relative to controls, in fish exposed to 10 mg/kg of Aroclor 5432, as well. However, this difference was not significant. Exposure to the higher chlorinated PCT formulation, Aroclor 5460, did not result in a statistically significant elevation of CYP1A or EROD

Figure 20. CYP1A and EROD levels in mummichogs injected

with the PCB formulation Aroclor 1254.

A.) CYP1A equivalents vs. dose

B.) EROD activity vs. dose

Standard deviation (±) indicated by error bars.

Solid line connects treatment means.

* Denotes statistical significance in ANOVA and

subsequent Tukey multiple comparison test (p=0.05),

in which data were log transformed.

Aroclor 1254
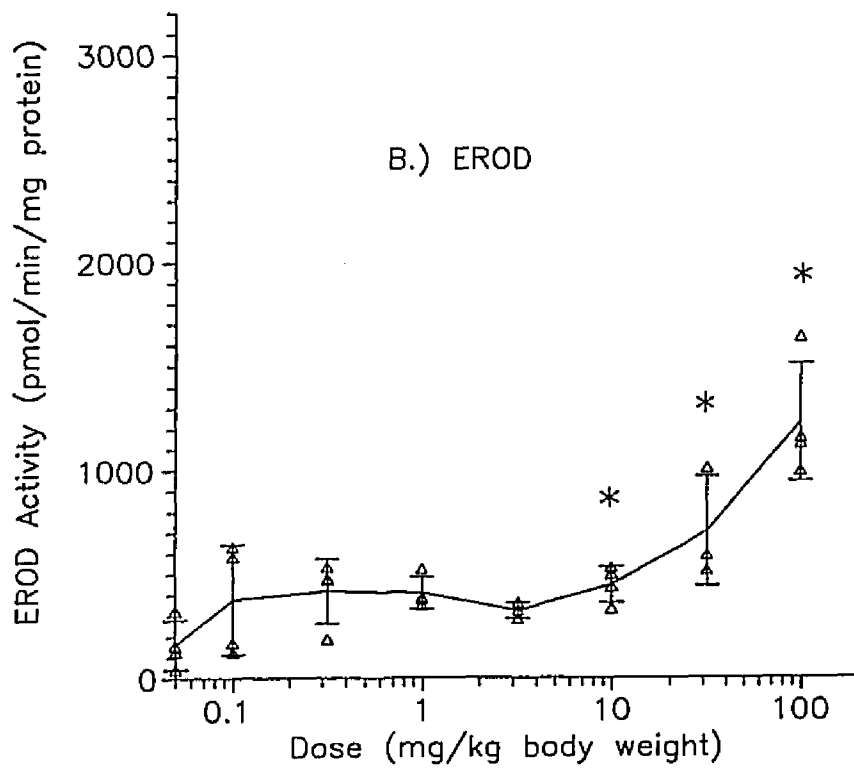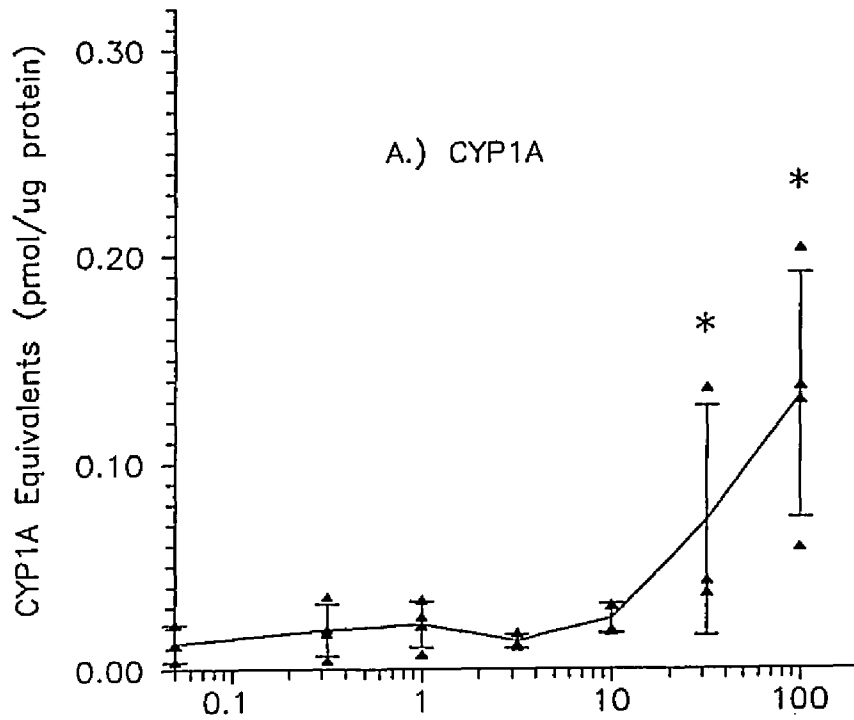
Figure 21. CYP1A and EROD levels in mummichogs injected
with the PCT formulation Aroclor 5432.

A.) CYP1A equivalents vs. dose

B.) EROD activity vs. dose

Standard deviation (±) indicated by error bars.
Solid line connects treatment means.
* Denotes statistical significance in ANOVA and
subsequent Tukey multiple comparison test (p=0.05),
in which data were log transformed.

Aroclor 5432
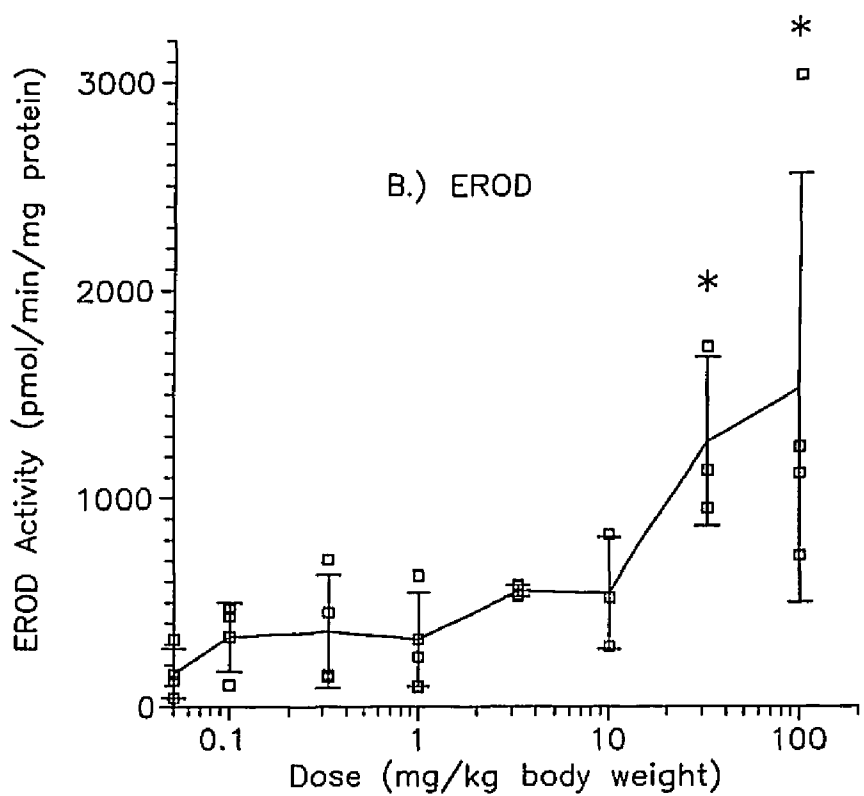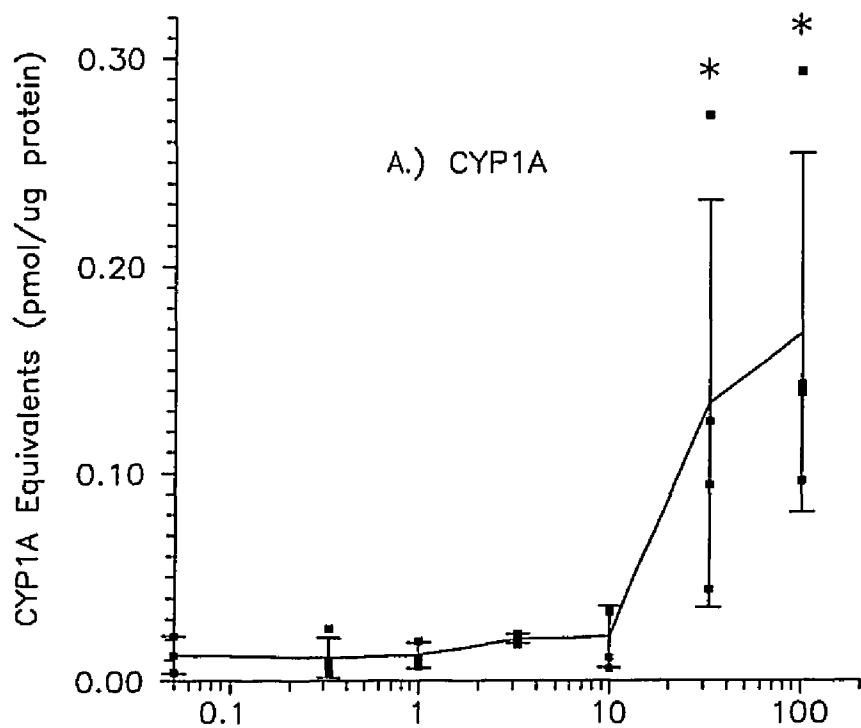
activity (Figure 22) (p>0.05).

Appendix I contains data tables of CYP1A and EROD results.

DISCUSSION

This work is believed to represent the first evidence of CYP1A induction in teleosts caused by the PCT formulation Aroclor 5432, and, similar to work in mammalian systems, suggests that the mechanisms of toxicity of this mixture may be similar to those of other halogenated aromatic compounds. As noted above, most of the biological effects of PCB and other HAH appear to be mediated through their binding to the Ah receptor. Induction of CYP1A proteins and associated EROD activity is one consequence of Ah receptor interaction. The presence of an Ah receptor in mummichogs has previously been confirmed (Hahn et al., 1992). The observation that Aroclor 5432 induces CYP1A and associated EROD activity, indicates that the biological effects of this mixture may also be partly dependent on Ah receptor interaction.

The results indicate that the potency of the PCT formulation Aroclor 5432 as an inducer of CYP1A in mummichogs is similar to that of the PCB formulation Aroclor 1254. In fish exposed to an Aroclor 5432 dose of 100 mg/kg, mean CYP1A and EROD were elevated 13-fold and 10-fold, respectively, relative to control fish (Table 4). Treatment with the same

Figure 22. CYP1A and EROD levels in mummichogs injected

with the PCT formulation Aroclor 5460.

A.) CYP1A equivalents vs. dose

B.) EROD activity vs. dose

Standard deviation (±) indicated by error bars.

Solid line connects treatment means.

* Denotes statistical significance in ANOVA and

subsequent Tukey multiple comparison test (p=0.05),

in which data were log transformed.

