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PCB Concentrations in Fishes from the Housatonic River, Connecticut, 1984–2006, and in Benthic Insects, 1978–2006

Report No. 07-08

Prepared for the

General Electric Company

by the

Patrick Center for Environmental Research
The Academy of Natural Sciences of Philadelphia
1900 Benjamin Franklin Parkway
Philadelphia, Pennsylvania 19103-1195

September 2007

GE
159 Plastics Avenue
Pittsfield, MA 01201
USA

October 3, 2007

Ms. Susan Peterson
Connecticut Department of Environmental Protection
Water Management Bureau
79 Elm Street
Hartford, CT 06106

Mr. Dean Tagliaferro
U.S. Environmental Protection Agency
c/o Weston Solutions, Inc.
10 Lyman Street
Pittsfield, MA 01201

Re: Housatonic River, Connecticut
Report on 2006 Fish Sampling and Benthic Insect Sampling

Dear Ms. Peterson and Mr. Tagliaferro:

Enclosed is a report entitled *PCB Concentrations in Fishes from the Housatonic River, Connecticut, 1984-2006, and in Benthic Insects, 1978-2006*, which was prepared on behalf of the General Electric Company (GE) by the Academy of Natural Sciences of Philadelphia. This report presents the results of the Academy's 2006 fish sampling and benthic insect sampling in the Housatonic River in Connecticut, and it includes comparisons of those results to the results from prior fish and benthic insect monitoring studies.

We would be glad to discuss this report with you. Please let me know if you have any questions or would like additional copies.

Very truly yours,

Kevin Mooney
Project Manager

Enclosure

cc: Susan Svirsky, EPA
Timothy Conway, EPA
Holly Inglis, EPA
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John Looney, CT AG
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EXECUTIVE SUMMARY

The Academy of Natural Sciences of Philadelphia (Academy) has conducted biennial fish surveys in the Connecticut portion of the Housatonic River since 1984. Benthic insects were monitored by the Connecticut Department of Environmental Protection (CTDEP) during 1978–1990 and have been monitored by the Academy since 1992. Data for both groups of organisms have documented a clear reduction in PCB concentrations in the biotic component of the river ecosystem since monitoring began.

Results of the Academy's 1994 study indicated a substantial reduction in PCB concentrations in brown trout, smallmouth bass, and benthic insects compared to 1992. Concentrations observed in the 1996–2004 studies were roughly similar to those in 1994 and, for fish, remained well below the levels in 1986–1992. For benthic insects, concentrations in 2001, 2002, and 2005 were among the lowest observed since monitoring began.

The 1994 biological monitoring study was the last of the biennial studies required by the 1990 Housatonic River Cooperative Agreement between CTDEP and the General Electric Company (GE). The 1996 and 1998 studies were conducted in order to determine whether the marked reduction in PCB concentrations observed in 1994 had persisted, and the results indicated that it largely had. A new Housatonic River Follow-up Cooperative Agreement was executed by GE and CTDEP in October 1999, requiring continuation of these biennial studies in 2000, 2002, and 2004. Although no cooperative agreement was in effect requiring monitoring in 2006, the biennial monitoring program was nevertheless continued in 2006, using the same study design as in previous years. The present report details results from the 2006 fish and benthic insect sampling.

Purpose of Study

The main purpose of the 2006 study was to compare PCB concentrations in brown trout, smallmouth bass, and benthic insects with levels observed in previous study years, and to compare PCB concentrations in smallmouth bass collected at four monitoring stations in 2006.

Sampling Stations

Sampling stations for this biological monitoring study were the same as in previous years. In upstream to downstream order, these were West Cornwall, Bulls Bridge, Lake Lillinonah, and Lake Zoar (see map in Fig. 1 of the report). An additional station (Falls Village, upstream of West Cornwall) was employed, at CTDEP's request, for supplemental sampling that was not part of the biennial monitoring program.

Taxa Monitored

The taxa sampled for long-term monitoring purposes were the same as in the 2000, 2002, and 2004 studies and included fish and benthic insects. The fish species were brown trout (collected only at West Cornwall) and smallmouth bass (collected at West Cornwall,

Bulls Bridge, Lake Lillinonah, and Lake Zoar). The benthic insect taxa (collected only at West Cornwall) consisted of filter-feeding caddisflies, predatory stoneflies, and predatory dobsonflies. In addition, at CTDEP's request, supplemental samples of northern pike were collected at Falls Village, Bulls Bridge, Lake Lillinonah, and Lake Zoar. All fish and benthic insect samples were collected during 2006.

PCB Analysis

Analytical Method

PCB analysis was based on the method of Mullin (1985), which allows specific quantitation of over 100 individual PCB congeners. This method permits both congener-based and Aroclor-based determinations of total PCB.

Quantitation of Total PCB

Total PCB was quantified by two procedures. The congener-based procedure sums the concentrations of all individual congeners (up to 121) quantitated by the analytical method. The Aroclor-based procedure is based instead on the concentrations of a much smaller number of congeners that are essentially unique to Aroclor 1254 or 1260. It extrapolates from these marker congeners to Aroclor concentrations, based on the relative proportions of the markers in each Aroclor, then sums the two Aroclor concentrations. Only the Aroclor-based procedure was used in the 1984–1990 studies, while both methods were used in the 1992–2006 studies.

Data Analysis and Rationale

Two basic types of differences in PCB concentrations are of interest in this study: differences among years and differences among stations. Year differences were assessed for both smallmouth bass and brown trout, using appropriate statistical techniques (see below). Station differences were assessed only for smallmouth bass, since it is the only species monitored at all sampling stations.

PCB concentrations in an individual fish can be influenced strongly by its age (or duration of exposure, which differs from age in fish that are stocked), sex, and lipid content. Since samples collected in different years or at different stations typically differ in their age, sex, and lipid distributions, observed differences in PCB concentrations among years or stations may simply reflect differences in these ancillary variables (e.g., unusually high lipid levels in a particular year) rather than real differences in PCB exposure. At the opposite extreme, real differences in exposure (e.g., a declining trend among years) may be masked by variability created by differences in these ancillary variables. Therefore, to the extent that inferences regarding differences in PCB exposure are of interest, it is important to identify and remove any statistically significant influence of these ancillary variables.

Given these facts, two criteria are paramount in choosing an appropriate statistical technique for analysis of the fish data: it must permit assessment of among-year and among-station variation, and it must permit detection and removal of the effects of

differences in ancillary variables (age, sex, lipid content). Analysis of covariance is a standard technique that satisfies both of these requirements, and it was therefore chosen as the basis for assessing the statistical significance of variation among stations and years for the fish data.

In contrast, tolerance limits for human consumption of fish and criteria for fish consumption advisories are based simply on the total PCB concentration of a fish fillet (on a wet weight basis), since this value indicates the amount of PCB consumed per unit mass of edible fish. Data for these purposes are therefore reported without adjusting for the effects of ancillary variables.

Results

Comparison of Fish Results with Previous Years

Overall, PCB concentrations in smallmouth bass and brown trout in 2006 were roughly similar to those found in 1994–2004 and well below the levels found in 1992 and most prior years. This pattern held for both Aroclor-based total PCBs (TPCB) and congener-based total PCBs (CTPCB).

For smallmouth bass, there was a clear pattern of low TPCB concentrations during 1994–2006 compared to 1992 and earlier. Similarly, CTPCB concentrations (which are only available for 1992–2006) were lower in 1994–2006 than in 1992. These patterns were confirmed statistically for both TPCB and CTPCB using analysis of covariance and pairwise comparisons between years. While these comparisons indicated some differences in temporal patterns among stations, they confirmed that concentrations in 2006 and other recent years (1994–2004) were significantly lower than those in 1988–1992.

For brown trout, TPCB and CTPCB concentrations in 2006 were similar to or lower than concentrations in 1994–2004 and were well below levels observed in 1992. This pattern was generally confirmed by analysis of covariance with pairwise comparisons between years.

Comparison of Fish Results among Stations

Visual comparison of smallmouth-bass total PCB concentrations at the four monitoring stations in 2006 indicates that, for both TPCB and CTPCB, concentrations at West Cornwall and Bulls Bridge were similar to each other, as were concentrations at Lake Lillinonah and Lake Zoar, but concentrations appeared higher at the two upstream stations (West Cornwall and Bulls Bridge) than at the two downstream stations (Lake Lillinonah and Lake Zoar). This general pattern was confirmed statistically using analysis of covariance with pairwise comparisons between stations, although TPCB at Lake Lillinonah was significantly different from that at Lake Zoar, while the CTPCB concentrations at those two stations were not significantly different. A similar pattern of downstream decrease in TPCB and CTPCB was found in previous biological monitoring studies.

Fish Exceeding the FDA Fish Consumption Tolerance Limit

For comparison with previous Housatonic River biological monitoring studies, an assessment was made of the percentage of fish with fillet PCB concentrations exceeding the U.S. Food and Drug Administration (FDA) fish consumption tolerance limit of 2.0 mg/kg wet weight. One of the 40 smallmouth bass samples (2.5%) in 2006 had a CTPCB concentration exceeding the FDA limit, while four (10%) had TPCB concentrations exceeding that limit. Among brown trout, 2 of 30 samples (7%) had CTPCB concentrations exceeding the FDA limit, while 3 of 30 samples (10%) had TPCB concentrations exceeding that limit. Proportions of both smallmouth bass and brown trout exceeding the FDA limit in 1994–2006 have been lower (sometimes zero for smallmouth bass) than in 1984–1992.

Supplemental Fish Sampling Results

Twenty northern pike samples were analyzed. One of those 20 northern pike (5%) had a CTPCB concentration greater than the FDA limit, while that fish plus one other (10%) had TPCB concentrations greater than the FDA limit. The maximum PCB concentration in northern pike in 2006 was 3.80 mg/kg wet weight TPCB. There was no clear pattern of downstream decrease in TPCB or CTPCB concentrations in northern pike. (The supplemental fish sampling for northern pike is discussed separately in Appendix J.)

Benthic Insect Results

PCB concentrations in predatory stoneflies in 2006 were similar to those in 1998, 2001, 2002, and 2005, while concentrations in filter-feeding caddisflies and predatory dobsonflies were similar to those in 2001 but appeared slightly higher than in 2002 and 2005. PCB concentrations in both predators and filter feeders were well below most of the values in 1978–1992. Rank correlation analysis of the entire data series for 1978–2006 revealed a highly statistically significant temporal trend of decreasing PCB concentrations in both filter feeders and predators.

Conclusions

Results of the 2006 fish monitoring study show that total PCB concentrations in brown trout and smallmouth bass were generally similar to those in the 1994–2004 studies and lower than the levels observed in 1992 and prior years. Concentrations in filter-feeding and predatory benthic insects in 2006 also were broadly similar to those in the 1994–2004 studies and well below the levels observed in 1992 and most prior years. Both insect groups showed a highly statistically significant temporal trend of decreasing total PCB concentration since 1978. These findings indicate that the substantial reduction in total PCB content of fishes and benthic insects that occurred after the 1992 study and was seen in the 1994–2004 studies has persisted into 2006.

QUALITY ASSURANCE STATEMENT

Study Number: 463

Study Title: PCB Concentrations in Fishes From the Housatonic River, Connecticut, 1984–2006, and in Benthic Insects, 1978–2006

This study was performed under the general provisions of the Patrick Center's Quality Assurance Implementation Plan (Rev. 1, June 1998). The final report has been determined to be an accurate reflection of the data obtained.

The dates that Quality Assurance activities on this study were completed are given below.

Data Reviews

Fisheries:	20 September 2007
Chemistry:	19 September 2007
Insects:	10 July 2007
Report Review:	11 July 2007

ARCHIVING: Raw data and the final report are filed in the Patrick Center's archives.

Robin S. Davis
Quality Assurance Unit
Patrick Center for Environmental Research
Academy of Natural Sciences

9/20/07
Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The Academy of Natural Sciences of Philadelphia (Academy) has conducted biennial fish surveys in the Connecticut portion of the Housatonic River since 1984. Benthic insects were monitored by the Connecticut Department of Environmental Protection (CTDEP) during 1978–1990 and have been monitored by the Academy since 1992. Data for both groups of organisms have documented a clear reduction in PCB concentrations in the biotic component of the river ecosystem since monitoring began.