Aroclor 5460

A.) CYP1A

B.) EROD
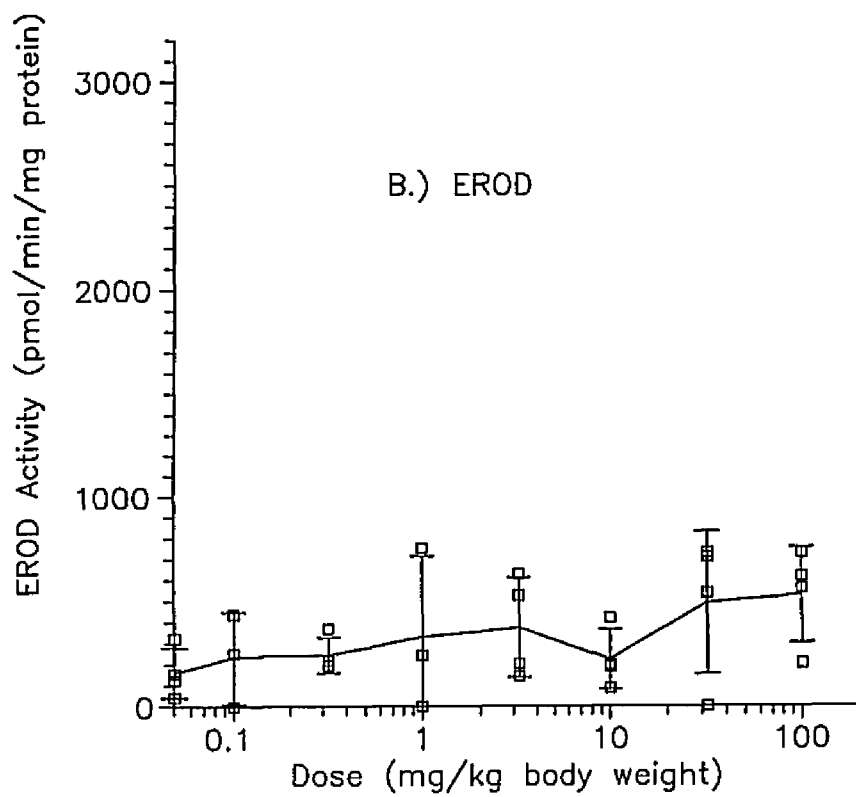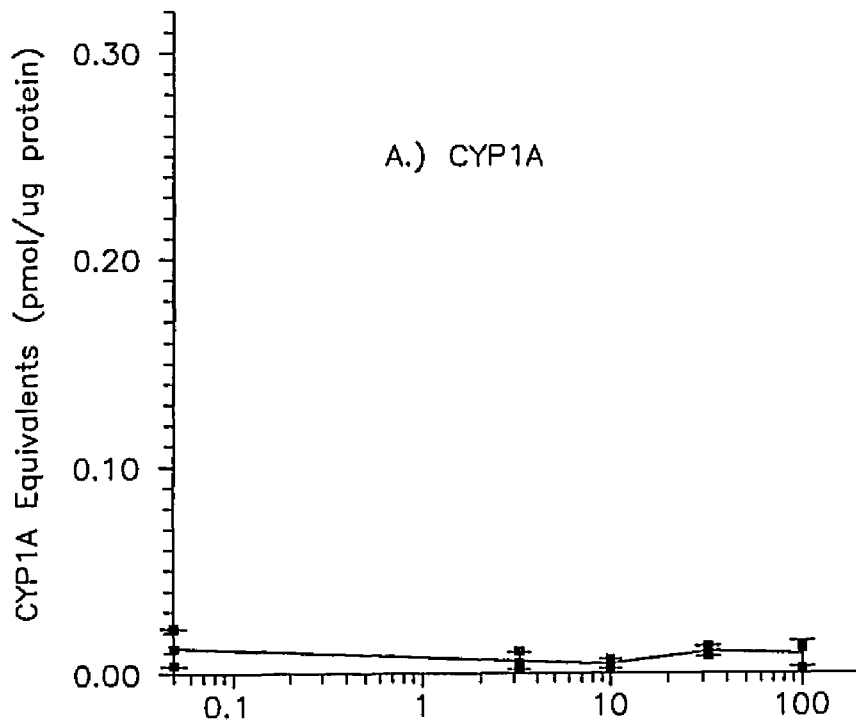
Table 4. Relative Induction Resulting from Injection of Aroclors 1254 and 5432 into Mummichogs.

Mixture	Dose (mg/kg)	Mean P4501A (pmol/ug)	Fold Induction[a]	Mean EROD Activity (pmol/min/mg)	Fold Induction[a]
Aroclor 1254	100	0.132	10	1230	8
	32	0.072	6	710	4
	10	0.025	2	450	3
Aroclor 5432	100	0.168	13	1532	10
	32	0.134	11	1270	8
	10	0.021	2	543	3

[a] Relative to corn oil-injected controls and rounded to nearest integer.

dose of Aroclor 1254 resulted in a 10-fold increase in CYP1A, and an 8-fold increase in EROD activity. In fish exposed to a 32 mg/kg dose of Aroclor 5432 mean CYP1A and EROD were elevated 11-fold and 8-fold, relative to controls. The 32mg/kg dose of Aroclor 1254 caused a 6-fold increase of CYP1A and a 4-fold increase in EROD activity.

The results from experiments with Aroclor 5460 differed from those of mammalian studies. Aroclor 5460 was not found to cause enzyme induction in fish, but has been reported to cause induction in mammals (Sosa-Lucero, 1973; Nilsen and Toftgård, 1981; Allen and Norback, 1973; Toftgård et al., 1980), although to a lesser extent than Aroclor 5432 (Toftgård et al., 1980). Further studies on these differences between mammals and fish, and between mixtures, would be useful.

Identification of a Planar PCB Congener in Aroclor 5432

As noted above, commercial PCT mixtures contain small amounts of PCB (Cooke et al., 1978). Thus, some of the induction caused by Aroclor 5432 may have been due to PCB components present. Coplanar PCB congeners have been shown to be the primary cause of enzyme induction in fish exposed to PCB mixtures (Melancon et al., 1981; Melancon and Lech, 1983; Gooch et al., 1989). The confirmation of the presence and subsequent quantification of coplanar PCB congeners in Aroclor 5432, or their removal from the mixture, would be useful to assess more accurately the effects of the PCB components in

the mixture.

PCB mixtures are complex formulations; there are 209 possible PCB congeners. Typical gas chromatographic methods commonly result in coelution of several congeners. This problem is amplified with PCT mixtures. Not only can PCT be *ortho-, meta-* and/or *para*-chlorinated, but the phenyl rings themselves can attain these conformations with respect to each other, resulting in more than 1000 possible congeners. Furthermore, variation in composition between synthesized batches may contribute to variation in determined congener composition for a given Aroclor mixture. The low concentration of planar PCB detected in most Aroclor mixtures (Schulz *et al.*, 1989; Duinker *et al.*, 1988; Kannan *et al.*, 1987) adds to the difficulty of accurate identification and quantification of these congeners in these complex mixtures. The resolution of PCT congeners from each other and from PCB components of the mixtures is, therefore, extremely difficult to produce, and has not, to date, been achieved.

In a preliminary experiment, attempted removal of the PCB components in the Aroclor 5432 mixture, using HPLC with a graphitic carbon column (50 mm x 4.6 mm Hypercarb, Shanden Scientific), was unsuccessful. Planar PCB components in Aroclor 5432 coeluted with PCT components. I was, therefore, unable to obtain a PCT fraction free of planar PCT for further dosing experiments. Furthermore, non-ortho-chlorinated PCT congeners are not commercially available, so dosing

experiments with these PCT congeners could not be conducted.

Therefore, using GC-MS, Aroclor 1254, 5432 and 5460 were examined for the presence of the planar PCB congeners 3,3',4,4'-tetrachlorobiphenyl (BZ 77), 3,3',4,4',5-pentachlorobiphenyl (BZ 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (BZ 169). This was done to assess whether these PCB are present and may contribute to enzyme induction in fish exposed to these mixtures.

PCB congeners were identified by their molecular ions and retention time. The non-planar PCB congener 2,3,3',4',6-pentachlorobiphenyl (BZ 110) coeluted with BZ 77 on the a DB5 column, preventing accurate quantitation of BZ 77 in the Aroclor mixtures. BZ 110 has been reported (Schulz et al., 1989) to comprise approximately 5% of Aroclor 1254; the magnitude of the signal from this congener masked the signal obtained from BZ 77 during GC-MS analysis. Reports in the literature confirm the difficulty in quantitating planar PCB, with reported values for BZ 77 in Aroclor 1254 ranging from below the quantitation limit to over 600 μg/kg (Schulz et al., 1989; Duinker et al., 1988; Kannan et al., 1987). BZ 126 coelutes with di-ortho chlorinated BZs 129 and 168, preventing accurate quantitation of this congener.

The use of a 100 m column partially coated with C18 (Chrompack, CPSIL CB5/C18) resolved the PCB congeners examined. However, column bleed from the C18 column degraded the MS source. This prevented the generation of a reproducible

calibration curve for planar PCB with the C18 column. Lack of resolution of PCT congeners with BZ 77 prevented use of this C18 column on another detector, such as an electron capture detector, which would have been less prone to column phase accumulation due to elevated detector temperature (300°C). Nevertheless, the presence of BZ 77 (Fig. 23) was confirmed in Aroclor 5432, as illustrated by the mass spectrum of this mixture at the BZ 77 retention time (Fig. 24). The molecular ion (m/e 292) and chlorine isotope peaks are present in the same ratios in both spectra. These spectra were obtained using the C18 column. The mass spectrum of Aroclor 5460 also contained a 292 ion at the retention time for BZ 77 (Fig. 25, C18 column). However, since this peak was so small, and there were no chlorine isotope peaks, the presence of BZ 77 in Aroclor 5460 cannot be confirmed.

Molecular ions for BZ 126 and 169 were also detected in Aroclor 5432 and 5460, at their respective retention times on the DB-5 and C18 columns. However, due to the coelution of several other PCT congeners at these retention times and a lack of an isotope peak profile, the presence of these congeners cannot be definitively confirmed.

The confirmed presence of BZ 77 in Aroclor 5432 is expected to have had an effect on CYP1A levels in mummichogs. However, the magnitude of the effect of the BZ 77 in Aroclor 5432 on the CYP1A induction results cannot be accurately estimated from this study.

Figure 23. NCI mass spectrum of 3,3',4,4'-tetrachlorobiphenyl

(BZ 77).

EXC18P3B PCB3B 1000PG -CI CH4
31-JAN-95 SCAN 2485 TIME 47.83 MIN.
100 % = 1034

TOTAL SCALE
3747 1*

120   140   160   180   200   220   240   260

292

280   300   320   340   360   380   400   420

452   557   595

440   460   480   500   520   540   560   580
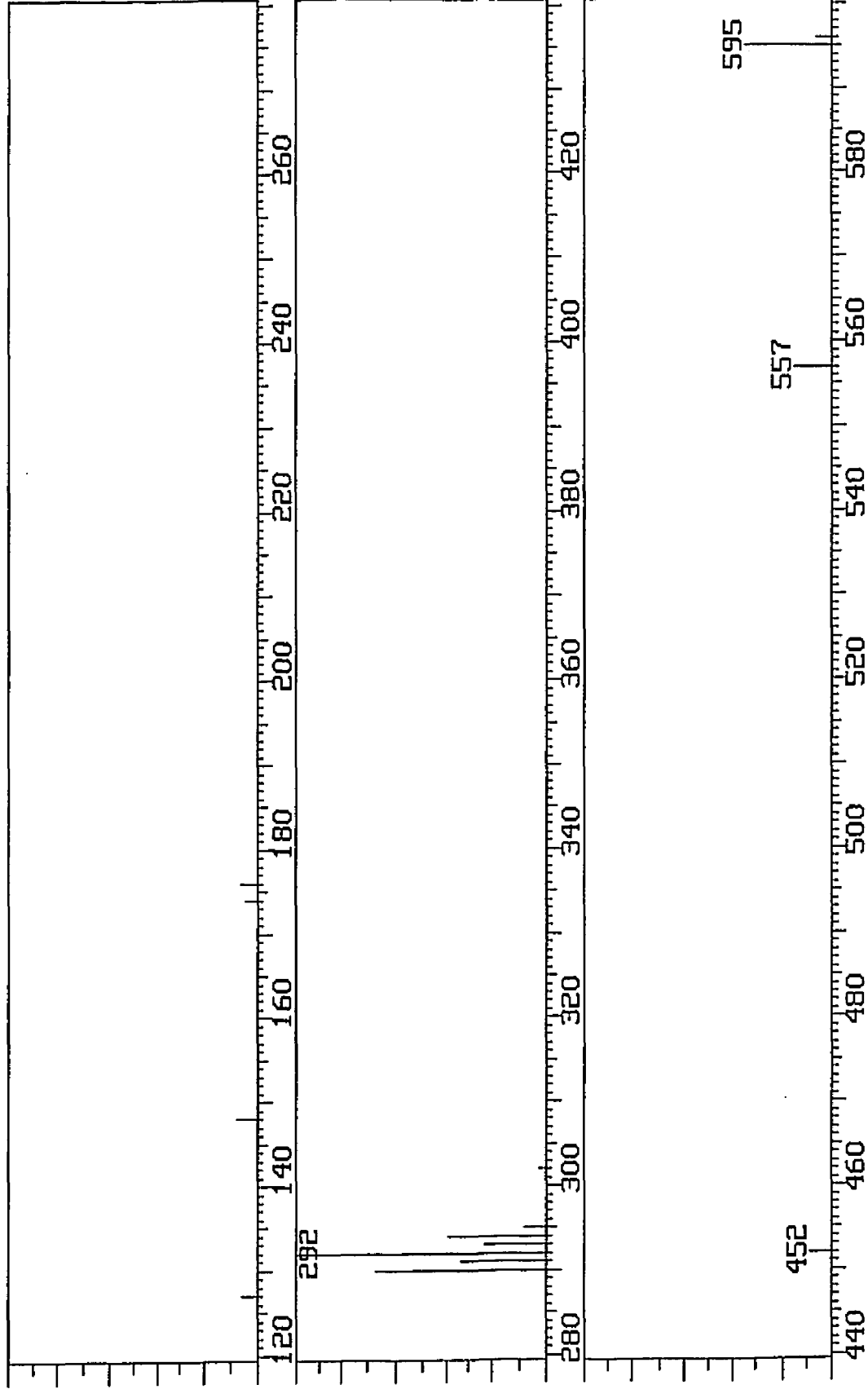
Figure 24. NCI mass spectrum of Aroclor 5432, at the retention
time of 3,3',4,4'-tetrachlorobiphenyl, confirming
the presence of this PCB congener in this mixture.

EXC18543Z 543Z 365UG/ML -CI CH4 SHORT COLLECTION 1.3UL
31-JAN-95 SCAN 2488 TIME 47.88 MIN.
100 % = 764

TOTAL SCALE
3366 1*

106

292

557

595
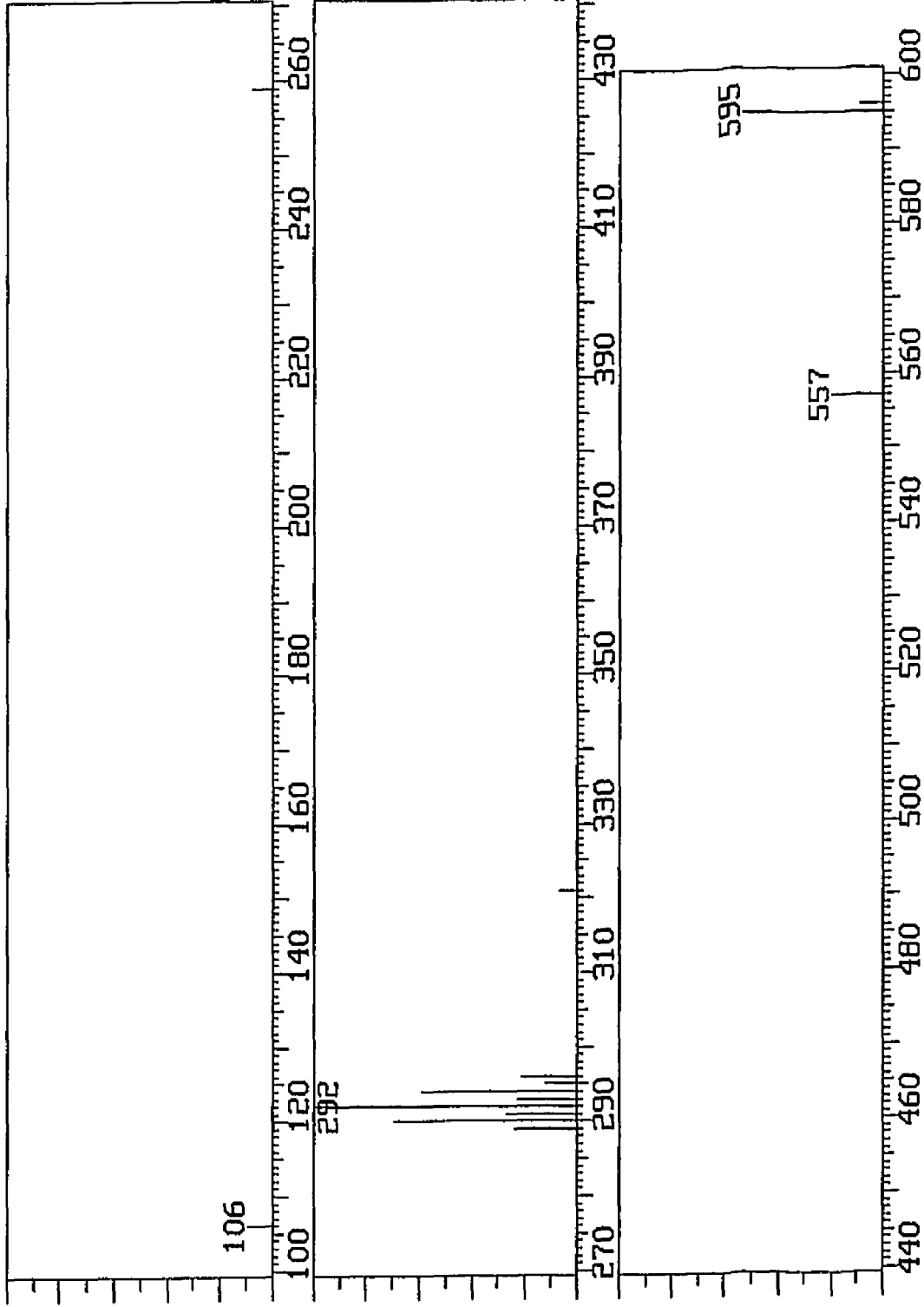
Figure 25. NCI mass spectrum of Aroclor 5460, at the retention
time of 3,3',4,4'-tetrachlorobiphenyl.

EXC18S460 5460 3246UG/ML 1.4UL -CI CH4
26-JAN-95 20:23:48 SCAN 2488 TIME 47.85 MIN.
100 % = 429

TOTAL SCALE
939 1*
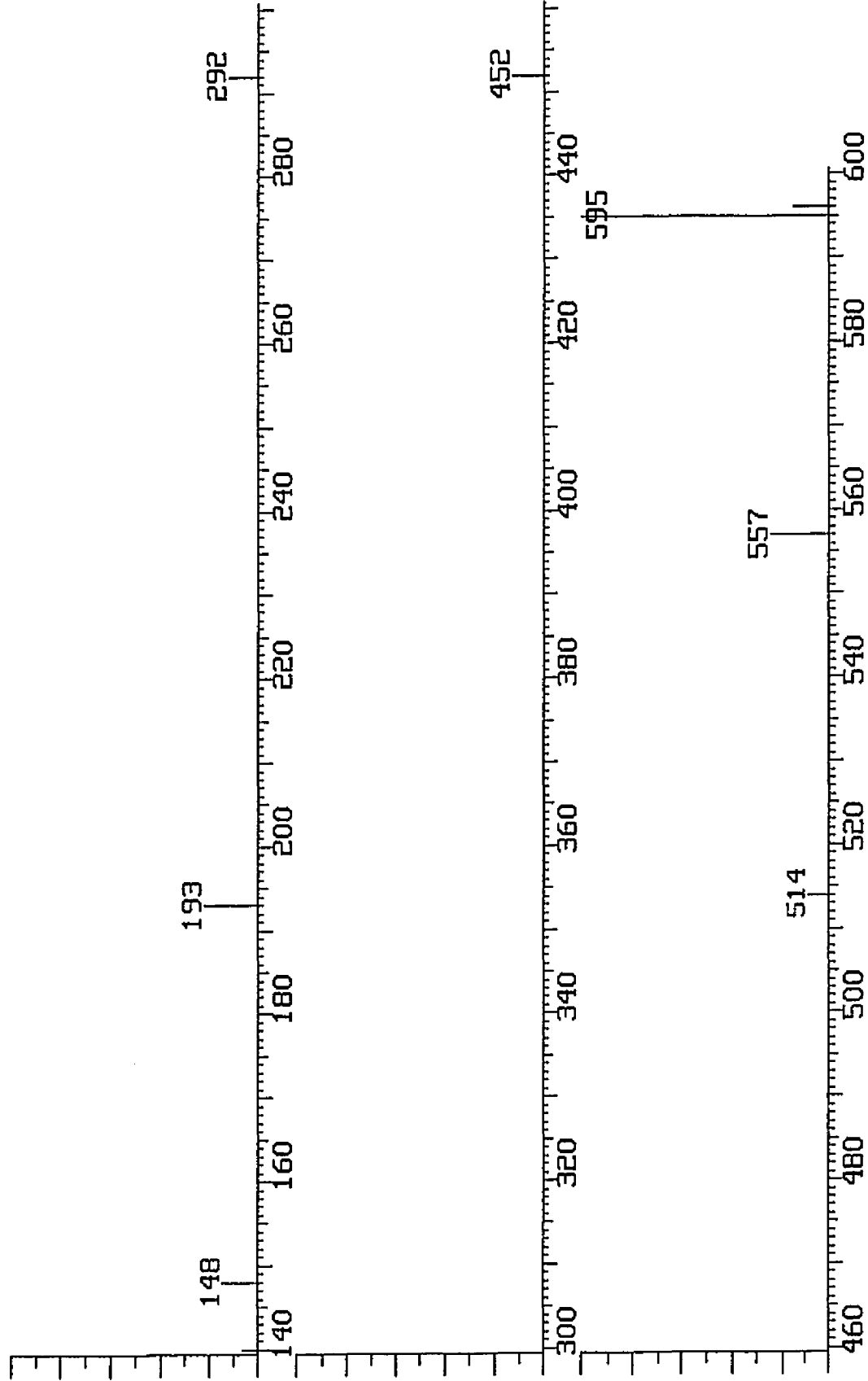
148
193
292

514
557
595
452

The use of multi-dimensional GC might make the quantitation of PCB congeners in PCT mixtures possible, enabling an estimation of the effects of planar PCB congeners on MO induction following Aroclor 5432 injection.

PCT were released to the environment as commercial mixtures. Therefore, information regarding the effects of the mixture, as a whole, is critical in evaluating the significance of releases of these mixtures to the environment.

Chapter V

Environmental Induction of Cytochrome P4501A

in Mummichogs Collected from an Estuarine Creek

ABSTRACT

Mummichogs (*Fundulus heteroclitus*) were collected at Tabbs Creek, a tributary of the Chesapeake Bay known to contain high sediment concentrations of the PCT formulation Aroclor 5432. Fish were collected along a gradient of increasing PCT contamination within Tabbs Creek, established in earlier studies. Samples were analyzed for environmental induction of hepatic CYP1A and EROD activity.