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The main objectives of the 2006 study were the following:

- *Measure PCB concentrations in selected Housatonic River fish.* As a continuation of prior studies, the species sampled and analyzed for total PCBs were brown trout at West Cornwall and smallmouth bass at West Cornwall, Bulls Bridge, Lake Lillinonah, and Lake Zoar (sampling locations are shown in Fig. 1). In addition, at the CTDEP's request, supplemental samples of northern pike were collected for PCB analysis from Falls Village (upstream from West Cornwall), Bulls Bridge, Lake Lillinonah, and Lake Zoar.
- *Measure PCB concentrations in selected benthic insects at West Cornwall.* As a continuation of prior studies, the insect taxa sampled and analyzed for total PCBs were filter-feeding caddisflies, predatory stoneflies, and predatory dobsonflies.
- *Compare PCB concentrations measured in brown trout and smallmouth bass with concentrations measured in previous years, and compare PCB concentrations measured in smallmouth bass spatially across the four stations sampled.*
- *Compare measured PCB concentrations for each benthic insect group with those measured in previous years.*

For maximal comparability with previous results, fish samples employed in the monitoring study were collected from the same locations and during the same seasonal time periods as in prior studies. The number of brown trout collected at West Cornwall and the number of smallmouth bass collected at all four stations were comparable to the numbers collected in the 1994, 1996, 2000, 2002, and 2004 studies and were greater than the numbers collected in 1998 (when the numbers of specimens were reduced at CTDEP's request). An attempt was also made to ensure that the size distribution of fish collected was generally consistent with previous studies.

The remainder of the text of this report describes study methods, summarizes the data, and presents the results of statistical analyses for species that are part of the long-term monitoring program (brown trout, smallmouth bass, and benthic insects). Sampling methods and PCB data for the supplemental samples of northern pike are detailed separately in Appendix J.

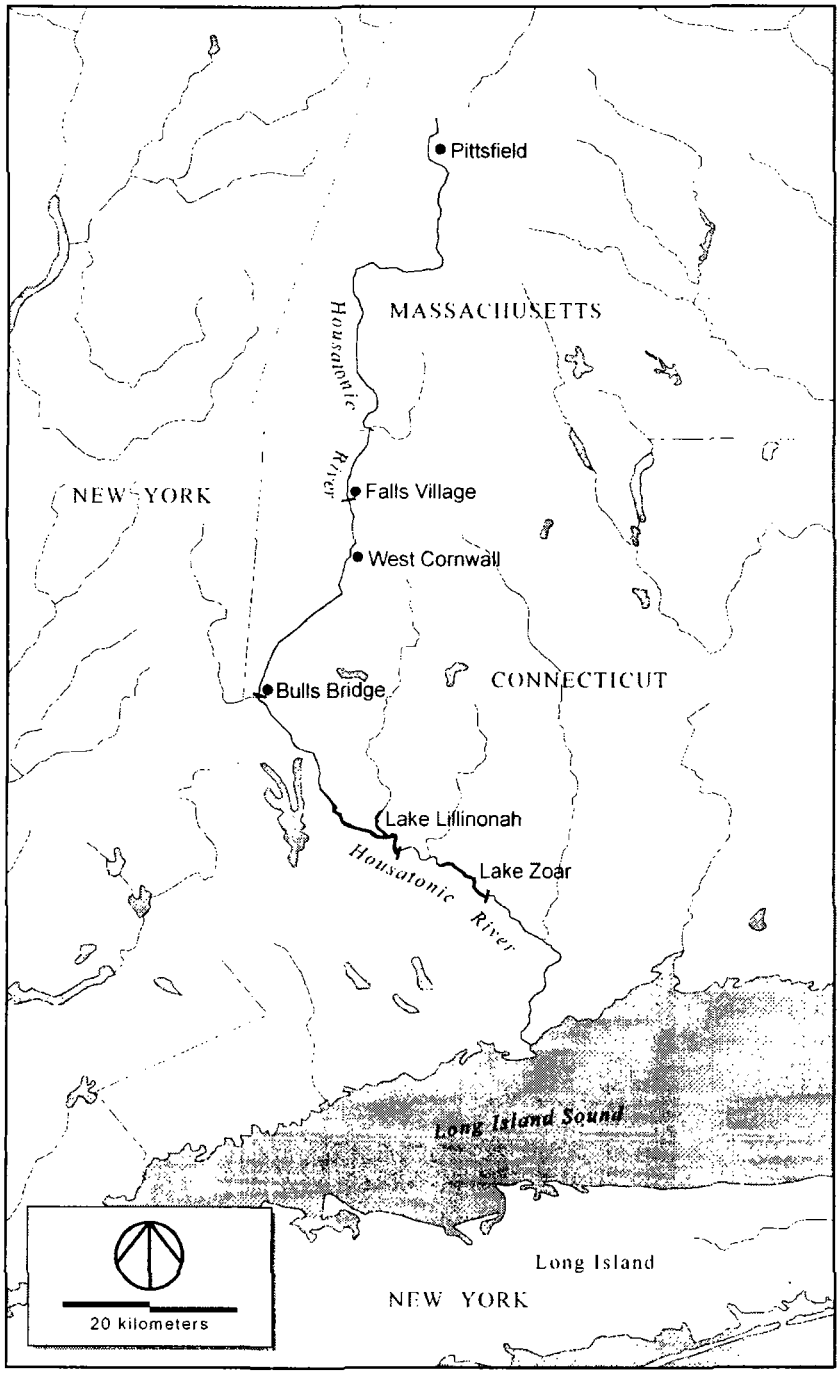

Figure 1. Map of the Housatonic River showing sampling stations for the 2006 fish and benthic insect collections in Connecticut. Smallmouth bass were collected at West Cornwall, Bulls Bridge, Lake Lillinonah, and Lake Zoar. Brown trout and benthic insects were collected only at West Cornwall. Supplemental samples of northern pike were collected at Falls Village, Bulls Bridge, Lake Lillinonah, and Lake Zoar. Approximate locations of dams at Falls Village, Bulls Bridge, Lake Lillinonah, and Lake Zoar are indicated by bars across the river.

SAMPLING DATES AND LOCATIONS

Fish and benthic insects employed in the monitoring study were collected from the same stations sampled in previous years. In upstream to downstream order, these are West Cornwall, Bulls Bridge, Lake Lillinonah, and Lake Zoar (Fig. 1). As in previous Academy studies, brown trout were collected only at West Cornwall, while smallmouth bass were collected at all four stations. Two main collecting trips for fish were made, one in August and one in October 2006. Fish collection dates and techniques for the four sampling stations are summarized in Table 1.

TABLE 1. Summary of sampling dates, methods, and locations for fish collections on the Housatonic River, Connecticut, in 2006. Symbols: BS = boat electroshocking, WS = walk along (shore) electroshocking, A = angling, GN = gill net.

Sampling Location	Sampling Dates in 2006	
	7-14 Aug	16–18 Oct
West Cornwall	WS	WS*
Bulls Bridge	A, BS, GN	A, GN
Lake Lillinonah	BS	BS
Lake Zoar	BS	BS

* Collection with State of Connecticut gear

West Cornwall

Holdover brown trout, 2006-stocked brown trout, and smallmouth bass were collected from several locations (including Turnip Island, Furnace Brook, and near the Covered Bridge) within the West Cornwall station and the Housatonic River Trout Management Area on 14 August by Academy personnel using walk-along electroshocking. A second sampling trip to the West Cornwall area was conducted on 17 October 2006. CTDEP provided assistance and walk-along shocking equipment during the collection of brown trout.

Benthic insect samples were collected on 19–20 June 2006 along the west bank of the river, upstream from the US 7 – CT 4 bridge and downstream from the mouth of Furnace Brook ("Church Hole") at Cornwall Bridge. This is the same site that was sampled in the 2004 study, and is located approximately 5 km downstream from the 2002 sampling site (the "Garbage Hole", 0.5 km downstream from the Covered Bridge at West Cornwall).

Bulls Bridge

Fish were collected at Bulls Bridge on 8–9 August and 17–18 October 2006 by boat electroshocking, gill netting, and angling. Gill nets were set overnight along rocky shorelines and adjacent to man-made structures (docks, bridge abutments, etc.). Angling

from shore and boat docks was used to catch additional fish samples. Boat electroshocking was conducted during the late afternoon and at night throughout the entire station, which extended from about 0.5 km above the State Route 341 bridge at the Kent School to an area 1.7 km downstream of the State Rt. 341 bridge.

Lake Lillinonah

Fish were collected at the Lake Lillinonah station by boat electroshocking on 7 August and 16 October 2006. Boat electroshocking was conducted in inlets or coves, around docks, and along rocky ledges and shorelines. Sampling was conducted from about 5.0 km below State Route 133 bridge to 5.0 km above State Route 133.

Lake Zoar

Fish were collected at the upper end of Lake Zoar (both banks) by boat electroshocking on 8 August and 18 October 2006. The lower end of the reservoir (both banks) was sampled by boat electroshocking on 8 August 2006. Typical habitat sampled by shocking included rock rip-rap, tree/brush snags, boat docks, and bridge pilings. Sampling in the upper end was conducted from the state boat ramp at Lakeside upstream to the spillway of the Shepaug Dam on 8 August and 18 October 2006. Sampling for fish in the lower section of the reservoir was conducted from about 0.6 km above Stevenson Dam to the vicinity of Jackson Cove, about 5.0 km above Stevenson Dam.

METHODS

Fish Collection and Handling

Brown trout and smallmouth bass were collected by Academy staff, with the assistance of the CTDEP Western Division Fisheries (West Cornwall only), by walk-along and boat electrofishing, gill netting, and angling. Two brown trout from the Burlington fish hatchery were provided by CTDEP for use in determining pre-stocking PCB levels. Table 2 shows the number of specimens of each species collected from each location.

TABLE 2. Number of specimens of each fish species collected from the Housatonic River in 2006 and analyzed for PCBs as part of the long-term monitoring program.

Species	Station					Total
	West Cornwall	Bulls Bridge	Lake Lillinonah	Lake Zoar	Burlington Hatchery	
Brown trout	30	—	—	—	2	32
Smallmouth bass	10	10	10	10	—	40
Total	40	10	10	10	2	72

Lakes Lillinonah and Zoar were sampled using a 17-ft electroshocking boat. A Smith-Root model 5.0 GPP electroshocker controller powered by a 5000 W generator was operated at pulsed DC output within the following ranges, depending on site and conditions: 180–250 volts, 20% pulse width, 80–100 pulses/sec, and 8–11 amps. Most boat shocking was conducted at night, though some late afternoon and early evening samples were taken. A Robin generator and Coffelt VVP unit operated at AC output fitted in a canoe was provided by CTDEP and was used for walk-along (tow-barge) electrofishing during daylight hours at West Cornwall. In addition, the Academy collected fish at West Cornwall using walk-along equipment powered by a Honda EG5000X generator and a VVP-15 electroshocker unit set at AC output. Gill nets and angling were the secondary collection techniques used at some of the stations.

During boat electroshocking, two persons collected the stunned fish with long-handled dip nets, while the boat operator controlled the boat and the electrical output of the shocker. Specimens were held in river water in a pre-cleaned metal tub (washed with Micro-90® cleaner and rinsed with river water for each location). Target specimens were identified and measured to ensure collection of appropriately sized fishes. The fish were then placed in a clean stainless steel pan (Micro-90® washed and river water rinsed for each location) that was set on wet ice inside a cooler. Samples were processed within 1 to 6 h from the time of capture. Specimens not required for chemical analysis were measured and released alive.

In addition to electroshocking, fish were also collected with gill nets and angling. Gill net sampling was performed using a 60-ft (18.3-m) net with 2 × 3 × 4-in (5.0 × 7.6 × 10.2-cm) mesh. Nets were set in the evening and collected in the morning, generally averaging

12 h of soak time. Once specimens were removed from the net, they were held in river water in a pre-cleaned (Micro-90® washed and river water rinsed) metal tub. After a set of nets was checked (approximately 45–60 min duration), specimens were identified and measured to ensure collection of appropriately sized fishes. The fish were then placed in a clean (Micro-90® washed and river water rinsed) stainless steel pan that was set on wet ice inside a cooler. The same handling procedure was followed for specimens collected by angling. Samples typically were processed within 1 to 6 h from the time of capture. Specimens not required for chemical analysis were measured and released alive. Angling was performed by standard rod and reel.

Two hatchery trout were provided by CTDEP. As in the 2002 and 2004 studies, these fish were taken from the Burlington hatchery in August 2006, concurrent with the August trout sampling trip. In studies prior to 2002, hatchery fish were taken in October (2000 study), August (1994–1998 studies), and May (1986 and 1988 studies).

At the field processing site, fish specimens required for chemical analysis were measured for total length to the nearest 0.1 cm with a standard metal ruler affixed to a pre-cleaned measuring board. Each specimen was assigned a unique field serial number, which was attached to the package containing the specimen and recorded in the field notes. Specimens were wrapped individually in clean, muffled aluminum foil. Fish were individually marked with either a Floy tag or a numbered metal tag. Floy tags were inserted into the head of specimens, while metal tags were placed in folds of the foil so as not to contact the skin. The outside of each foil pack was labeled with an index card bearing information on date of capture, species, locality of capture, and serial number. The foil pack and index card were secured with freezer tape and stored on dry ice in clean coolers (Micro-90® washed). Specimens were maintained frozen on dry ice and transported to the Academy's Philadelphia laboratories. Chain-of-custody forms were prepared in the field and accompanied samples to Philadelphia; they were also used to verify transfer of specimens from state collecting crews to Academy field personnel.

Upon arrival at the Academy's laboratories in Philadelphia, sample data were entered into the Fisheries Section database, and specimens were placed in freezers until laboratory processing. Chain-of-custody forms were used to track samples from Academy field personnel to fisheries laboratory personnel, and then to Academy chemistry laboratory personnel for processing or storage.

Fishes were handled in both the field and lab according to Academy Standard Operating Procedure P-14-04 (Fish Preservation, Fixation, and Curation, Rev. 2) and quality control procedures. Specimens were prepared using clean equipment, and contact between specimens or with uncleaned laboratory surfaces was avoided to minimize chances of contamination.

Benthic Insect Collection and Handling

Benthic insects were collected by agitating the substrate and capturing dislodged animals with a triangle net as the current swept them downstream, or by picking them from the surfaces of rocks with forceps. Individual insects were rinsed in river water to remove any attached substrate particles.

Aquatic insect samples were placed in I-Chem Superfund Analyzed glass jars bearing a label on the outside. At the field site, sample jars were placed on ice in a cooler as they were filled. Samples were then frozen for transport to the Academy's laboratories in Philadelphia. Upon arrival, samples were transferred to a freezer and stored frozen until preparation for PCB analysis.

Preparation of Fillet Samples

Fishes to be analyzed for PCBs were partially thawed, after which total length (± 0.1 cm) and weight (± 0.1 g) were measured and identifications were confirmed. Brown trout from West Cornwall were examined for fin clips, and observed marks were recorded. (Marks have been used to distinguish among the various size classes of trout that are stocked in a given year: fingerling, yearling, and adult.) During sample preparation, external and internal anomalies, presence of parasites, etc. were noted. Laboratory methods followed Academy Standard Operating Procedure P-14-12 (Preparation of Fish Samples for Contaminant Analysis). Lengths measured in the lab were used in all analyses. When possible, sex of specimens was determined by gross macroscopic examination. Each fish was given a four-digit analysis number prefixed by "F–" (e.g., F–0538) that was used for tracking the fillet through chemical analyses.

A cleaned glass filleting plate and a cleaned and rinsed stainless steel fillet knife or scalpel blade were used for each specimen. Prior to filleting the fish, excess mucous and debris were rinsed from the fish with deionized water and/or wiped with a Kimwipe®. Following standard practice based on typical human food-preparation customs, skin and scales were left on trout fillets, while smallmouth bass fillets were prepared with scales removed but skin retained. The left fillet was used for chemical analysis. Fillet weight was recorded and otoliths from all target specimens were removed and preserved in 95% ethanol for subsequent age analysis. The entire fillet (including the flesh covering the abdominal cavity) was minced and placed into pre-cleaned 2000-class jars. The fillets were transferred to the Academy Chemistry Section along with a chain-of-custody form. The remains were wrapped in aluminum foil, labeled, and refrozen, permitting examination or analysis of additional material, if necessary.

Cleaning of the glass plates and fillet knives at the end of each laboratory session included the following steps:

1. Wash with dilute Micro-90® cleaner and thoroughly rinse in deionized water.
2. Rinse in acetone and hexane, then rinse with dichloromethane and air dry.
3. Cover plate and knife with muffled aluminum foil to avoid contamination prior to use.

Fish Aging

Ages of fish were estimated using otoliths, which are ear-bones found in the brain of fish. Comparison of otolith annuli (year) counts with total lengths and known stocking dates (analysis of fin clips) helped in verifying ages of some brown trout. CTDEP stocks brown trout in the Housatonic River in the Trout Management Area (TMA) at West Cornwall. For stocked brown trout, the time of residence in the river (river age) is more meaningful

than total age for assessing exposure to PCBs. The brown trout collected in 2006 included yearling and adult fish stocked from the Burlington hatchery.

In contrast to some previous years, relatively few marked stocked trout were found in 2006. Seven fish had right maxillary clips, marking them as adult fish stocked in the spring of 2006. Other brown trout were identifiable as fish stocked in 2006 based on a combination of size (relative to data provided by the State on size ranges at the time of stocking) and otolith structure. While most of the unmarked fish stocked in 2006 were probably yearling fish (which were not marked at the time of stocking), some adult fish stocked in 2006 may have lost the mark by regrowth of the maxillary. Discrimination of these groups is complicated by overlap in length due to differential growth rates after stocking and by irregular development of otolith bands of trout in hatcheries. Errors in assignment of fish to these two groups would not affect the primary analysis, since that analysis is based on length of time in the river after stocking. In past studies, holdover trout have been distinguished principally by marks (fin clips and elastomer dye) and length. In 2006, one holdover fish stocked in the spring of 2005 was identifiable by marks (maxillary and adipose clip), size, and otolith structure. Two fish were identified as stocked in the fall of 2005 by marks (ventral fin clip), size, and otolith structure. Four unmarked fish were identified as stocked in the fall of 2005 by similarity of size and otolith structure to the two marked fish.

The largest pair of otoliths (sagitta) was dissected from the fish in the laboratory during the filleting procedure and placed in small vials of 95% ethanol. One of the sagitta was embedded with fast-cure epoxy resin and dried. Thin transverse sections were cut through the otolith with a Buehler Isomet low-speed saw. Three to five of these thin sections per fish were affixed to a microscope slide with immersion oil. Sections were examined under a dissecting microscope at 12–50x magnification. Specimens that were more difficult to age were examined under a compound microscope (50–400x magnification).

When viewing sectioned otoliths, annuli (annual marks) are visible as pronounced dark bands, containing within them thin, faint bands representing other cycles of growth. Age was estimated by counting the pronounced bands, with the innermost band assumed to represent the first winter-spring transition (between age 0+ and 1+). Ages were determined independently by two fisheries biologists who read the otoliths and compared results. Exact agreement occurred for 88% of the smallmouth bass and 90% of the brown trout. A mutually agreed upon determination was reached for discrepancies in age after re-examining the otoliths and consulting with a third experienced reader.

Analysis of PCBs

The method of PCB analysis was identical to that employed in the 2002 and 2004 studies. The laboratory method used for treatment of fish is based on the Academy's Standard Operating Procedure P-16-77, "Extraction and Cleanup of Fish Tissue for PCB and Pesticide Analysis" (Appendix A). Fish tissues and insect samples were ground using a Tissuemizer®, and the homogenized samples were stored frozen until extraction for PCBs. Samples were thawed and 5 g of the homogenate was sub-sampled using a stainless steel spatula. Approximately 30 g of Na_2SO_4 (manufactured by J.T. Baker, previously muffled at 450°C for 4 hours) was added to the sub-sample to eliminate water.

The dried sample was placed in a Soxhlet extractor with pre-cleaned glass wool and extracted in a 1:1 hexane-acetone (manufactured by J.T. Baker, pesticide residue grade) mixture for a minimum of 18 h. The extracts were sub-sampled for gravimetric lipid determination. For this, a known volume of the 1:1 hexane-acetone extract was transferred to a pre-weighed aluminum pan. The solvent was evaporated in a fume hood for at least 24 h. The residue remaining (lipid) was weighed and percent lipid was calculated (wet weight basis).

Lipids were removed from sample extracts by treatment with concentrated trace metal grade sulfuric acid (manufactured by J.T. Baker). The organic phase was further cleaned by solid-liquid chromatography using florisil sep-pak columns (manufactured by Burdick and Jackson). The PCBs were eluted from this column using pesticide residue grade hexane.

PCB identification was congener-specific, based on the Academy's Standard Operating Procedure P-16-84 Rev. 2, "Quantification of Individual Polychlorinated Biphenyl Congeners (PCBs), Chlorinated Pesticides and Industrial Compounds by Capillary Column Gas Chromatography" (Appendix B). Congener-specific PCBs were analyzed using a Hewlett Packard 6890 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 5% phenylmethyl silicon capillary column. The identification and quantification of PCB congeners followed the '610 Method' in which the identities and concentrations of each congener in a mixed Aroclor standard (25:18:18 mixture of Aroclors 1232, 1248, and 1262) were determined by calibration with individual PCB congener standards. Congener identities in the sample extracts were based on their chromatographic retention times relative to the internal standards added. In cases where two or more congeners could not be chromatographically resolved, the combined concentrations were reported.

Statistical Methods

Measures of PCB Concentrations

The primary analytical measure used for summarizing and analyzing data was total PCB concentration on a wet weight basis. This measure is relevant to regulatory thresholds, such as fish consumption advisory limits. Total PCB concentration was estimated by two methods. The first was based on measuring the concentrations of selected congeners that are essentially unique to Aroclor 1254 and 1260, extrapolating to Aroclor concentrations from the relative proportions of these congeners in each Aroclor, and then summing the two Aroclor concentrations. The resulting estimate of Aroclor-based total PCB concentration is denoted TPCB. The second measure was calculated by summing concentrations of all of the identifiable PCB congeners. The resulting estimate of congener-based total PCB concentration is denoted CTPCB.

The TPCB method was the only one used in the 1984–1990 monitoring studies, while both TPCB and CTPCB methods were used in the 1992–2006 studies. In a previous study, the two estimates of total PCB were compared using the 1992, 1994, and 1996 data and were found to be highly correlated in all three years (ANSP 1997). This correlation was confirmed by regression analysis of the relationship between the TPCB and CTPCB

data for 2006 (Appendix C). Thus, CTPCB is a good surrogate measure for TPCB. In analyses that included all monitoring years, only TPCB was used, while analyses that included only years 1992–2006 were conducted using CTPCB values, since CTPCB values are expected to provide a more accurate measure of total PCB concentrations than do TPCB values. This procedure is consistent with previous monitoring reports.

Variables that Influence PCB Uptake

PCB concentrations in fishes can be influenced by a variety of factors other than a fish's level of exposure. Influential variables include a fish's river age, lipid content, and sex.

The river age of a fish is the time the fish has spent in the river. For stocked brown trout in the Housatonic River, PCB exposure occurs primarily in the river rather than the hatchery. Therefore, river age is a more meaningful indicator of exposure than is total age. For smallmouth bass, which are not stocked, river age is identical to total age.

Since PCBs partition preferentially into lipid, a fish's PCB uptake rate and steady-state burden are likely to be influenced by its lipid content. Lipid content often differs between sexes, with females having higher lipid levels than do males.

Statistical Analyses

One of the major goals of this study was to assess differences in PCB concentrations among years and stations. Because the composition of samples collected in different years or at different stations unavoidably differs somewhat with respect to variables that influence PCB uptake (e.g., river age, lipid content, and sex), differences among samples with respect to these variables could produce statistically significant year or station effects that are not caused by differences in PCB exposure. At the opposite extreme, differences with respect to these variables could mask the effects of real differences in PCB exposure. It is therefore desirable to identify and remove the effects of these confounding variables when they are statistically significant.

Analysis of covariance (ANCOVA), as implemented by the General Linear Model (GLM) procedure in Statistica, was the primary statistical technique used for year and station comparisons. Year, sex[1], and station were incorporated in ANCOVA models as discrete effects. River age and lipid content (both log-transformed) were incorporated as covariates. Statistical significance of effects and covariates was assessed by the p value associated with the F value of the corresponding Type III sum of squares[2] (the Type III sum of squares is discussed in SAS 1985). The statistical significance of variation among years, among stations, and among treatment interactions was assessed.