Environmental induction of CYP1A was observed along the gradient of PCT contamination within Tabbs Creek. CYP1A levels in fish collected nearest the PCT outfall, sites TA and TB, were significantly greater than levels in fish collected at site TC, the site furthest downstream, and from control fish collected at an uncontaminated field site (p=0.05). The mean EROD activity at TB was significantly different from the Kings Creek control activity. EROD levels in fish collected from TA were the lowest in Tabbs Creek, despite the fact that the mean CYP1A protein levels were highest at this site, and that CYP1A is the source of EROD activity. The low EROD activity of the TA fish indicates an inhibition of enzyme activity in fish collected near the outfall. Turnover number (activity/pmol CYP1A) illustrates a decrease in catalytic efficiency of fish in Tabbs Creek with a decrease in distance from the PCT outfall. Similar inhibition of EROD activity at high xenobiotic doses has been reported by several other researchers.

Despite the fact that PCT is present at high concentrations within the creek, and that induction by PCT has been demonstrated in the laboratory, the induction observed in mummichogs collected from Tabbs Creek can not be attributed solely to the PCT contamination within Tabbs Creek. Other known enzyme inducers have been detected in Tabbs Creek, including PAH and PCB.

Regardless of the inability to determine the inducing agent, the observed environmental induction in fish collected from Tabbs Creek is a cause for concern. Induction and inhibition of monoxygenase activity may affect the ability of the fish to enzymatically respond to additional contaminant inputs, and may signal the development of Ah receptor-mediated toxicity. Tabbs Creek is currently undergoing remedial investigation under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). The results of this study contributed to the evaluation of potential options for remediation under CERCLA at Tabbs Creek.

Introduction

Many reports have been published concerning environmental induction of cytochrome P4501A and resultant EROD activity from a variety of environments (Elskus and Stegeman, 1989; Stegeman et al., 1990; Van Veld et al., 1990; Addison et al., 1994) There have been several reports of hepatic enzyme induction in fish environmentally exposed primarily to polychlorinated biphenyls (PCB) (Addison and Edwards, 1988; Stegeman et al., 1988). However, there have been no previous reports of environmental induction of fish in polychlorinated terphenyl (PCT)-contaminated environments.

PCT are present at high levels in Tabbs Creek, but other enzyme inducing xenobiotics have also been detected in the creek. Polycyclic aromatic hydrocarbons (PAH) and PCB have been reported at the upper end of the creek, near the PCT outfall. The concentration Benzo(a)pyrene was reported to be 1794 μg/kg, and several other PAH were detected at similar levels (Hale and Smith, 1988).

Materials and Methods

Male mummichogs were collected from four sites on July 11 through 14, 1994, using baited minnow traps. Three of the sites were along the Tabbs Creek PCT gradient (TA, TB and TC [Figure 26]). TA was the site closest to the PCT outfall, as

Figure 26. Map of sites in Tabbs Creek sampled for
environmental induction study.
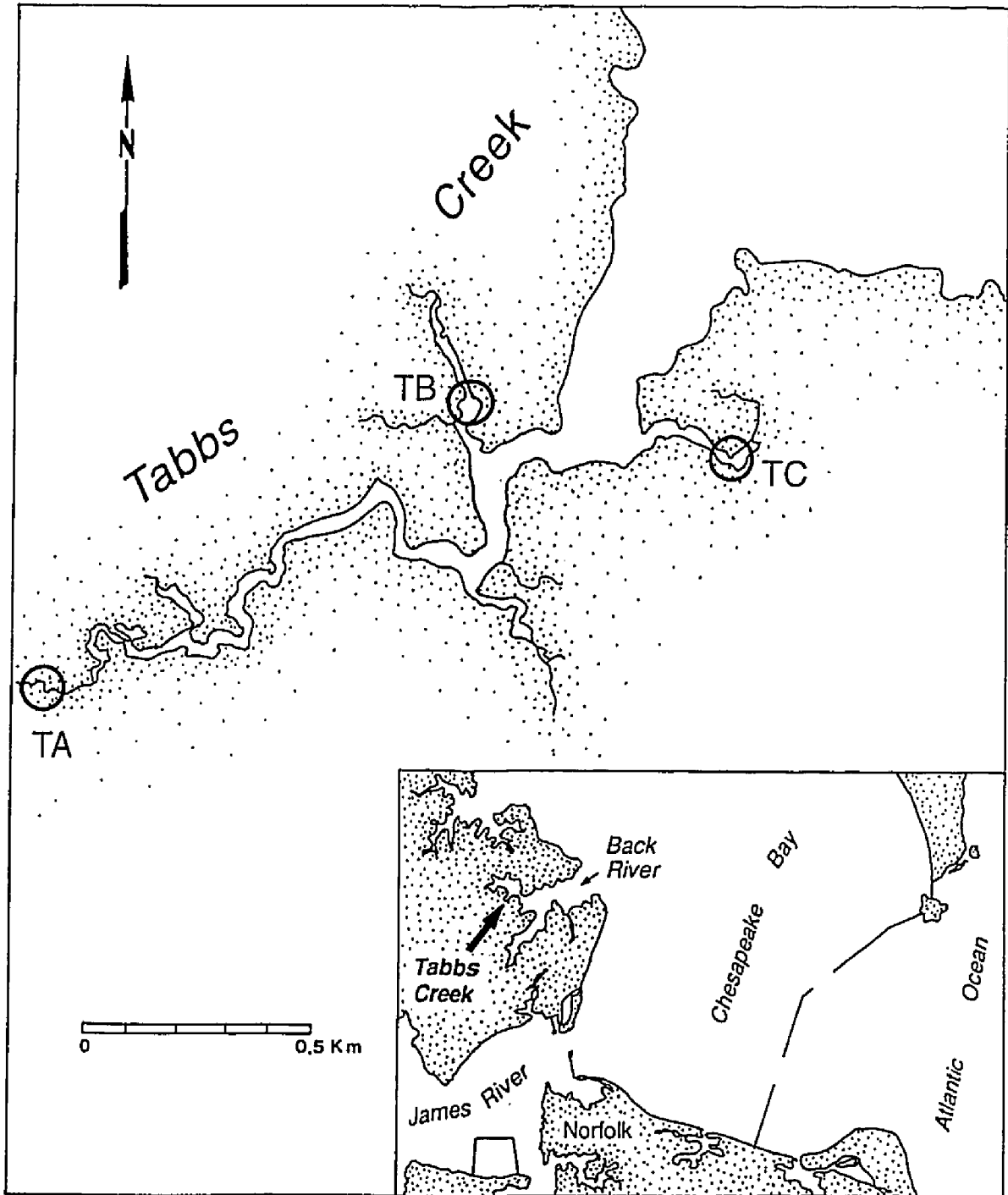
described in Chapter II. The collection of fish from TA was made at and upstream of the collection for the TC1 site of Chapter II. Site TB was near TC3 of Chapter II, but was slightly off the main creek channel. TC was approximately 0.5 km up a creek tributary that branched from the main creek between sites TC3 and TC4 of the monitoring study described in Chapter II. The fourth site was a control site, Kings Creek, which is located outside of the Back River basin. Kings Creek was the collection site for uninduced fish used in the induction study of Chapter III.

The Tabbs Creek fish were dissected in the field. Livers were removed, rinsed with an ice-cold KCl solution and placed in liquid nitrogen. Kings Creek fish were transported back to the lab, due to the proximity of the site to the lab, and were treated similarly. All samples were transferred to a -80C freezer and held there until analysis (within 1 month).

Samples were analyzed by the methods described in Chapter III, except that two fish were composited per replicate. There was difficulty in collecting larger mummichogs, particularly at TA. Because Edwards (1988) found no significant relationship between P450 or EROD and liver or body weight in male fish, size differential should have no significant effect on results.

Results

Mean CYP1A equivalents at TA, TB, and TC were 0.064, 0.063, and 0.035 pmol/μg (n=12). Mean CYP1A was 0.019 at the control site (n=12). CYP1A levels in fish collected at the sites nearest the PCT outfall, TA and TB, were significantly greater than levels in fish collected at site TC, and at the control site (p=0.05) (Figure 27). CYP1A concentrations were not significantly different between sites TA, at the outfall, and site TB, several river kilometers downstream from the outfall. Mean CYP1A equivalents in fish from TA and TB were elevated approximately three-fold over the Kings Creek control levels.

The mean EROD activities at sites TA, TB and TC were 920, 1370, and 1120, respectively (n=12). The mean EROD of the Kings Creek control was 890 (n=12). Only the EROD activity at TB was significantly different from the Kings Creek control activity (Fig. 28). The mean EROD levels in fish collected from TA were the lowest in Tabbs Creek, despite the fact that the mean CYP1A protein levels were highest at this site, and that CYP1A is the source of EROD activity. The low mean EROD activity of the TA fish indicates an inhibition of enzyme activity in fish collected near the outfall.

The graph of turnover number (activity/pmol CYP1A) illustrates a decrease in catalytic efficiency of CYP1A in Tabbs Creek fish with a decrease in distance from the PCT outfall, near TA (Fig. 29). This decrease in catalytic

Figure 27. CYP1A levels in mummichogs collected from Tabbs
Creek.

Error bars indicate standard error. * Denotes
statistical significance in ANOVA and subsequent
Tukey multiple comparison test (p=0.05),
in which data were log transformed.

CYP1A Levels in
Tabbs Creek Mummichogs
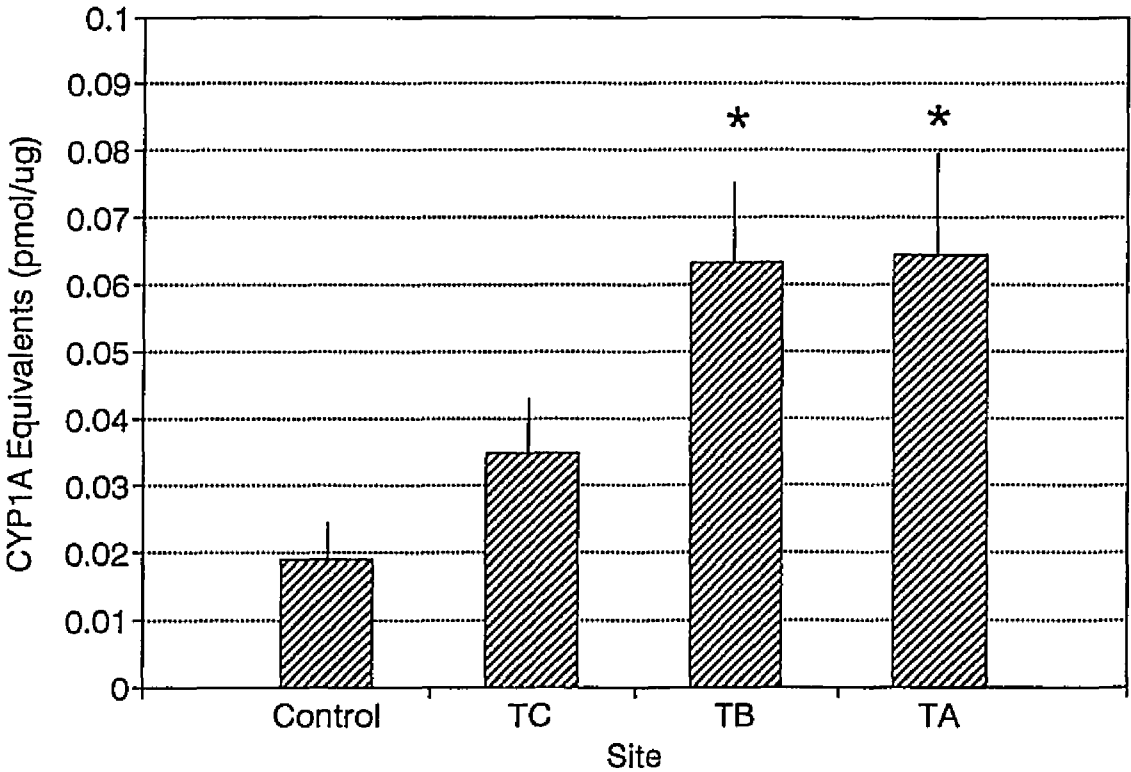
Figure 28. EROD activity in mummichogs collected from Tabbs
          Creek.