Statistical distributions of TPCB and CTPCB were strongly positively skewed and thus were inappropriate for analyses that assume a normal distribution, such as ANCOVA.

1 Sex could not be determined by macroscopic examination in a small number of smallmouth bass specimens taken in previous years. These specimens were found not to influence statistical outcomes and were dropped from analyses that included sex as a covariate.

2 Using the Type III sums of squares assesses the contribution of each effect after all other effects in the model have been incorporated.

Therefore, following standard statistical practice (e.g., Sokal and Rohlf 1969), TPCB and CTPCB data were log-transformed prior to statistical analysis. The purpose of this transformation is to produce variables whose variance is independent of the mean (homogeneous variance) and whose variation about the mean is approximately normally distributed (Gaussian residuals). These properties are important in ensuring the validity of standard statistical methods such as ANCOVA. Additionally, for positively skewed data, the geometric mean is known to be a better measure of central tendency than is the arithmetic mean and therefore was used in graphical presentations of data.

ANCOVA was used to test for statistically significant differences among stations and years for smallmouth bass and brown trout. Models were designed to examine among-year differences at West Cornwall for brown trout, and to examine both among-year and among-station differences for smallmouth bass. ANCOVAs included main effects (station, year, and sex), covariates (log river age and log lipid, where "lipid" is percent lipid on a wet-weight basis), and interaction terms for main effects and covariates. Following standard statistical practice, covariates that were not statistically significant were dropped from the model, and the ANCOVA was repeated to assess significant effects and interactions. With regard to lipid-normalization, this means that PCB levels were adjusted (or normalized) for associated lipid levels in the final model only when ANCOVA indicated that PCB concentrations were influenced significantly by lipid content.

The removal of non-significant terms from a statistical model pools variance associated with the removed effects with residual error. Because this procedure increases both the sums of squares and degrees of freedom of the residual error, it can either increase or decrease the mean squares error. An alpha level of 0.05 was used to remove non-significant terms (Sokal and Rohlf 1969); this pooling did not greatly affect significance of other effects in the analyses performed. In general, once significant main effects were included in models, the significance of interactions did not depend on which other interaction terms were included (e.g., significance of a station-year interaction did not depend on inclusion of station-sex, year-sex, or lipid-station interactions, although they did depend on the inclusion of year and station main effects).

Least-squares means associated with each treatment level were examined to determine differences among mean total PCB levels. The least-squares mean adjusts for covariate effects and thus provides an estimate of PCB content independent of river age, sex, and lipid content (or other influential variables). When probability levels generated from an ANCOVA indicated a significant station or year effect, pairwise multiple comparisons were used to identify significant differences between pairs of least-squares means, using the Tukey unequal sample size HSD (honest significant difference) criterion. Thus, any differences detected by these tests represented differences in PCB concentration after accounting for the effects of age, sex, and lipid content.

RESULTS

Summary of the 2006 Monitoring Data for Brown Trout and Smallmouth Bass

Thirty brown trout collected from West Cornwall and two brown trout from the Burlington hatchery were analyzed for PCB content (stocking dates are summarized in Appendix D). The specimens from West Cornwall consisted of 5 males and 25 females. Forty smallmouth bass from four stations were analyzed for PCB content, including 13 males and 27 females. The (arithmetic) mean and range of CTPCB concentrations and lipid-normalized CTPCB concentrations are summarized in Table 3. Hatchery trout had a geometric mean CTPCB level of 0.01 mg/kg (wet) and were not used in the statistical analyses.

TABLE 3. Arithmetic means and ranges of congener-based total PCB estimates (mg/kg wet weight) in brown trout and smallmouth bass collected in 2006. In the "Male/Female" column, the first and second numbers listed for each site (e.g., 14/13) are the numbers of male and female specimens.

Species and Station	Number of Specimens	Riverage Group	Male / Female	River Age		CTPCB		CTPCB/LIPID	
				Mean	Range	Mean	Range	Mean	Range
Brown trout									
W. Cornwall	30	all	5/25	0.6	0.3-1.5	1.21	0.43-3.86	49	5-119
W. Cornwall	12	<0.34	3/9	0.3	0.33	1.17	0.87-1.54	37	10-84
W. Cornwall	11	0.34-0.9	1/10	0.5	0.5	1.14	0.73-2.25	69	30-119
W. Cornwall	6	0.91-1.4	1/5	0.9	0.9	1.00	0.43-1.74	27	5-59
W. Cornwall	1	1.5	0/1	1.5	—	3.86	—	112	—
Smallmouth bass									
W. Cornwall	10	all	2/8	5.1	3-8	0.89	0.51-1.83	71	38-141
Bulls Bridge	10	all	2/8	6.6	3-11	1.08	0.43-1.71	81	47-152
L. Lillinonah	10	all	6/4	4.4	3-5	0.35	0.24-0.49	31	9-60
L. Zoar	10	all	3/7	6.2	5-10	0.58	0.16-2.25	33	12-75

Comparison with Previous Years

Smallmouth bass and brown trout were the primary fish species of interest in the 2006 monitoring study. Comparisons among years were therefore restricted to these two species, excluding hatchery trout. (A tabular comparison of average PCB content in all species of fishes collected in 1984–2006, without adjustment for the influence of covariables, can be found in Appendix E.)

Smallmouth Bass

Visual inspection of sample (geometric) means for smallmouth bass suggests that TPCB concentrations were lower during 1994–2006 than during 1986–1992 (Fig. 2A; Table 4).

This pattern is also suggested by the lipid-normalized TPCB data (Fig. 2B) and by the CTPCB data (Table 4, 1994–2006 versus 1992).

ANCOVA confirmed this apparent pattern for both TPCB and CTPCB. (Statistically significant main effects, covariates, and interactions in the ANCOVA models are summarized in Appendix F.) For the analysis involving TPCB data from all stations combined, the least squares means and pairwise comparisons show a trend from higher concentrations in 1988–1992 to lower concentrations in the more recent years. The adjusted mean TPCB concentration in 2006 was not statistically significantly different from that in 2004 or from those in 1994–1998, although it was higher than those in 2000 and 2002, which had the lowest means among all years (Table 5). However, concentrations in recent years (1994–2006) were significantly lower than those in 1988–1992 (Table 5). Pairwise comparisons of the CTPCB concentration also show the highest concentration in 1992, followed by lower concentrations in more recent years, with no significant differences between the 2006 mean and those in 2000 through 2004 or those in 1994 and 1996 (Table 5).

When stations were tested separately for differences between years, there was a general pattern of decrease after 1992, but there were some differences in the temporal patterns among stations (Table 6). (Statistically significant main effects, covariates, and interactions in the ANCOVA models are summarized in Appendix F.) At West Cornwall, TPCB concentrations in 2006 were not significantly different from those in 2004 or in 1994 through 2000 (although the 2006 mean was higher than that in 2002), and the concentrations in those recent years (1994–2004) were lower than in 1988–1992. The same general pattern was seen at Bulls Bridge, although the trend is not as regular. Pairwise comparisons for that station showed that TPCB concentrations in 2006 were statistically significantly higher than in 2000 and 2002 (though the magnitude of increase is small), but were not significantly different from those in 2004 and were similar to or lower than the concentrations in 1998 and prior years. At Lake Lillinonah, TPCB concentrations in 2006 were not significantly different from those in 1994–2004, and the concentrations in 2000–2004 were lower than those in 1984–1988 and 1992. At Lake Zoar, TPCB concentrations in 2006, while lower than those in 1990 and 1992, were not significantly different from those in any other years.

Brown Trout

Visual inspection of sample (geometric) means for brown trout suggests that mean TPCB and CTPCB concentrations in 2006 were similar to mean concentrations in 1994–2004 and well below the mean concentrations in 1992 (and prior years, for TPCB) (Fig. 3A; Table 4; Appendix G). The same pattern is suggested by the lipid-normalized data for 1984–2004 (Fig. 3B).

This apparent pattern was generally confirmed by ANCOVA. (Statistically significant main effects, covariates, and interactions in the ANCOVA models are summarized in Appendix F.) Pairwise comparisons showed that TPCB concentrations in 2006 were higher than in 1996, but not significantly different from those in the other recent years (1994 and 1998–2004), and that TPCB concentrations in 1994–2006 were significantly

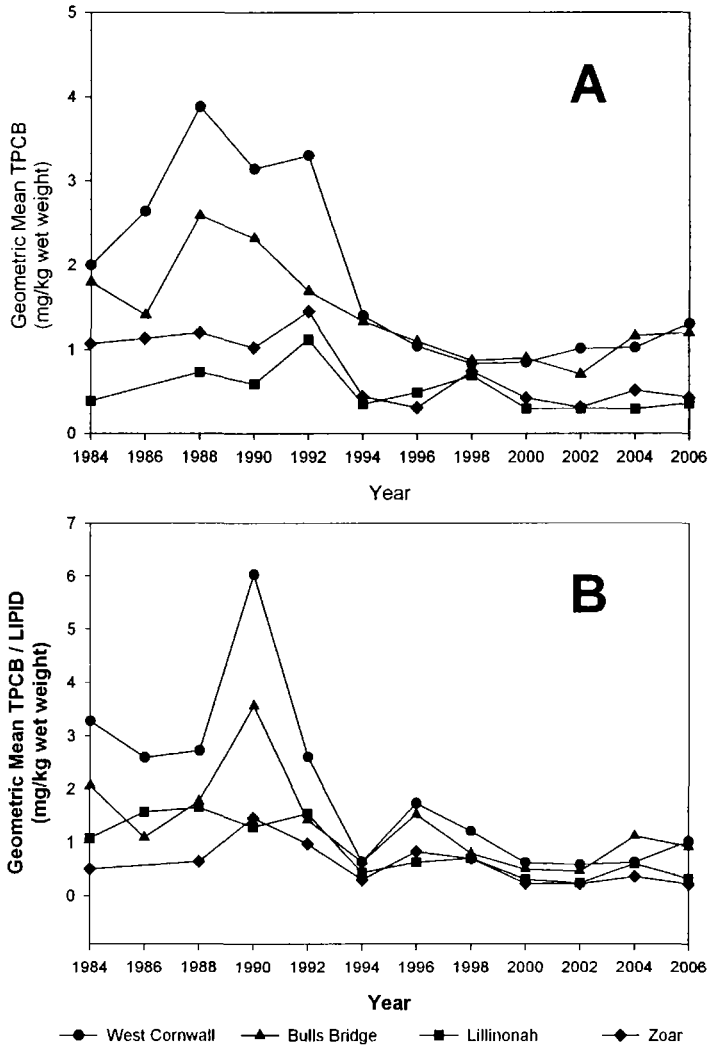

Figure 2. Trends in PCB concentrations in smallmouth bass at four sampling stations on the Housatonic River, 1984–2006. Panel A — Geometric means (unadjusted) of TPCB. Panel B — Geometric means (unadjusted) of lipid-normalized TPCB (TPCB divided by proportion lipid). The pronounced peak in lipid-normalized TPCB in 1990 is due to unusually low lipid levels rather than high TPCB levels (e.g., see Appendix F in ANSP 1995).

TABLE 4. Geometric means (unadjusted) of congener-based total PCB estimates (CTPCB) and Aroclor-based estimates (TPCB) for fish collected in the Housatonic River, CT, 1984–2006.

Year	Brown Trout		Smallmouth Bass			
	W. Cornwall	Hatchery	W. Cornwall	Bulls Bridge	Lillinonah	Zoar
CTPCB						
2006	1.12	0.01	0.83	0.98	0.34	0.37
2004	1.59	0.09	0.88	1.00	0.44	0.25
2002	1.60	0.30	1.04	0.73	0.32	0.31
2000	1.43	0.03	0.86	0.91	0.45	0.27
1998	2.22	0.12	0.72	0.87	0.78	0.69
1996	1.35	—	0.94	0.98	0.28	0.46
1994	1.11	0.42	1.27	1.19	0.41	0.34
1992	6.33	—	2.49	1.29	1.11	0.88
TPCB						
2006	1.40	0.01	1.03	1.26	0.44	0.46
2004	1.85	0.09	1.02	1.16	0.51	0.29
2002	1.55	0.29	1.01	0.71	0.31	0.30
2000	1.41	0.04	0.85	0.9	0.42	0.3
1998	3.02	—	0.83	0.87	0.74	0.69
1996	1.41	—	1.04	1.10	0.31	0.49
1994	1.22	0.43	1.40	1.33	0.44	0.35
1992	8.07	—	3.3	1.69	1.45	1.12
1990	5.30	—	3.14	2.32	1.02	0.59
1988	4.80	—	3.88	2.59	1.20	0.73
1986	5.51	—	2.64	1.41	1.13	—
1984	2.30	—	2.00	1.80	1.07	0.39

TABLE 5. Results of smallmouth bass multiple-comparison tests for pairwise differences between least squares means (LSMs) for years or stations, based on the natural logarithm of TPCB for 1984–2006 (excluding 1986) (left column) and the natural logarithm of CTPCB for 1992–2006 (right column) after adjusting for the effects of covariates. Untransformed LSMs can be estimated from the values reported in this table as follows: $y = e^x$, where x is the LSM reported in this table and y is the corresponding untransformed LSM. Years or stations with the same "Group" letter code are not statistically significantly different from one another at $\alpha = 0.05$. These groups are summarized in the bottom table of each column, where years and stations are grouped (with parentheses) from left to right in order of decreasing LSM.

ln(TPCB)

Year comparisons

Year	LSM	Group
1984	0.1464	b
1988	0.5010	a
1990	0.7727	a
1992	0.5194	a
1994	-0.3190	bc
1996	-0.4243	c
1998	-0.1983	bc
2000	-0.9298	d
2002	-0.9699	d
2004	-0.4290	c
2006	-0.4148	c

Station comparisons

Station	LSM	Group
B	0.2252	a
C	0.3096	a
L	-0.4389	b
Z	-0.7306	c

Summary

Effect	Significance Groups*
Years	(90 92 88) (84 98 94) (98 94 06 96 04) (00 02)
Stations	(C B) L Z

ln(CTPCB)

Year comparisons

Year	LSM	Group
—		
—		
—		
1992	0.3074	a
1994	-0.3300	bc
1996	-0.6196	cd
1998	-0.1473	b
2000	-0.7336	d
2002	-0.8173	d
2004	-0.5172	c
2006	-0.5668	cd

Station comparisons

Station	LSM	Group
B	-0.0719	a
C	-0.0435	a
L	-0.7224	b
Z	-0.8743	b

Summary

Effect	Significance Groups*
Years	(92) (98 94) (94 04 06 96) (06 96 00 02)
Stations	(C B) (L Z)

*Listed in order of decreasing LSM

TABLE 6. Results of smallmouth bass multiple-comparison tests for pairwise differences between least squares means (LSMs) for years at each sampling station, based on the natural logarithm of TPCB for 1984–2006 (excluding 1986) after adjusting for the effects of covariates (see Table 8 for the corresponding untransformed LSMs). Years or stations with the same "Group" letter code are not statistically significantly different from one another at $\alpha = 0.05$. These groups are summarized in the bottom table, where years are grouped (with parentheses) from left to right in order of decreasing LSM.

W. Cornwall

Year	LSM	Group
1984	0.1060	de
1986	1.0252	abc
1988	1.1414	ab
1990	1.2793	a
1992	1.3304	a
1994	-0.0368	de
1996	-0.1406	de
1998	-0.0463	de
2000	-0.3604	de
2002	-0.5516	e
2004	0.1525	bcd
2006	0.1407	cd

Bulls Bridge

Year	LSM	Group
1984	0.6693	abc
1986	0.3106	cde
1988	0.9445	ab
1990	1.2703	a
1992	0.5666	abcd
1994	0.3582	bcde
1996	-0.0370	defg
1998	-0.1441	efg
2000	-0.5688	fg
2002	-0.5692	g
2004	0.1038	cdef
2006	0.2630	cde

Lake Lillinonah

Year	LSM	Group
1984	0.1231	ab
1986	0.1249	abc
1988	0.0790	abcde
1990	-0.0177	abcd
1992	0.3316	a
1994	-0.5156	bcdef
1996	-1.0767	ef
1998	-0.2123	abcde
2000	-0.9898	ef
2002	-1.1648	f
2004	-0.6396	cdef
2006	-0.8477	def

Lake Zoar

Year	LSM	Group
1984	-0.6292	bcd
1986	—	
1988	-0.3645	abc
1990	0.0526	a
1992	-0.1351	ab
1994	-1.2547	bcd
1996	-0.5953	abcd
1998	-0.3792	abc
2000	-1.6271	d
2002	-1.2476	cd
2004	-1.2502	cd
2006	-1.0479	abcd

Summary

Station	Significance Groups*
W. Cornwall	(92 90 88 86) (88 86 04) (86 04 06) (04 06 84 94 98 96 00) (84 94 98 96 00 02)
Bulls Bridge	(90 88 84 92) (88 84 92 94) (84 92 94 86 06 04) (92 94 86 06 04 96) (94 86 06 04 96 98) (04 96 98 00) (96 98 00 02)
Lake Lillinonah	(92 84 86 88 90 98) (84 86 88 90 98 94) (86 88 90 98 94 04) (90 98 94 04 06) (98 94 04 06 00 96) (94 04 06 00 96 02)
Lake Zoar	(90 92 88 98 96 84 06) (92 88 98 96 84 06 94) (88 98 96 84 06 94 02 04) (96 84 06 94 02 04 00)

*Listed in order of decreasing LSM

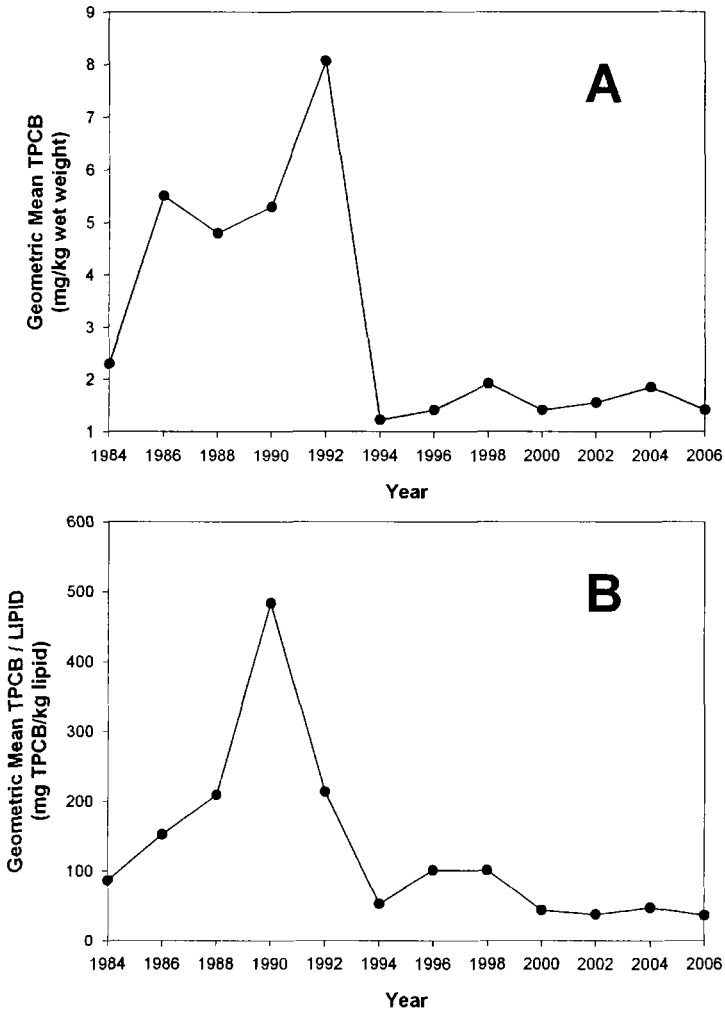

Figure 3. Trends in PCB concentrations in brown trout collected from West Cornwall, 1984–2006. Panel A — Geometric means (unadjusted) of TPCB. Panel B — Geometric means (unadjusted) of lipid-normalized TPCB (TPCB divided by proportion lipid). The pronounced peak in lipid-normalized TPCB in 1990 is due to unusually low lipid levels rather than high TPCB levels (e.g., see Appendix F in ANSP 1995).

TABLE 7. Results of brown trout multiple-comparison tests for pairwise differences between least squares means (LSMs) for years at West Cornwall, based on the natural logarithm of TPCB for 1984–2006 (left column) and the natural logarithm of CTPCB for 1992–2006 (right column) after adjusting for the effects of covariates (see Table 8 for the corresponding untransformed LSMs). Years or stations with the same "Group" letter code are not statistically significantly different from one another at $\alpha = 0.05$. These groups are summarized in the bottom table, where years are grouped (with parentheses) from left to right in order of decreasing LSM.

ln(TPCB)

Year	LSM	Group
1984	0.951	cd
1986	1.319	c
1988	1.624	bc
1990	1.914	ab
1992	2.054	a
1994	0.539	def
1996	-0.097	g
1998	0.599	def
2000	0.339	ef
2002	0.252	fg
2004	0.674	de
2006	0.344	ef

ln(CTPCB)

Year	LSM	Group
—		
—		
—		
—		
1992	0.823	a
1994	0.475	abc
1996	0.604	abc
1998	0.728	ab
2000	0.433	bcd
2002	0.336	cd
2004	0.699	ab
2006	0.118	d

Summary

Measure	Significance Groups*
ln(TPCB)	(92 90) (90 88) (88 86) (84 04 98 94) (04 98 94 06 00) (98 94 06 00 02) (96)
ln(CTPCB)	(92 98 04 96 94) (98 04 96 94 00) (96 94 00 02) (00 02 06)

*Listed in order of decreasing LSM

TABLE 8. Untransformed least-squares means (LSMs) corresponding to the LSMs of transformed TPCB and CTPCB concentrations shown in Figures 2 and 3 and listed in Tables 6 and 7. Values in this table have units of mg/kg wet weight and are related to those in Figures 2 and 3 and in Tables 6 and 7 as follows: $y = e^x$, where x is a value in Figures 2 and 3 and y is the corresponding value in this table. All smallmouth bass LSMs are for TPCB, while LSMs for both TPCB and CTPCB are presented for brown trout.

Year	2006	2004	2002	2000	1998	1996	1994	1992	1990	1988	1986	1984
Smallmouth Bass												
W. Cornwall	1.15	1.16	0.58	0.70	0.95	0.87	0.96	3.78	3.59	3.13	2.79	1.11
Bulls Bridge	1.30	1.11	0.57	0.57	0.87	0.96	1.43	1.76	3.56	2.57	1.36	1.95
Lillinonah	0.43	0.53	0.31	0.37	0.81	0.34	0.60	1.39	0.98	1.08	1.13	1.13
Zoar	0.35	0.29	0.29	0.20	0.68	0.55	0.29	1.14	1.05	0.69	—	0.53
Brown Trout												
W. Cornwall (TPCB)	1.41	1.96	1.29	1.40	1.82	0.91	1.72	7.80	6.78	5.07	3.74	2.59
W. Cornwall (CTPCB)	1.13	2.01	1.40	1.54	2.07	1.83	1.61	2.28	—	—	—	—

lower than those in 1986–1992 (Table 7). Pairwise comparisons of the CTPCB concentrations in brown trout show that 2006 had the lowest adjusted mean concentration of any year, and that the mean concentration in 2006 was not significantly different from those in 2000 and 2002 but was significantly lower than those in 1992–1998 and 2004 (Table 7; see also Table 4).

Comparison among Stations

Visual inspection of mean TPCB and CTPCB concentrations for smallmouth bass in 2006 indicates that concentrations appear higher at the two upstream stations (West Cornwall and Bulls Bridge) than at the two downstream stations (Lake Lillinonah and Lake Zoar) (Table 4; Fig. 2). A similar pattern of downstream decrease in TPCB and CTPCB was found in previous biological monitoring studies.

Using a statistical model that included data from all years, analysis of covariance revealed the following statistically significant station differences in mean TPCB and CTPCB concentrations: Pairwise comparisons indicated that, for both TPCB and CTPCB, West Cornwall and Bulls Bridge PCB concentrations did not differ significantly, but both were greater than at Lake Lillinonah and Lake Zoar (Table 5). Mean TPCB concentrations at Lake Lillinonah were significantly greater than those at Lake Zoar, while the difference was not significant for CTPCB (Table 5).