          Error bars indicate standard error. * Denotes

          statistical significance in ANOVA and subsequent

          Tukey multiple comparison test (p=0.05),

          in which data were log transformed.

EROD Activity in
Tabbs Creek Mummichogs
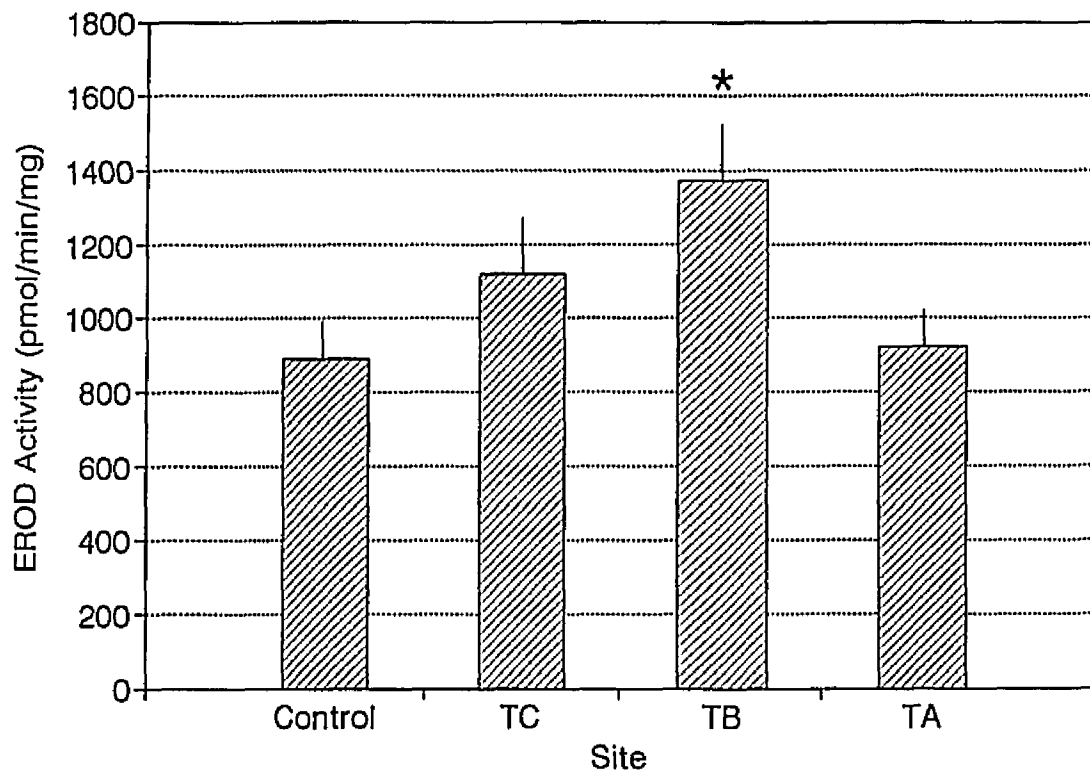
Figure 29. Catalytic efficiency of mummichogs collected from
Tabbs Creek.

Error bars indicate standard error. * Denotes
statistical significance in ANOVA and subsequent
Tukey multiple comparison test (p=0.05),
in which data were log transformed.

Catalytic Efficiency
Tabbs Creek Mummichogs
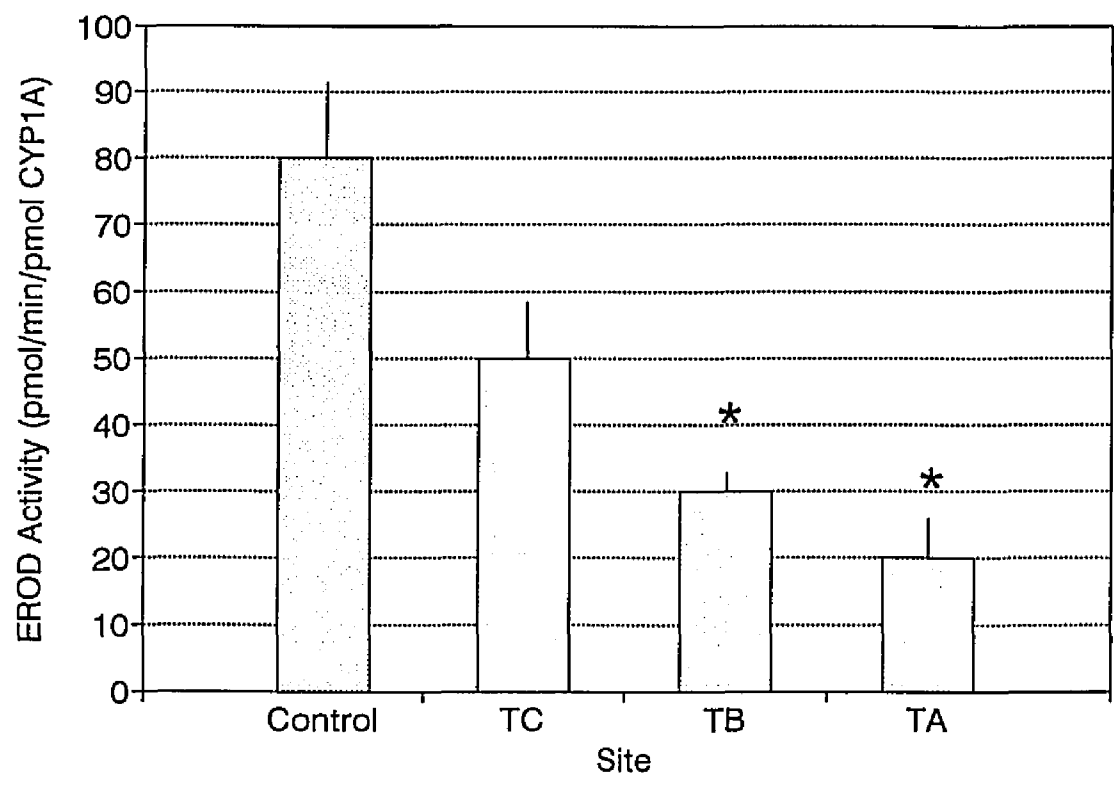
efficiency with increasing proximity to the outfall illustrates enzyme inhibition at TA. The catalytic efficiencies of TA and TB fish were significantly different from that of TC and Kings Creek control fish.

Appendix II contains data tables of CYP1A and EROD and catalytic efficiency reported in this chapter.

Discussion

Environmental induction of CYP1A was observed along a gradient of PCT contamination within Tabbs Creek. Despite the fact that PCT is present at high concentrations within the creek, and that induction by PCT was demonstrated in the laboratory, the induction observed in mummichogs collected from Tabbs Creek can not be attributed solely to the PCT contamination within Tabbs Creek. As noted earlier, other known enzyme inducers have been detected in Tabbs Creek, including PAH (eg. benzo(a)pyrene) and PCB (Hale and Smith, 1988).

There may be several factors contributing to the large coefficient of variation within the sites, including the natural variability of CYP1A and EROD levels between fish. The demonstrated heterogeneity of PCT distribution in Tabbs Creek sediments within a site, and, therefore, of exposure of the fish, may also have contributed to CYP1A variability. The variability of PCT levels in a small area was illustrated by

the large intrasite standard deviation reported in Chapter II. The movement of fish within in a larger area, away from the site, may have caused additional variability in exposure to inducers.

Inhibition of enzyme activity has been reported in both laboratory and field studies. A decrease in EROD activity was detected in rainbow trout (*Oncorhynchus mykiss*) and carp exposed to relatively high doses (100 mg/kg) of Aroclor 1254 (Melancon and Lech, 1983). An inhibition of EROD activity in fish cells exposed to 0.1 mg/kg 3,3′,4,4′-tetrachlorobiphenyl (BZ #77) has also been reported (Hahn *et al.*, 1993) These authors found that P4501A levels continued to increase at doses inhibitory to EROD activity. Whether this dose-dependent decrease in catalytic efficiency of P4501A was due to binding of inducing agents to the P450 catalytic site or other factors had not been determined. Gooch *et al.* (1989) reported decreased EROD activity at high doses of BZ 77, and found the decreased induction to be due to competitive inhibition by this PCB congener.

Monosson and Stegeman (1991) reported environmental inhibition of CYP1A in winter flounder exposed to PCB, and possibly PAH, in Narragansett Bay, as demonstrated by a decrease in EROD with an increase in CYP1A levels, and consequently a decrease in turnover number. In contrast, Elskus and Stegeman (1989) found no decrease in catalytic efficiency, based on total P450 levels, in mummichogs

environmentally exposed to PCB and PAH, despite an 1000-fold increase in total PCB and 60-fold increase in total PAH at the contaminated site relative to the control site. The authors noted that the contaminated site, Seekonk River, Rhode Island, is among the most contaminated sites in the coastal US, according to a NOAA study conducted in the mid-1980's. The inhibition of enzyme activity in fish collected at TA may have been due to a reduction in the functioning of other components of the MO system, or, more likely, to competitive inhibition of EROD activity by PCT or other inducers remaining in the microsomal fraction, or to other factors.

The observed environmental induction in fish collected from Tabbs Creek is a cause for concern, indicating a potential compromise in the environmental fitness of organisms in the creek. Induction and inhibition of MO activity may affect the ability of the fish to enzymatically respond to additional contaminant inputs, and may signal the development of Ah receptor-mediated toxicity. Tabbs Creek is currently undergoing remedial investigation under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), commonly referred to as the Environmental Protection Agency Superfund. The results of this study contributed to the evaluation of potential options for remediation under CERCLA at Tabbs Creek. The work described herein demonstrates the negative ecological impact PCT may be having on organisms within Tabbs Creek.

Chapter VI

Conclusions

Aroclor 5432, a mixture of polychlorinated terphenyls (PCT), was detected in several biological species collected from Tabbs Creek: saltmarsh cordgrass (*Spartina alterniflora*), American oysters (*Crassostrea virginica*), red-jointed fiddler crabs (*Uca minax*), wharf crabs (*Sesarma reticulatum*) and mummichogs (*Fundulus heteroclitus*). This tidal creek, located in the southern Chesapeake Bay region, contains high sediment concentrations of PCT. Species from several phyla were analyzed in order to examine PCT bioavailability and accumulation in physiologically and ecologically different organisms. PCT concentrations in sediment, saltmarsh cordgrass, native oysters, and fiddler crabs decreased with distance downstream from the PCT outfall. Residues in transplanted oysters and mummichogs showed a more variable trend with distance downstream. The organism with the highest mean concentration (18,300 μg/kg dry weight) was the native oyster, a benthic filter feeder. This work confirms the bioavailability of PCT to a wide variety of aquatic organisms.