Fish Exceeding the FDA Fish Consumption Tolerance Limit

Previous reports on the Housatonic River biological monitoring studies have included an assessment of the percentage of fish with total PCB concentrations in fillets exceeding the U.S. Food and Drug Administration (FDA) fish consumption tolerance limit of 2.0 mg/kg wet weight. For comparison with those prior assessments, a similar assessment was conducted for fish collected in 2006.

One smallmouth bass, a 47.5 cm female, collected from Lake Zoar in 2006 had a CTPCB concentration above the FDA limit, and four had a TPCB concentration exceeding that level: the same 47.5 cm fish from Zoar, a 36.3 cm long, 8-year old male fish from West Cornwall, a 37.3 cm long, 7-year old male and a 37.2 cm long, 5-year old female from Bulls Bridge. Among brown trout, 2 of 30 samples (7%) had CTPCB concentrations exceeding the FDA limit. These included the only holdover fish from the spring, 2005, stocking (a 37.3-cm fish collected in October) and a 30.5 cm fish stocked in the spring of 2006 and caught in October. Three of the 30 brown trout samples (10%) had TPCB concentrations exceeding the FDA limit. These included the same two fish with CTPCB greater than 2 mg/kg and a 36.7-cm long holdover fish stocked in the fall of 2005 and caught in August.

The percentages of brown trout and smallmouth bass with total PCB concentrations *less* than the FDA limit in each study year are shown in Table 9. As shown in that table, the percentages of brown trout with TPCB and CTPCB concentrations less than 2.0 mg/kg wet weight in 2006 were greater than the percentages found in prior years. Since 1994, the percentages have fluctuated but have been substantially above the percentages in prior years. For smallmouth bass, the percentage of fish with TPCB and CTPCB

TABLE 9. Summary of percentages of brown trout and smallmouth bass at each sampling station with total PCB concentrations less than 2.0 mg/kg wet weight. All percentages except those in parentheses are based on TPCB. Values in parentheses are based on CTPCB (available for years 1992–2006) and are given only where different from those based on TPCB.

Year	Brown Trout	Smallmouth Bass			
	W. Cornwall	W. Cornwall	Bulls Bridge	Lillinonah	Zoar
2006	90 (93)	90 (100)	80 (100)	100 (100)	90 (90)
2004	63 (87)	90 (100)	100	100	100
2002	73 (70)	100	100	100	100
2000	86	100	100	100	100
1998	60	100	100	100	90
1996	60 (70)	100	100	100	100
1994	86 (92)	69 (77)	100	100	100
1992	0 (2)	14 (21)	75 (88)	75 (88)	71
1990	0	17	17	100	100
1988	0	8	21	88	88
1986	4	31	58	77	—
1984	50	38	50	92	100

concentrations less than 2.0 mg/kg wet weight in 2006 ranged from 80% (TPCB at Bulls Bridge) to 100%. Overall, the percentages of smallmouth bass with PCB concentrations less than the FDA limit have remained high since 1994.

In addition to the results for brown trout and smallmouth bass samples in the long-term monitoring study, the 2006 study also included 20 supplemental samples of northern pike from Falls Village, Bulls Bridge, Lake Lillinonah, and Lake Zoar (5 samples from each station). As discussed in more detail in Appendix J, one of the 20 samples (5%) had a CTPCB concentration (3.02 mg/kg wet weight) greater than 2.0 mg/kg wet weight, and two (10%) had TPCB concentrations (3.80 and 2.08 mg/kg wet weight) greater than that level.

Benthic Insects

Benthic aquatic insect larvae were collected in the general vicinity of West Cornwall in June 2006 and were analyzed for total PCB and lipid. Three taxonomic groups were sampled: filter-feeding caddisflies (family Hydropsychidae), predatory dobsonflies (family Corydalidae; the aquatic larvae are also known as hellgrammites), and predatory stoneflies (family Perlidae). The amount of material collected in the field was sufficient to permit analysis of two composite samples for each group. The results are summarized in Table 10.

Historical data on total PCB concentrations in Housatonic River benthic insects are shown in Figure 4 (CTPCB) and Figure 5 (TPCB) (the Academy's data for 1992–2006 are tabulated in Appendix H). CTPCB concentrations in stoneflies in 2006 were similar

TABLE 10. PCB and lipid levels in aquatic insects collected from the Housatonic River in the vicinity of West Cornwall in June 2006. CTPCB denotes congener-based total PCB concentrations, while TPCB denotes Aroclor-based total PCB concentrations. Lipid-normalized values are given in units of mg CTPCB or TPCB in wet tissue per kg lipid in wet tissue. Values for all three insect taxa are geometric means of two composite samples (arithmetic means are similar and are not shown).

Taxon	Proportion Lipid (kg lipid / kg wet tissue)	Tissue Concentration (mg PCB / kg wet tissue)		Lipid-normalized Concentration (mg PCB / kg lipid)	
		CTPCB	TPCB	CTPCB	TPCB
Caddisflies (Hydropsychidae)	0.039	1.33	1.61	20.69	21.21
Dobsonflies (Corydalidae)	0.045	1.46	1.93	32.24	42.62
Stoneflies (Perlidae)	0.031	0.64	0.66	34.36	41.74

to those in 1998, 2001, 2002, and 2005 (Fig. 4). Concentrations in caddisflies and dobsonflies were similar to those in 2001 but appear slightly higher than those in 2002 and 2005. All three taxa exhibited lower CTPCB concentrations than in 1992–1996. When dobsonfly and stonefly TPCB concentrations were averaged to obtain a single estimate for predators, TPCB concentrations in both filter feeders and predators in 2006 appeared slightly higher than the corresponding values in 2002 and 2005, similar to those in 2001, and well below most of the values in 1978–1992 (Fig. 5).

The historical data series shown in Figure 5 suggests decreasing trends in TPCB concentrations in both filter feeders and predators. Kendall's test of rank correlation was used to determine whether there is statistically sound evidence for these apparent trends. The results indicate highly statistically significant decreasing trends in both groups of benthic insects (Table 11).

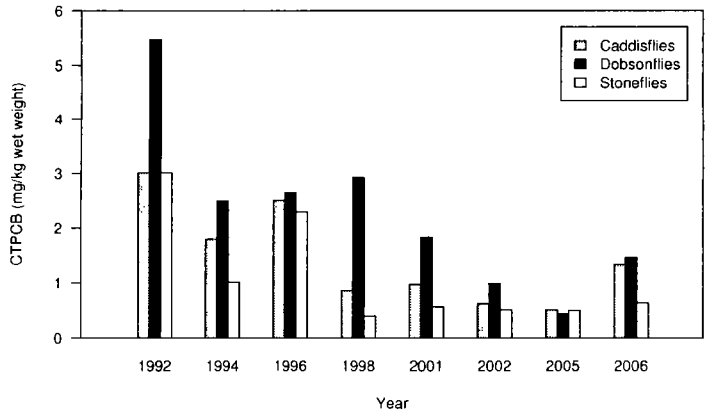

Figure 4. Total congener-based PCB concentrations (CTPCB) in benthic aquatic insects from West Cornwall, 1992–2006. Caddisflies are filter feeders, while dobsonflies and stoneflies are predators. Values are geometric means of replicate composite samples for each group. Plotted values are tabulated in Appendix H.

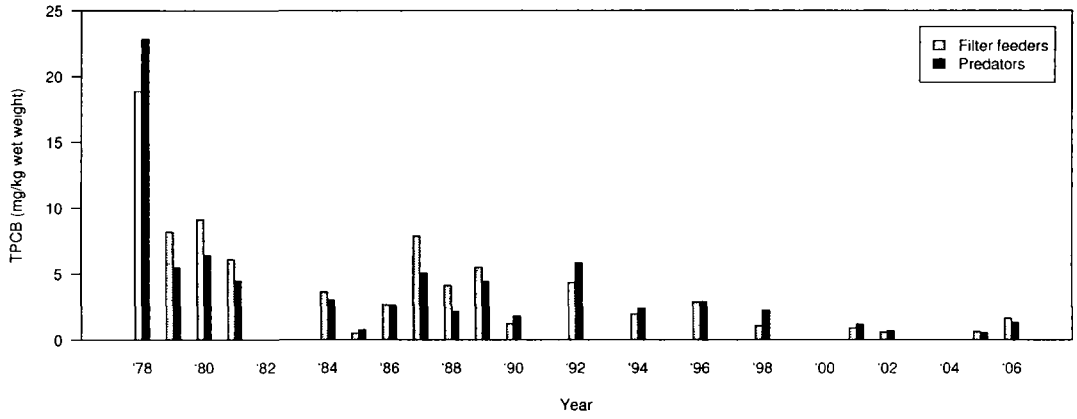

Figure 5. Historical data series of total Aroclor-based PCB concentrations (TPCB) in benthic aquatic insects, 1978–2006. Filter feeders include only hydropsychid caddisflies, while predators include both dobsonflies and perlid stoneflies. Values for predators are arithmetic means of separate values for dobsonflies and stoneflies.

TABLE 11. Results of Kendall's test of rank correlation between TPCB and study year for filter-feeding and predatory insects, 1978–2006. Reported *p* values are for one-tailed tests of the null hypothesis that the true correlation is zero, with the alternative hypothesis that the true correlation is negative. Since the same test is applied to two groups, each *p* value should be compared with Bonferroni-adjusted error rate $\alpha/2 = 0.025$ to ensure an experiment-wise error rate of $\alpha = 0.05$. Note that *p* is much less than 0.025 for both insect groups, providing strong evidence that the true correlation between TPCB and study year is negative in both cases.

Insect Group	Number of Studies	Correlation Coefficient (Kendall's τ)	*p* Value
Filter feeders	19	-0.58	0.0002
Predators	19	-0.57	0.0002

Precision, Accuracy, and Detection Limit Analyses

Methods used to assess precision, accuracy, and detection limits were the same as in the 2002 and 2004 studies and are described below.

Detection Limits

Matrix blanks were generated to monitor possible laboratory contamination and to calculate the detection limits for PCBs. Each matrix blank, consisting of approximately 30 g of clean Na_2SO_4, was analyzed using the same procedures as the samples. Chromatograms of most blanks were devoid of significant peaks, suggesting that little contamination through laboratory exposure occurred.

The detection limit on a mass (ng) basis for each congener (or co-eluting congeners) was estimated as the blank area plus three times the standard deviation of the average blank

peak areas. These values were then compared to the resulting mass obtained from each chromatographic peak representing an analyte. When an analyte mass was found to be less than the detection limit for that analyte (on a mass basis), that value was reported as "BDL". The method detection is commonly reported on a mass of analyte per mass sample basis (ng/g wet weight) by dividing the detection limit (ng) by the average wet mass extracted (ca. 5 g). The matrix blank-based detection limits for PCBs ranged from 0.01 ng/g (congener 129) to 9.05 ng/g (congener 3). Based on the matrix blanks, the average detection limit for total PCBs was 0.44 ng/g. The sum of the individual detection limits (ng/g wet weight basis) was 9.2 ng/g.

Surrogate Recoveries

Analyte loss through analytical manipulations was assessed by the addition of surrogate PCB congeners 14, 65 and 166 to all samples prior to extraction by Soxhlet apparatus. Average recoveries of congeners 14, 65 and 166 were 90 ± 10%, 100 ± 13% and 108 ± 23%. With a lower standard deviation, constant recoveries regardless of contaminant concentration, and no known interferences, congeners 14 and 65 are the most reliable for assessing analyte loss. Reported values for PCB concentration in this study were not corrected for analyte loss.

Duplicate and Triplicate Analyses

Relative percent difference (RPD) values for duplicates analyses were low, with an average of 19%. Relative standard deviation (RSD) values for triplicate analyses were also low, with an average of 15%. These results indicate a high degree of precision.

Standard Reference Materials

For this study, National Institute for Standards and Technology (NIST) standard reference material (SRM 1946, Lake Superior Fish Tissue) was used to evaluate extraction efficiency and analytical accuracy. With the exception of congeners 18, 63, 99, 158, 201, and 206, average percent recoveries were 123%. The average recovery for those six congeners was 523%, and these are typically those that represent the lowest concentrations within the SRM matrix. As concentration decreases within a sample, the associated standard error (a measure of the ability to accurately quantify the true concentration) increases. This trend is observed in our evaluation of the SRM concentrations and is typical for PCB analysis. Despite the high recoveries for the subset of six congeners, the Academy analysis not only predicts the PCB patterns within the SRM but estimates the magnitude of most congeners accurately, as well (Fig. 6).

Method Spikes

Analyte loss for all PCB congeners was determined through method spikes, using a 25:18:18 mixture of Aroclors 1232, 1248, and 1262 into a blank matrix (one containing no biological matrix). The average percent recovery of spiked congeners was 111%. The average relative standard error for method spikes was ±11%.

Combining Congeners

In previous studies, congeners 31 and 28 were reported separately. These two congeners were reported as '31+28' in the 2006 study. Similarly, congeners 41 and 71 were reported separately in previous studies but as '41+71' in the 2006 study. The two congeners in each of these pairs typically elute at very similar retention times and consequently are not well resolved. In previous studies, we were able to quantify them separately, but chromatograms produced in the 2006 study showed that the two congeners in each pair had eluted unusually closely together. As a result, estimating where one peak ended and the other began could not be done accurately. We therefore chose to treat each pair as a single peak and to report them as coeluting congeners. This procedure yields an accurate estimate of the sum of the two congener concentrations in each pair.

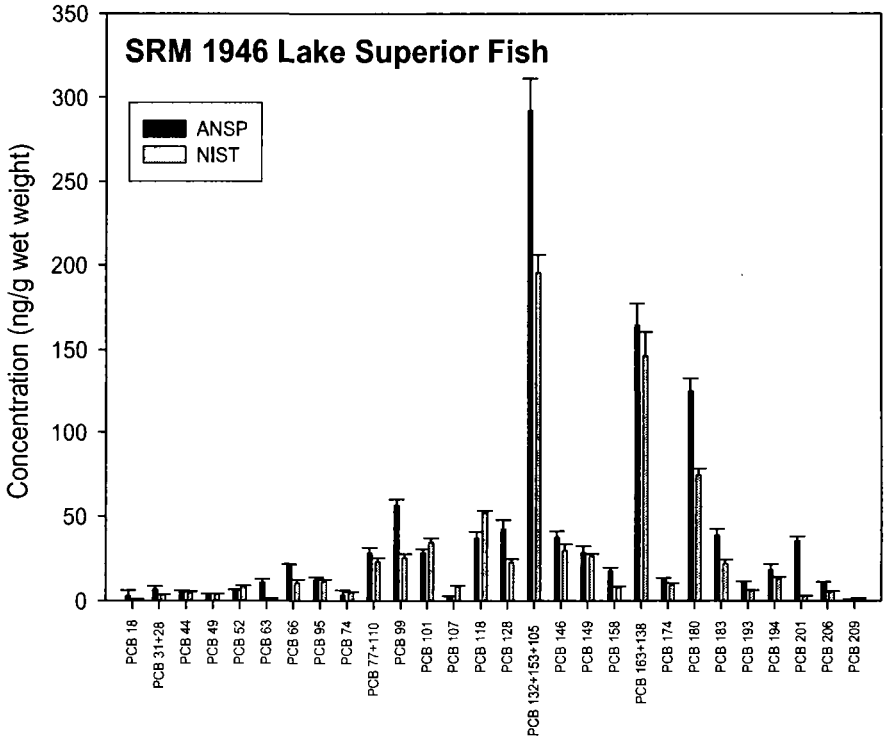

Figure 6. Comparison of Academy and NIST PCB values for SRM 1946 (error bars represent standard deviations).

DISCUSSION

The results of this study of PCB concentrations in fish and benthic insects of the Connecticut portion of the Housatonic River consist of among-year and among-station comparisons of smallmouth bass at four sampling stations, and among-year comparisons of brown trout and benthic insects at a single sampling station (West Cornwall).

For smallmouth bass, there was a clear pattern of low TPCB concentrations during 1994–2006 compared to 1992 and earlier. Similarly, CTPCB concentrations (which are only available for 1992–2006) were lower in 1994–2006 than in 1992. These patterns were confirmed statistically for both TPCB and CTPCB using analysis of covariance and pairwise comparisons between years. There were some differences in the temporal patterns among stations, though these statistical analyses confirmed that the concentrations in 2006 and other recent years were lower than the concentrations in 1992 and prior years. Within the lower concentrations of the 1994–2006 period, the adjusted mean TPCB concentrations in 2006 in smallmouth bass at West Cornwall and Bulls Bridge were significantly higher than that in 2002 (and 2000 for Bulls Bridge), but were not significantly different from that in 2004 and were similar to or lower than the concentrations in 1998 and prior years. For smallmouth bass at Lake Lillinonah and Lake Zoar, the adjusted mean TPCB concentrations in 2006 were not significantly different from those in 1994–2004 and were generally lower than those in prior years.

Visual inspection of the 2006 TPCB and CTPCB data for smallmouth bass also indicates a pattern of decreasing concentrations from the two upstream stations (West Cornwall and Bulls Bridge) in a downstream direction to Lake Lillinonah and Lake Zoar, as in previous monitoring studies. This pattern was confirmed statistically, using analysis of covariance, which showed that, for both TPCB and CTPCB, concentrations at the two upstream stations did not differ significantly from each other but were higher than those at Lake Lillinonah and Lake Zoar.

For brown trout, TPCB and CTPCB concentrations in 2006 were lower than or equal to concentrations in 1994–2004 and were well below levels observed in 1992. This pattern was generally confirmed by analysis of covariance with pairwise comparisons between years. In the case of TPCB, pairwise comparisons showed that the mean 2006 concentration was not significantly different from those in 1994 and 1998-2004 and that concentrations in 1994–2006 were significantly lower than those in 1986–1992. For CTPCB, the mean 2006 concentration was not significantly different from those in 2000 and 2002 and was significantly lower than those in 1992–1998 and 2004. In fact, the mean for 2006 was slightly lower than that in the other recent years.

Historically, PCB concentrations in fish of the Connecticut portion of the Housatonic River exhibited a pattern of high values in the late 1970s, a substantial decrease around 1980, and subsequently variable behavior at concentrations well below those of the late 1970s (ANSP 1997). After unusually low levels were observed in 1984, higher levels were found in 1986–1992. There was a substantial decrease in PCB concentrations in

1994, and that decrease largely persisted in 1996 and 1998. The 2000, 2002, 2004, and 2006 data all show PCB concentrations roughly similar to those in 1994–1998.

The same temporal pattern is reflected in the percentage of fish with fillet PCB concentrations exceeding the FDA tolerance limit of 2.0 mg/kg wet weight. In the 1984–1992 studies, smallmouth bass with concentrations exceeding that limit were relatively common at most stations, with the exceedance percentage typically being highest at West Cornwall and decreasing downstream. In 1994–1998, smallmouth bass exceeding the limit were rare. In the 2000, 2002, and 2004 studies, none of the smallmouth bass collected from the 4 stations had a CTPCB concentration exceeding the limit (although one bass in 2004 had a TPCB concentration exceeding that level). In 2006, four smallmouth bass had TPCB concentrations exceeding the limit (one of which also had a CTPCB concentration exceeding the limit). Among brown trout, nearly all the fish collected from West Cornwall in the years 1986–1992 had PCB concentrations exceeding the FDA limit. Since then, the percentage of trout exceeding the limit has decreased substantially. In the 2006 study, only 7% of the specimens from West Cornwall had CTPCB concentrations that exceeded the FDA limit, and 10% of the specimens from West Cornwall had TPCB concentration above that limit. The percentages of trout exceeding the FDA limit depend partly on the proportion of holdover trout in the samples, since in recent years, these represent many of the fish which exceed the FDA limit. In 2006, there was only one holdover trout in the samples from the previous spring stocking, and six stocked in the fall of 2005.

Analysis of benthic insect samples showed that PCB concentrations in predatory stoneflies in 2006 were similar to those in 1998, 2001, 2002, and 2005, while concentrations in filter-feeding caddisflies and predatory dobsonflies were similar to those in 2001 but appeared slightly higher than in 2002 and 2005. PCB concentrations in both predators and filter feeders were well below most of the values in 1978–1992, and rank correlation analysis of the entire data series for 1978–2006 revealed a highly statistically significant temporal trend of decreasing PCB concentrations in both filter feeders and predators.

In summary, results of the 2006 fish monitoring study show that total PCB concentrations in brown trout and smallmouth bass were generally similar to those observed in the 1994–2004 studies, and were well below the levels observed in 1992 and most prior years. Similar patterns hold for both filter-feeding and predatory benthic insects, which also show a highly statistically significant temporal trend of decreasing total PCB concentration over the monitoring period, 1978–2006. These findings indicate that the substantial reduction in PCB content of fishes and benthic insects that occurred after the 1992 study and was seen in the 1994–2004 studies has persisted into 2006.

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APPENDICES

APPENDIX A

SOP No. P-16-77: Extraction and Cleanup of Fish Tissue for PCB and Pesticide Analysis.

ACADEMY OF NATURAL SCIENCES
ENVIRONMENTAL RESEARCH DIVISION

Procedure No. P-16-77
Rev. 1 (4/95)

EXTRACTION AND CLEANUP OF FISH TISSUE FOR PCB AND PESTICIDE ANALYSIS

EXTRACTION AND CLEANUP OF FISH TISSUE FOR PCB AND PESTICIDE ANALYSIS

Prerequisite: Use of this method requires a working knowledge of the inherent hazards and possible routes of contamination in working with organic solvents. Also, a working knowledge of glassware cleaning and standard residue analysis techniques is required.

1.0 METHOD

This method includes instructions for extracting PCBs and pesticides from fish tissue. Also, specific criteria for gas chromatography (ECD-capillary) and quantitation on a congener and compound specific basis is included. For basic instructions on gas chromatography see SOP No. P-16-84.

2.0 SUMMARY

The fish tissue is combined with sodium sulfate, Soxhlet extracted and concentrated to 10 ml. One ml of this extract is taken and analyzed for lipid content. The remainder of the extract is mixed with concentrated acid to destroy the lipid and other biogenic material and finally cleaned up by Florisil sep-pak chromatography.

3.0 STANDARDS

3.1 PCB Standard

Mixture of Aroclors 1232, 1248, and 1262 in a 25:18:18 ratio. Individual Aroclor concentrations of 250 ng/ml (Aroclor 1232), 180 ng/ml (Aroclor 1248), and 180 ng/ml (Aroclor 1262) are recommended for total PCB concentration of 610 ng/ml.

3.2 Pesticide Standard

Mixed pesticide standard containing 19 organochlorine pesticides and industrial compounds.

3.3 Internal Standard

80 ng of 2,4,6-trichlorobiphenyl (PCB 30) and 60 ng of 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB 204).

3.4 Surrogate Standard

210 ng of 3,5-dichlorobiphenyl (PCB 14), 50 ng of 2,3,4,4',5,6-hexachlorobiphenyl (PCB 166), and 200 ng of delta hexachlorocyclohexane (δ-HCH).

4.0 APPARATUS

4.1 Glassware (all cleaned using SOP No. P-16-37)

For Extraction: Soxhlet extractors (200 ml), Allihn condensers, 500-ml round bottom flasks, glass thimbles (4 cm x 11 cm) .

For Sample Preparation: 250-ml beakers, stainless steel spatula, 10-ml volumetric flasks, syringe with stainless steel needle, 12 ml vials with Teflon lined screw caps.

4.2 Glass wool for extraction.

4.3 Rotary Evaporator for sample reduction.

4.4 Sodium Sulfate (pre-extracted overnight in dichloromethane).

4.5 Waters Florisil Sep-pak cartridges.

4.6 Sulfuric Acid

4.7 Tekmar Tissuemizer.

4.8 Heating mantles and voltage controllers for extraction.

4.9 Teflon boiling chips (pre-extracted overnight in dichloromethane).

5.0 SAMPLE PREPARATION

5.1 Frozen fish fillets are allowed to thaw and are finely ground using the Tekmar Tissuemizer.

5.2 At the time of analysis, 10.0 g of thawed fish sample is weighed and placed into a 250-ml beaker. The sample is then combined with sodium sulfate in a 1:6 ratio (sample: sodium sulfate) and mixed with a clean spatula until the sample is homogenized.