Mummichogs injected with Aroclor 5432 exhibited significantly elevated levels of hepatic cytochrome P4501A and associated EROD activity. Induction resulting from Aroclor 5432 of the same order of magnitude as that caused by

equivalent doses of Aroclor 1254. Treatment with Aroclor 5460 did not result in significant induction. This work represents the first report of hepatic cytochrome P4501A induction caused by Aroclor 5432 in teleosts and, similar to work in mammalian systems, suggests that the effects of this mixture may be mediated through Ah receptor binding.

Because commercial PCT mixtures contain small amounts of PCB, PCB components may have contributed to the induction observed for Aroclor 5432. The planar PCB congener 3,3',4,4'-tetrachlorobiphenyl (BZ 77) was detected in an Aroclor 5432 standard by GC-MS operating in NCI mode. Gooch et al. (1989) found that a 0.1 mg/kg body weight dose of BZ 77 caused EROD induction, with EROD activities greater than 11-fold of control activities. Melancon and Lech (1983) reported enzyme induction in fish dosed with 0.01 mg/kg BZ 77. Therefore, the presence of BZ 77 in Aroclor 5432 may have contributed to the elevated P4501A and EROD levels observed in the study described in Chapter IV. From the studies described herein, it is not possible to determine if the PCT themselves are causing the observed MO induction, or if the induction is a result of other components in the mixture, or a combination of components.

Due to the number and complexity of compounds in commercial PCT mixtures, the resolution of PCT congeners from each other and from PCB components of the mixtures is extremely difficult to produce, and has not, to date, been

attained. The use of a column partially coated with C18 resolved the PCB congeners examined, but resulted in coelution of BZ 77 with PCT congeners in Aroclor 5432. Column bleed from the C18 column degraded the MS source, preventing the generation of a reproducible calibration. The use of multi-dimensional GC might make the quantitation of PCB congeners in PCT mixtures possible.

Environmental induction was observed in mummichogs collected from PCT contaminated Tabbs Creek. This study revealed CYP1A induction at the two most contaminated sites along a PCT gradient. Fish at the upper creek site also exhibited inhibition of EROD activity. Definitive environmental induction by PCT cannot be established due to the presence of other enzyme inducing pollutants in Tabbs Creek.

Appendix I

CYP1A and EROD Levels in Mummichogs

Injected with Aroclor 1254, 5432 or 5460

Aroclor 1254
CYP1A and EROD Levels in Mummichogs Following
Aroclor 1254 Injection. Data Table for Chapter IV.

Sample Name	Dose (mg/kg)	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/µg)	Mean CYP1A (SD)
1254 A	100	9	1640	1230 (240)	0.2031	0.1323 (0.0511)
1254 B	100	17	1160		0.1303	
1254 C	100	20.B	1130		0.0589	
1254 D	100	38.B	990		0.1371	
1254 A	32	38	1010	700 (220)	0.1361	0.0721 (0.0454)
1254 B	32	11	520		0.0371	
1254 C	32	16.B	590		0.0430	
1254 D	32	19.B	---		---	
1254 A	10	24	330	450 (80)	0.0307	0.0249 (0.0062)
1254 B	10	33	500		0.0182	
1254 C	10	39.B	440		0.0314	
1254 D	10	49.B	530		0.0191	
1254 A	3.2	35	---	320 (30)	---	0.0136 (0.0027)
1254 B	3.2	3	320		0.0174	
1254 C	3.2	33.B	360		0.0110	
1254 D	3.2	31.B	280		0.0124	

Aroclor 1254
CYP1A and EROD Levels in Mummichogs Following
Aroclor 1254 Injection. Data Table for Chapter IV.

Sample Name	Dose (mg/kg)	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/μg)	Mean CYP1A (SD)
1254 A	1.0	43	530	410 (70)	0.0338	0.0218 (0.0096)
1254 B	1.0	13	380		0.0253	
1254 C	1.0	9.B	350		0.0071	
1254 D	1.0	43.B	390		0.0211	
1254 A	0.32	22	530	420 (130)	0.0174	0.0191 (0.0108)
1254 B	0.32	31	190		0.0046	
1254 C	0.32	8.B	480		0.0195	
1254 D	0.32	42.B	470		0.0351	
1254 A	0.1	27	590	380 (230)	---	Not Applicable
1254 B	0.1	32	170		---	
1254 C	0.1	14.B	130		---	
1254 D	0.1	22.B	630		---	

SD denotes standard deviation. Dashes mark samples lost, unanalyzed or of insufficient quantity for analysis.

Aroclor 5432

CYP1A and EROD Levels in Mummichogs Following
Aroclor 5432 Injection. Data Table for Chapter IV.

Sample Name	Dose (mg/kg)	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/μg)	Mean CYP1A (SD)
5432 A	100	4	3040	1530	0.2935	0.1677
5432 B	100	42	1250	(890)	0.1425	(0.0750)
5432 C	100	37.B	720		0.0959	
5432 D	100	18.B	1120		0.1387	
5432 A	32	45	1130	1270	0.2722	0.1338
5432 B	32	34	---	(330)	0.0941	(0.0850)
5432 C	32	23.B	1730		0.1248	
5432 D	32	7.B	950		0.0441	
5432 A	10	10	520	540	0.0350	0.0214
5432 B	10	28	820	(220)	0.0334	(0.0129)
5432 C	10	30.B	---		0.0112	
5432 D	10	10.B	290		0.0062	
5432 A	3.2	23	530	560	0.0216	0.0204
5432 B	3.2	27.B	590	(20)	0.0201	(0.0020)
5432 C	3.2	12.B	580		0.0226	
5432 D	3.2	3.B	540		0.0175	

Aroclor 5432

CYP1A and EROD Levels in Mummichogs Following
Aroclor 5432 Injection. Data Table for Chapter IV.

Sample Name	Dose (mg/kg)	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/µg)	Mean CYP1A (SD)
5432 A	1.0	7	100	320 (190)	0.0070	0.0124 (0.0051)
5432 B	1.0	26	240		0.0192	
5432 C	1.0	24.B	320		0.0111	
5432 D	1.0	45.B	630		---	
5432 A	0.32	6	140	360 (240)	0.0064	0.0113 (0.0083)
5432 B	0.32	30	450		0.0094	
5432 C	0.32	6.B	710		0.0254	
5432 D	0.32	41.B	150		0.0041	
5432 A	0.1	46	430	330 (140)	---	Not Applicable
5432 B	0.1	14	100		---	
5432 C	0.1	32.B	340		---	
5432 D	0.1	4.B	470		---	

SD denotes standard deviation. Dashes mark samples lost, unanalyzed or of insufficient quantity to analyze.

Aroclor 5460
CYP1A and EROD in Mummichogs Following
Aroclor 5460 Injection. Data Table for Chapter IV.

Sample Name	Dose (mg/kg)	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/µg)	Mean CYP1A (SD)
5460 A	100	15	730	530	---	0.0095
5460 B	100	29	210	(190)	0.0022	(0.0052)
5460 C	100	25.B	620		0.0126	
5460 D	100	36.B	560		0.0137	
5460 A	32	2	<30	500	---	0.0108
5460 B	32	25	540	(280)	0.0083	(0.0020)
5460 C	32	47.B	710		0.0131	
5460 D	32	15.B	730		0.0110	
5460 A	10	5	200	220	0.0022	0.0046
5460 B	10	16	90	(120)	0.0068	(0.0018)
5460 C	10	34.B	420		0.0048	
5460 D	10	11.B	190		---	
5460 A	3.2	41	630	380	0.0108	0.0061
5460 B	3.2	8	530	(210)	0.0054	(0.0035)
5460 C	3.2	13.B	200		---	
5460 D	3.2	48.B	140		0.0022	

Aroclor 5460
CYP1A and EROD in Mummichogs Following
Aroclor 5460 Injection. Data Table for Chapter IV.

Sample Name	Dose (mg/kg)	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/µg)	Mean CYP1A (SD)
5460 A	1.0	29.B	---	340	---	Not Applicable
5460 B	1.0	40.B	240	(300)	---	
5460 C	1.0	21.B	<30		---	
5460 D	1.0	26.B	750		---	
5460 A	0.32	37	220	240	---	Not Applicable
5460 B	0.32	1	190	(70)	---	
5460 C	0.32	17.B	200		---	
5460 D	0.32	44.B	370		---	
5460 A	0.1	21	440	240	---	Not Applicable
5460 B	0.1	36	<30	(170)	---	
5460 C	0.1	1.B	250		---	
5460 D	0.1	5.B	---		---	

SD denotes standard deviation. Dashes mark samples below lowest calibration standard, lost, unanalyzed or of insufficient quantity to analyze.

Controls

CYP1A and EROD in Corn Oil and Uninjected Mummichogs.
Data Table for Chapter IV

Sample Name	Tank No.	EROD Activity (pmol/min/mg)	Mean EROD (SD)	CYP1A (pmol/μg)	Mean CYP1A (SD)
Corn Oil A	19	320		0.0220	
Corn Oil B	39	160	160	0.0039	0.0100
Corn Oil C	35.B	40	(100)	0.0018	(0.0080)
Corn Oil D	2.B	130		0.0121	
No Injection A	12	---		---	
No Injection B	40	300	270	0.0401	0.0218
No Injection C	46.B	80	(150)	---	
No Injection D	28.B	440		0.0035	

SD Denotes standard deviation. Dashes mark samples lost, unalayzed or
of insufficient quantity to analyze.

Appendix II

CYP1A and EROD Levels in Fish Collected from Tabbs Creek

for Environmental Induction Study

CYP1A Levels, EROD Activity and Catalytic Efficiency in Mummichogs
Collected at Tabbs Creek Site TA.

Sample	EROD (pmol/min/mg)	CYP1A Equivalents (pmol/ug)	Catalytic Efficiency (pmol/min/pmol CYP1A)
TA1	1320	0.1679	10
TA2	1490	0.1596	10
TA3	420	0.0414	10
TA4	470	0.0117	40
TA5	920	0.0485	20
TA6	1070	0.089	10
TA7	640	0.0091	70
TA8	790	0.0281	30
TA9	920	0.0534	20
TA10	1340	0.0967	10
TA11	710	0.0265	30
TA12	950	0.0420	20
Mean	920	0.0645	20
Sample SD	340	0.0534	20
CV	37 %	83 %	100

Note: CV is coefficient of variation

CYP1A Levels, EROD Activity and Catalytic Efficiency in Mummichogs
Collected at Tabbs Creek Site TB.

Sample	EROD (pmol/min/mg)	CYP1A Equivalents (pmol/ug)	Catalytic Efficiency (pmol/min/pmol CYP1A)
TB1	880	0.0291	30
TB2	650	0.0156	40
TB3	1370	0.0992	10
TB4	1830	0.1160	20
TB5	1290	0.0521	20
TB6	2290	0.1224	20
TB7	1270	0.0604	20
TB8	760	0.0138	60
TB9	2170	0.1112	20
TB10	1600	0.0800	20
TB11	1240	0.0278	40
TB12	1110	0.0324	30
Mean	1370	0.0633	30
Sample SD	520	0.0409	10
CV	38 %	65 %	33%

Note: CV is coefficient of variation.

CYP1A Levels, EROD Activity and Catalytic Efficiency in Mummichogs
Collected at Tabbs Creek Site TC.

Sample	EROD (pmol/min/mg)	CYP1A Equivalents (pmol/ug)	Catalytic Efficiency (pmol/min/pmol CYP1A)
TC1	640	0.0235	30
TC2	730	0.0138	50
TC3	150	0.0029	50
TC4	1070	0.0419	30
TC5	900	0.0079	110
TC6	1480	0.0306	50
TC7	930	0.0114	80
TC8	1260	0.0362	30
TC9	2140	0.1062	20
TC10	1580	0.0566	30
TC11	1240	0.0382	30
TC12	1300	0.0485	30
Mean	1120	0.0348	(45=) 50
Sample SD	510	0.0281	30
CV	46 %	81 %	50

Note: CV is coefficient of variation.

CYP1A Levels, EROD Activity and Catalytic Efficiency in Mummichogs
Collected at the Kings Creek Control Site.

Sample	EROD (pmol/min/mg)	CYP1A Equivalents (pmol/ug)	Catalytic Efficiency (pmol/min/pmol CYP1A)
K1	500	0.0045	110
K2	330	0.0047	70
K3	670	0.0054	120
K4	1010	0.0299	30
K5	710	0.0076	90
K6	690	0.0062	110
K7	800	0.0056	140
K8	780	0.0060	130
K9	1250	0.0324	40
K10	1190	0.0348	30
K11	1150	0.0242	50
K12	1570	0.0669	20
Mean	890	0.0190	80
Sample SD	350	0.0193	40
CV	39 %	102 %	50%

Note: CV is the coefficient of variation.

LITERATURE CITED

Addison, R.F., D.E. Willis and M.E. Zinck. 1994. Liver microsomal mono-oxygenase induction in winter flounder (*Pseudopleuronectes americanus*) from a gradient of sediment PAH concentrations at Sydney Harbour, Nova Scotia. *Mar. Environ. Res.* 37:283-296.

Addison, R.F. and A.J. Edwards. 1988. Hepatic microsomal mono-oxygenase activity in flounder *Platichthys flesus* from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper. *Mar. Ecol. Prog. Ser.* 46:51-54.

Addison, R.F., M.E. Zinck and D.E. Willis. 1978. Induction of hepatic mixed-function oxidase (MFO) enzymes in trout (*Salvelinus fontinalis*) by feeding Aroclor 1254 or 3-methylcholanthrene. *Comp. Biochem. Physiol.* C 61:323-325.

Allen J.R., and D.H. Norback. 1973. Polychlorinated biphenyl- and triphenyl-induced gastric mucosal hyperplasia in primates. *Science* 179:498-499.

Andrews, J.D. 1982. Anaerobic mortalities of oysters in Virginia caused by low salinities. *J. Shellfish Res.* 2(2):127-132.

Ankley, G.T., V.S. Blazer, R.E. Reinert and M. Agosin. 1986. Effects of Aroclor 1254 on cytochrome P-450-dependent monooxygenase, glutathione s-transferase, and UDP-glucuronosyltransferase activities in channel catfish liver. *Aquat. Toxicol.* 9:91-103.

Bend, J.R. and M.O. James. 1978. Xenobiotic metabolism in marine and freshwater species, In *Biochemical and Biophysical Perspectives in Marine Biology* (D.C. Malins and J.R. Sargent, Eds.) pp. 125-188. Academic Press, NY.

Black, S.D. and M.J. Coon. 1987. P-450 cytochromes: structure and function. *Advances in Enzymal and Related Areas of Molecular Biology* 60:35-87.

Bombick, D.W., J. Jankun, K. Tullis and F. Matsumura. 1988. 2,3,7,8-tetrachlorodibenzo-p-dioxin causes increases in expression of c-erb-A and levels of protein-tyrosine kinases in selected tissues of responsive mouse strains. *Proc. Natl. Acad. Sci.* 85:4128-4132.

Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.

Bursey, C.R. 1982. Factors influencing the distribution and co-occurrence of *Sesarma reticulatum* (Decapoda:Grapsidae) and *Uca pugnax* (Decapoda:Ocypodidae) in a Virginia salt marsh. *Am. Zool.* 22:950.

Butner, A. and B.H. Brattstrom. 1960. Local movement in Menidia and *Fundulus*. *Copeia* 1960(2):139-141.

Code of Federal Regulations. 1984. Volume 40, Part 704. See also *Federal Register* 49(59):11181-11184.

Code of Federal Regulations. 1988. Volume 21, Part 109, pp. 93-94.

Cooke, M., N. Graham, A.M. Prescott and D.J. Roberts. 1978. Analysis of polychlorinated napthalenes, polychlorinated biphenyls and polychlorinated terphenyls via carbon skeleton chromatography. *J. Chromatogr.* 156:293-299.

Dalh, A.R., W.M. Hadley, F.F. Hahn, J.M. Benson and R.O. McClellan. 1982. Cytochrome P-450-dependent monooxygenases in olfactory epithelium of dogs: possible role in tumorogenicity. *Science* 216:57-59.

De Kok, A., R.B. Geerdink, G. De Vries and U.A.Th. Brinkman. 1982. An evaluation of chromatographic methods for the analysis of polychlorinated terphenyls in environmental samples. *Int. J. Environ. Anal. Chem.* 12:99-122.

Denison, M.S., J.M. Fisher and J.P. Whitlock, Jr. 1989. Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. *J. Biol. Chem.* 264(28):16478-16482.

Doguchi, M. and S. Fukano. 1975. Residue levels of polychlorinated terphenyls, polychlorinated biphenyls and DDT in human blood. *Bull. Environ. Contam. Toxicol.* 13(1):57-63.

Duinker, J.C., D.E. Schulz and G. Petrick. 1988. Multidimensional gas chromatography with electron capture detection for the determination of toxic congeners in polychlorinated biphenyl mixtures. *Anal. Chem.* 60:478-482.

Edwards, A.J., R.F. Addison, D.E. Willis amd K.W. Renton. 1988. Seasonal variation of hepatic mixed function oxidases in winter flounder (*Pseudopleuronectes americanus*). *Mar. Environ. Res.* 26:299-309.

Elskus, A.A. and J.J. Stegeman. 1989. Induced cytochrome P-450 in *Fundulus heteroclitus* associated with environmental contamination by polychlorinated biphenyls and polynuclear aromatic hydrocarbons. *Mar. Environ. Res.* 27:31-50.

Freudenthal, J. and P.A. Greve. 1973. Polychlorinated terphenyls in the environment. *Bull. Environ. Contam. Toxicol.* 10(2):108-111.

Fries, G.F. and G.S. Marrow. 1973. Polychlorinated terphenyls as potential contaminants of animal products. *J. Assoc. Offic. Anal. Chem.* 56:1002-1007.

Gooch, J.W., A.A. Elskus, P.J. Kloepper-Sams, M.E. Hahn and J.J. Stegeman. 1989. Effects of *ortho-* and non-*ortho-* substituted polychlorinated biphenyl congeners on the hepatic monooxygenase system in scup (*Stenotomus chrysops*). *Toxicol. Appl. Pharmacol.* 98:422-433.

Gray, E.H. 1942. Ecological and life history aspects of the red-jointed fiddler crab *Uca minax* (Le Conte), region Solomon's Island, Maryland. *Ches. Biol. Lab. Publ.* 51.

Gruger, E.H., M.M. Wekall, P.T. Numoto and D.R. Craddock. 1977. Induction of hepatic aryl hydrocarbon hydroxylase in salmon exposed to petroleum dissolved in seawater and to petroleum and polychlorinated biphenyls, separate and together, in food. *Bull. Environ. Contam. Toxicol.* 17:512-520.

Gulley, D.D., A.M. Boelter and H.L. Bergman. *Toxstat®, version 3.2,* University of Wyoming, Laramie, WY.

Hahn, M.E., T.M. Lamb, M.E. Schultz, R.M. Smolowitz and J.J. Stegeman. 1993. Cytochrome P4501A induction and inhibition by 3,3′,4,4′-tetrachlorobiphenyl in an Ah receptor-containing fish hepatoma cell line (PLHC-1). *Aq. Toxicol.* 26:185-208.

Hahn, M.E., A. Poland, E. Glover and J.J.Stegeman. 1992. The Ah receptor in marine animals: Phylogenetic distribution and relationship to cytochrome P4501A inducibility. *Mar. Environ. Res.* 34:87-92.

Hale, R.C., J. Greaves, G.G. Vadas, E. Harvey, and K. Gallagher. 1991a. Distribution of polychlorinated terphenyls in tributaries of the southern Chesapeake Bay, In *Aquatic Toxicology and Risk Assessment: Fourteenth Volume, ASTM STP 1124* (M.A. Mayes and M.G. Barron, Eds.) pp. 305-312. American Society for Testing and Materials, Philadelphia, PA.

Hale, R.C., E. Bush, K. Gallagher, J.L. Gundersen, and R.F. Mothershead II. 1991b. Separation of polychlorinated terphenyls from lipoidal material by preparative gel permeation chromatography and gas chromatography. *J. Chromat.* 539:149-156.

Hale, R.C., J. Greaves, K. Gallagher, and G.G. Vadas. 1990. Novel chlorinated terphenyls in sediment and shellfish of an estuarine environment. *Environ. Sci. Technol.* 24:1727-1731.

Hale, R.C. and C.L. Smith. 1988. Analysis of Effluents and Associated Sediments and Tissue for Toxic Organic Compounds 1987-1988. Final Report to State Water Control Board.

Hamelink J.L., R.C. Waybrant and R.C. Ball. 1971. A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. *Trans. Am. Fish. Soc.* 100(2):207-214.

Hassell, K.D., and D.C. Holmes, D.C. 1977. Polychlorinated terphenyls (PCT) in some British birds. *Bull. Environ. Contam. Toxicol.* 17(5):618-621.

Heilmann, L.J., Y.-Y Sheen, S.W. Bigelow and D.W. Nebert. 1988. The trout P450IA1 gene: cDNA and deducted protein sequence, expression in liver, and evolutionary significance. *DNA* 7:379-385.

Higgins, P.J. 1980. Effects of food availability on the valve movements and feeding behavior of juvenile *Crassostrea virginica* (Gmelin). I. Valve movements and periodic activity. *J. Exp. Mar. Biol. Ecol.* 45:229-244.

Hill, D.W., E. Hejtmancik and B.J.Camp. 1976. Induction of hepatic microsomal enzymes by Aroclor 1254 in *Ictalurus punctatus* (Channel Catfish). *Bull. Environ. Contam. Toxicol.* 16:495-502.

Hughes, E.H. and E.B. Scherr. 1983. Subtidal food webs in a Georgia estuary: $\delta^{13}C$ Analysis. *J. Exp. Mar. Biol. Ecol.* 67(3):227-242.

Israel, D.I. and J.P. Whitlock, Jr. 1983. Induction of mRNA specific for cytochrome P_1-450 in wild type and variant mouse hepatoma cells. *J. Biol. Chem.* 258(17):10390-10394.

Jamieson, J.W.S. 1977. Polychlorinated terphenyls in the environment. Report for the Environmental Protection Service, Canada. Report EPS-3-EC-77-22.

Jensen, A.A. and K.J. Jørgensen. 1983. Polychlorinated terphenyls (PCTs) use, levels, and biological effects. *Sci. Tot. Environ.* 27:231-250.

Jones, P.B.C., D.R. Galeazzi, J.M. Fisher and J.P. Whitlock, Jr. 1985. Control of cytochrome P_1-450 gene expression by dioxin. *Science* 227:1499-1502.

Kannan, N., S. Tanabe, T. Wakimoto and R. Tatsukawa. 1987. Coplanar polychlorinated biphenyls in Aroclor and Kanechlor mixtures. *J. Assoc. Off. Anal. Chem.* 70(3):451-454.

Kloepper-Sams, P.J., S.S. Park, H.V. Gelboin and J.J. Stegeman. 1987. Specificity and cross-reactivity of monoclonal and polyclonal antibodies against cytochrome P-450E of the marine fish scup. *Arch. Biochem. Biophys.* 253:268-279.

Klotz, A.V., J.J. Stegeman and C. Walsh. 1983. An aryl hydrocarbon hydroxylating hepatic cytochrome P-450 from the marine fish (*Stenotomus chrysops*). *Arch. Biochem. Biophys.* 226:578-592.

Klotz, A.V., J.J. Stegeman and C. Walsh. 1984. An alternative 7-ethoxyresorufin *O*-deethylase activity assay: A continuous visible spectrophotometric method for measurement of cytochrome P-450 monooxygenase activity. *Anal. Biochem.* 140:138-145.

Kneib, R.T., A.E. Stiven and E.B. Haines. 1980. Stable carbon isotope ratios in *Fundulus heteroclitus* (L.) muscle tissue and gut contents from a North Carolina *Spartina* marsh. *J. Exp. Mar. Biol. Ecol.* 46(1):89-98.

Landers, J.P. and N.J. Bunce. 1991. Review article: the Ah receptor and the mechanism of dioxin toxicity. *Biochem. J.* 276:273-287.

Landfair, S.W. 1995. Toxic Substances Control Act. In *Environmental Law Handbook* (T.F.P. Sullivan, Ed.) pp. 353-408. Government Institutes, Inc., Rockville, MD.

Lech, J.J., M.J. Vodicnik, C.R. Elcombe. 1982. Induction of monooxygenase activity in fish. In *Aquatic Toxicology* (L.J. Weber, Ed.) pp. 107-148. Raven Press, NY.

Lotrich, V.A. 1975. Summer home range and movements of *Fundulus heteroclitus* (Pisces:Cyprinodontidae) in a tidal creek. *Ecology* 56(1):191-198.

Macek, K.J. and S. Korn. 1970. Significance of the food chain in DDT accumulation by fish. *J. Fish. Res. Board Can.* 27(8):1496-1498.

Madhukar, B.V., D.W. Brewster and F. Matsumura. 1984. Effects of *in vivo*-administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster. *Proc. Natl. Acad. Sci.* 81:7407-7411.

Maurer, D. and L. Watling. 1973. Studies on the oyster community in Delaware: the effects of the estuarine environment on the associated fauna. *Intern. Rev. Gesamten. Hydrobiol.* 58(2):161-201.

Melancon, M.J., C.R. Elcombe, M.J. Vodicnik and J.J. Lech. 1981. Induction of cytochromes P450 and mixed-function oxidase activity by polychlorinated biphenyls and β-napthoflavone in carp (*Cyprinus Carpio*). *Comp. Biochem. Physiol.* C69:219-226.

Melancon, M.J. and J.J. Lech. 1983. Dose-effect relationship for induction of hepatic monooxygenase activity in rainbow trout and carp by Aroclor 1254. *Aquat. Toxicol.* 4:51-61.

Monosson, E. and J.J. Stegeman. 1991. Cytochrome P450E (P4501A) induction and inhibition in winter flounder by 3,3',4,4'-tetrachlorobiphenyl: comparison of response in fish from Georges Bank and Narragansett Bay. *Environ. Toxicol. Chem.* 10:764-774.

Montague, C.L. 1980. A natural history of temperate western Atlantic fiddler crabs (Genus *Uca*) with reference to their impact on the salt marsh. *Contr. Mar. Sci. Univ. Tex.* 23:25-55.

Moorthy, B., S. Chen, D. Li and K. Randerath. 1993. 3-methylcholanthrene-inducible liver cytochrome(s) P450 in female Sprague-Dawley rats: possible link between P450 turnover and formation of DNA adducts and I-compounds. *Carcinogenesis* 14(5):879-886.

Narbonne, J.F., J.L. Gallis. 1979. *In vivo* and *in vitro* effect of phenoclor DP6 on drug metabolizing activity in mullet liver. *Bull. Environ. Contam. Toxicol.* 23: 338-343.

Nebert, D.W. 1989. The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. *Crit. Rev. Toxicol.* 20:153-174.

Nilsen, O.G. and R. Toftgård. 1981. Effects of polychlorinated terphenyls and paraffins on rat liver microsomal cytochrome P-450 and in vitro metabolic activities. *Arch. Toxicol.* 47:1-11.

Okey, A.B., G.P. Bondy, M.E. Mason, G.F. Kahl, H.J.Eisen, T.M. Guenthner and D.W. Nebert. 1979. Regulatory gene product of the Ah locus: Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. *J. Biol. Chem.* 254:11636-11648.

Oliver, B.G. and A.J. Niimi. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environ. Sci. Technol.* 22:388-397.

Omura, T. and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* 239:2370.

Park, S.S., H. Miller, A.V. Klotz, P.J. Kloepper-Sams, J.J. Stegeman and H.V. Gelboin. 1986. Monoclonal antibodies to liver microsomal cytochrome P-450E of the marine fish *Stenotomus versicolor* (scup): Cross reactivity with 3-methylcholanthrene induced rat cytochrome P-450. *Arch. Biochem. Biophys.* 249:339-349.

Parkinson, A., R. Cockerline and S. Safe. 1980. Polychlorinated biphenyl isomers and congeners as inducers of both 3-Methylcholanthrene- and Phenobarbitone-type microsomal enzyme activity. *Chem.-Biol. Interact.* 29:277-289.

Poland, A. and J.C. Knutson. 1982. 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* 22:517-554.

Poland, A. and E. Glover. 1980. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: segregation of toxicity with the Ah locus. *Mol. Pharmacol.* 17:86-94.

Poland, A. and E. Glover. 1977. Chlorinated biphenyl induction of aryl hydrocarbon hydroxylase activity: a study of the structure-activity relationship. *Molec. Pharmacol.* 13: 924-938.

Prinslow, T.E., I. Valiela, and J.M. Teal. 1974. The effect of detritus and ration size on the growth of *Fundulus heteroclitus* (L.). *J. Exp. Mar. Biol. Ecol.* 16(1):1-10.

Rasmussen, J.B., D.J. Rowan, D.R.S. Lean, and J.H. Carey. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. *Can. J. Fish. Aquat. Sci.* 47:2030-2038.

Reidenbaugh, T.G. 1983. Productivity of cordgrass *Spartina alterniflora*, estimated from live standing crops, mortality, and leaf shedding in a Virginia salt marsh. *Estuaries* 6(1):57-65.

Renberg, L., G. Sunstrom, and L. Reutergardh. 1978. Polychlorinated terphenyls (PCT) in Swedish white-tailed eagles and in grey seals, a preliminary study. *Chemosphere* 6:477-482.

Reyes, H., S. Reisz-Porszasz and O. Hankinson. 1992. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* 256:1193-1195.

Safe, S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *CRC Crit. Rev. Toxicol.* 21:51-88.

Safe, S. 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annu. Rev. Pharmacol. Toxicol.* 26:371-399.

Schulz, D.E., G. Petrick and J.C. Duinker. 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environ. Sci. Technol.* 23:852-859.

Shaw, G.R. and D.W. Connell. 1982. Factors influencing concentrations of polychlorinated biphenyls in organisms from an estuarine ecosystem. *Aust. J. Mar. Freshw. Res.* 33:1057-1070.

Shirai, T., Y. Miyata, K. Nakanishi, G. Murasaki and N. Ito. 1978. Hepatocarcinogenicity of polychlorinated terphenyl (PCT) in ICR mice and its enhancement by hexachlorobenzene (HCB). *Cancer Lett.* 4:271-275.

Sivarajah, K., C.S. Franklin and W.P. Williams. 1978. The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. *J. Fish Biol.* 13:401-409.

Skaare, J.U., E.G. Jensen, A. Goksøyr and E. Egaas. 1991. Response of xenobiotic metabolizing enzymes of rainbow trout (*Oncorhynchus mykiss*) to the mono-*ortho* substituted polychlorinated PCB congener 2,3′,4,4′,5-pentachlorobiphenyl, PCB-118, detected by enzyme activities and immunochemical methods. *Arch. Environ. Contam. Toxicol.* 20:349-352.

Sosa-Lucero, J.C., F.A. de la Iglesia, and G.H. Thomas. 1973. Distribution of a polychlorinated terphenyl (PCT) (Aroclor 5460) in rat tissues and effect on hepatic microsomal mixed function oxidases. *Bull. Environ. Contam. Toxicol.* 10(4):248-256.

Stegeman, J.J. and M.E. Hahn. 1994. Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species, In *Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives* (D.C. Malins and G.K. Ostrander, Eds.) pp. 87-206. CRC Press, Boca Raton, FL.

Stegeman, J.J., K.W. Renton, B.R. Woodin, Y-S Zhang and R.F. Addison. 1990. Experimental and environmental induction of Cytochrome P450E in fish from Bermuda waters. *J. Exp. Mar. Biol. Ecol.* 138:49-67.

Stegeman, J.J. 1989. Cytochrome P450 forms in fish: catalytic, immunological and sequence similarities. *Xenobiotica* 19(10): 1093-1110.

Stegeman, J.J., B.R. Woodin and A. Goksøyr. 1988. Apparent cytochrome P-450 induction as an indication of exposure to environmental chemicals in the flounder, *Platichthys flesus*. *Mar. Ecol. Prog. Ser.* 46:55-60.

Stegeman, J.J., F.Y. Teng, and E.A. Snowberger. 1987. Induced cytochrome P450 in winter flounder (*Pseudopleuronectes americanus*) from coastal Massachusetts evaluated by catalytic assay and monoclonal antibody probes. *Canadian J. Fish. Aquat. Sci.* 44:1270-1277.

Toftgård, R., O.G. Nilsen, and H. Glaumann. 1980. Polychlorinated terphenyls are mixed type inducers of rat liver microsomal cytochrome P-450. In *Biochemistry, Biophysics, and Regulation of Cytochrome P-450* (J.-Å. Gustafsson, J. Carlstedt-Duke, A. Mode, J. Rafter, Eds.) pp. 227-230. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands.

Truitt, R.V. 1931. The oyster and the oyster industry of Maryland. *Ches. Biol. Lab. Publ.* 4.

Valiela, I., J.E. Wright, J.M. Teal, and S.B. Volkmann. 1977. Growth, production and energy transformations in the salt-marsh killifish *Fundulus heteroclitus*. *Mar. Biol.* 40(2):135-144.

Van Veld, P.A., D.J. Westbrook, B.R. Woodin, R.C. Hale, C.L. Smith, R.J. Huggett and J.J. Stegeman. 1990. Induced cytochrome P-450 in intestine and liver of spot (*Leiostomus xanthurus*) from a polycyclic aromatic hydrocarbon contaminated environment. *Aquat. Toxicol.* 17:119-132.

Watanabe, I., T. Yakushiji, and N. Kunita. 1980. Distribution differences between polychlorinated terphenyls and polychlorinated biphenyls in human tissue. *Bull. Environ. Contam. Toxicol.* 25:810-815.

Wu, L. and J.P. Whitlock, Jr. 1992. Mechanism of dioxin action: Ah recptor-mediated increase in promoter accessibility *in vivo*. *Proc. Natl. Acad. Sci.* 89:4811-4815.

Zitko, V., O. Hutzinger, W.D. Jamieson, and P.M.K. Choi. 1972. Polychlorinated terphenyls in the environment. *Bull. Environ. Contam. Toxicol.* 7(4):200-201.

Vita

Kathryn Gallagher

Born in Red Bank, New Jersey, March 31, 1962. Graduated from Mater Dei High School, New Monmouth, New Jersey in 1980. Received B.S. in Biology with a concentration in Marine Biology and Limnology from San Francisco State University, San Francisco, California in 1986. Entered master of arts program at the College of William and Mary, School of Marine Science, Gloucester Point, Virginia in 1988. Bypassed masters degree and entered doctoral program at the College of William and Mary in November 1992.