5.3 The sample mixture is transferred to a glass thimble with glass wool at the bottom and placed into the Soxhlet extractor. At this point the surrogate standard is added. The sample is then extracted overnight (refluxing at least 16 h at 4-6 cycles/h) with 350 ml of 1:1 hexane:acetone mixture.

5.4 The sample extract is then transferred quantitatively from the original 500-ml round bottom flask to a clean flask, with two 25-ml aliquots of hexane. This is done because during extraction, fish and sodium sulfate collect at the bottom of the flask. The extract is reduced to approximately 5 ml using a rotary evaporator, exchanged three times with 25-ml aliquots of hexane, and finally evaporated to 5 ml. Between exchanges the sample is checked for water. If water is present, it is removed with a pasteur pipet.

5.5 The sample extract is then diluted to 10 ml with hexane using a 10-ml volumetric flask. The lipid content of the sample is determined at this point by placing a 1.0-ml aliquot of the extract in a preweighed aluminum pan. This is allowed to sit at room temperature overnight to dry. The pan is reweighed and the % lipid calculated.

$$\%\ Lipid = \frac{g\ of\ lipid}{total\ sample\ wt.\ (g)} \times 1000$$

5.6 The remaining sample extract is concentrated under a stream of ultra high purity (UHP) nitrogen to approximately 2 ml. It is then washed with an equal volume of sulfuric acid and stored in the refrigerator at 4°C overnight or until separation occurs. In cases where lipid content is high it may be necessary to add more sulfuric acid and hexane. The sample extract is returned to the refrigerator to separate. The hexane phase is transferred to another vial, and the acid phase is washed 2-3 times more with 1-2 ml of hexane, combining all hexane washes. The sample extract (in hexane) is then reduced to approximately 2 ml under a stream of UHP nitrogen.

5.7 The sample extract is cleaned by Florisil column chromatography using Waters sep-pak cartridges. The column is pre-rinsed with approximately 10 ml of hexane which is discarded. The sample is then passed through the column. All deliveries to the sep-pak column are made using a glass Luer-lock syringe. To collect the hexane fraction for PCB analysis, the column is then rinsed with four bed volumes of hexane and collected into a 10-ml volumetric flask, and the volume adjusted to 10 ml. After the hexane has run through the syringe an equal amount of dichloromethane is run through the sep-pak to obtain the fraction for pesticide analysis. The dichloromethane fraction is blown down to ~1 ml under N_2 then combined with an equal amount of hexane. This is repeated three more times, and the remaining sample is adjusted to 10 ml with hexane. The sample is then transferred to a 12-ml vial. The sample is now ready for analysis.

6.0 STANDARDS

(For specific volumes and directions see Organic Standards Preparation Logbook.) The following concentrations are recommended based on past GC performance and levels of contaminants typically observed in recent projects.

Working Standards:

PCB Standard: 250 ng/ml of Aroclor 1232, 180 ng/ml of Aroclor 1248, and 180 ng/ml of Aroclor 1262 to yield a total PCB concentration of 610 ng/ml.

Pesticide Standard: Mixed pesticide standard with 19 organochlorine pesticides and industrial compounds of environmental interest.

Surrogate Standard:

210 ng of 3,5 dichlorobiphenyl (PCB 14), 50 ng of 2,3,4,4',5,6 hexachlorobiphenyl (PCB 166), and 200 ng of delta hexachlorocyclohexane (δ-HCH) are added to the sample before extraction.

Internal Standard:

80 ng of 2,4,6 trichlorobiphenyl (PCB 30) and 60 ng of 2,2',3,4,4',5,6,6'- octachlorobiphenyl (PCB 204) are added to the 10-ml sample just before analysis on the GC.

7.0 QA/QC

7.1 Laboratory duplicate, laboratory blanks, and standard reference materials (SRMs) are extracted and analyzed at a frequency of 5 to 10% depending on requirements specified by the contract. Blank spikes are extracted and analyzed at an unspecified frequency to evaluate method performance. Surrogate recoveries provide some measure of method performance for individual sample matrices. Analyte recoveries for SRMs reflect method performance for a variety of compounds in a given type of matrix. Hence, SRMs are used in lieu of conventional matrix spikes in this procedure.

8.0 AROCLOR QUANTITATION

Aroclor 1254 is quantitated as the sum of congeners 52, 49, 44, 41, 74, 70+76, 95+66, 91, 60+56, 84, 101, 99, 83, 97, 87, 85, 110, 82 divided by 0.5252.

Aroclor 1260 is quantitated as the sum of congeners 178, 187, 183, 185, 174, 177, 171, 172, 180, 170, 201, 203+196 divided by 0.3747.

APPENDIX B

SOP No. P-16-84, Rev. 2: Quantitation of Individual Polychlorinated Biphenyl Congeners (PCBs), Chlorinated Pesticides and Industrial Compounds by Capillary Column Gas Chromatography.

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-84
Rev. 6 (6/05)

QUANTIFICATION OF INDIVIDUAL POLYCHLORINATED BIPHENYL CONGENERS (PCBs), CHLORINATED PESTICIDES AND INDUSTRIAL COMPOUNDS BY CAPILLARY COLUMN GAS CHROMATOGRAPHY

Quantification of Individual Polychlorinated Biphenyl Congeners (PCBs), Chlorinated Pesticides and Industrial Compounds by Capillary Column Gas Chromatography

1. SCOPE AND APPLICATION

1.1. This method describes the analysis and quantification of polychlorinated biphenyls (PCBs), selected chlorinated pesticides and industrial compounds by capillary column gas chromatography (GC) with an electron capture detector (ECD). PCBs are quantified on a congener specific basis using this method. The compounds that can be determined by this method are listed in Appendices A and B.

1.2. The selection of compounds of interest may be specified in the project protocol, may be based on existing site data or based on initial screening of samples.

1.3. The analysis is preceded by extraction and clean-up as stated in the relevant SOP for each particular matrix.

1.4. Standards.

1.4.1. A PCB standard is composed of a mix of Aroclors which is composed of most congeners that would be found in environmental samples. Individual congeners of environmental interest not found in the Aroclor mix or found in amounts just above the limit of quantification may be added to the standard. The congeners can be summed for a total PCB (*t*-PCB) value.

1.4.2. A mixed pesticide standard is composed of a mixture of 30 organochlorine pesticides and industrial compounds that are found in environmental samples. Other chlorinated organic compounds of environmental interest may be added to the standard.

2. SUMMARY OF METHOD.

2.1. This method describes a procedure to determine PCBs and pesticides by capillary column gas chromatography (GC) with electron capture detection (ECD). Before using this method, refer to the appropriate sample extraction and clean-up techniques. The clean-up technique (Procedure Nos. P-16-109 and P-16-111) can generate several eluent fractions of different polarity which are analyzed separately to minimize interferences. The first fraction is eluted using a non-polar eluent (petroleum ether). This fraction contains all PCB congeners and some chlorinated pesticides and industrial compounds. The second fraction is eluted with a moderately polar eluent (50:50 dichloromethane:petroleum ether). This fraction contains the

remaining chlorinated pesticides and industrial compounds. Other more polar fractions may follow.

2.2. Samples are quantified on a congener-specific basis using a standard mixture of Aroclors 1232, 1248, and 1262. This mixture may be supplemented with individual congeners of particular environmental interest. Organochlorine pesticides and industrial compounds are quantified using a separate standard containing 31 such compounds of interest. Confirmation of selected analytes may be performed on a second capillary column possessing a different stationary phase.

3. APPARATUS AND MATERIALS.

3.1. Gas Chromatography.

3.1.1. Agilent 6890N GC with dual split/splitless injection ports equipped for capillary columns.

3.1.2. Column.

3.1.2.1. Column: J & W Scientific DB-5 capillary column, part number 122-5062, (5% -phenyl) - methylpolysiloxane stationary phase, 60-m x 0.25-mm I.D., 0.25-μm film thickness, or equivalent.

3.1.3. HP G2397A electron capture detectors (ECDs), or equivalent.

3.1.4. Agilent 7683 Series autosampler (optional).

3.1.5. Dell Computer with version 10 of Agilent's Chemstation software.

3.2. Gases.

3.2.1. Make-up gas - 5% methane/95% argon.

3.2.2. Carrier gas - helium or hydrogen (preferred).

4. REAGENTS, SOLVENTS, AND STANDARDS.

4.1. Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used if it is determined that the reagent is of

sufficiently high purity to permit its use without compromising the accuracy of the determination.

4.2. Solvents.

4.2.1. Hexane - Pesticide quality or equivalent.

4.2.2. Dichloromethane - Pesticide quality or equivalent.

4.3. Standards.

4.3.1. Standards of the Aroclors, individual congeners (for surrogates and internal standards) and organochlorine pesticides of interest are purchased from a commercial supplier.

4.3.2. Surrogate standards- 3,5-dichlorobiphenyl (PCB 14), 2,3,5,6- tetrachlorobiphenyl (PCB 65), and 2,3,4,4',5,6-hexachlorobiphenyl (PCB 166) which are used in the analysis of the nonpolar clean-up fraction and dibutylchlorendate which is used in the analysis of the moderately polar clean-up fraction are purchased from a commercial supplier. Other surrogates may be used in conjunction with or in place of the above as required for special applications.

4.3.3. Internal standards- 2,4,6-trichlorobiphenyl (PCB 30) and 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB 204) are purchased from a commercial supplier as certified standards. Other internal standards may be used in addition to or in place of the above if appropriate for a particular application.

4.4. Performance standards.

4.4.1. PCB standard: A mixed congener standard that contains most congeners that would be found in environmental samples is made by mixing Aroclors 1232, 1248, and 1262 in a 25:18:18 ratio (250, 180, 180 ng/ml recommended for a total concentration of 610 ng/mL) (Appendix A). This mix is supplemented with individual congeners of environmental interest which are not found or are found in very low amounts in these Aroclors. Other congeners of interest may also be added to the mixture. This standard will also contain surrogate standards (see Section 4.6 below) and internal standards (see Section 4.7 below). The absolute concentration may be changed to accommodate individual detector sensitivities, but their same relative proportions should be maintained. This standard solution will be used to check instrument performance, reproducibility, and sensitivity. An example of an acceptable standard chromatogram is shown in Figure 1.

4.4.2. Pesticide standard: The above PCB standard will also contain 11 chlorinated pesticides and industrial compounds which elute partially or completely in the nonpolar fraction of sample clean-up with the PCBs. A mixed pesticide standard (MPS) which contains 30 chlorinated pesticides and industrial compounds (including the above 11 from the PCB standard) that would be found in environmental samples is used to quantify analytes eluting in the moderately polar clean-up fraction (Appendix B). This standard will also contain a surrogate standard (See Section 4.6 below) and internal standards (see Section 4.7 below). The absolute concentration may be changed to accommodate individual detector sensitivities, but their same relative proportions should be maintained. This standard solution will be used to check instrument performance, reproducibility, and sensitivity. Examples of acceptable standard chromatograms are shown in Figures 2 and 3.

4.5. Calibration standards: Calibration standards will be used to generate response factors for quantitation (see Section 5.4). The standards shall have the same composition as the performance standard (see above), but may differ in total concentration. Concentrations of the calibration standards shall be chosen based on the type of matrix being analyzed, its expected PCB concentration, and the method chosen for instrument calibration (see Section 5.4).

4.6. Surrogate standards: A surrogate standard will be used to monitor analytical recoveries of PCB congeners. Four surrogate standards may be added to each sample, matrix spike, and blank before extraction. The surrogates for the PCB analysis are PCB congeners 3,5- dichlorobiphenyl (PCB 14), 2,3,5,6- tetrachlorobiphenyl (PCB 65), and 2,3,4,4',5,6- hexachlorobiphenyl (PCB 166). These congeners will also serve as surrogates for the pesticides and industrial compounds that elute in the nonpolar fraction of sample clean-up. Recommended concentrations in the 610 ng/mL performance standard (Section 4.4.1 above) are 25, 5, and 5 ng/mL, respectively. The surrogate for the chlorinated pesticides and industrial compounds analysis eluting in the moderately polar fraction of sample clean-up is delta HCH. The recommended concentration in the MPS performance standard (Section 4.4.2 above) is 20 ng/mL. Other surrogates may be used in conjunction with or in place of the above as required for special applications.

4.7. Internal standards: Internal standards are used in the quanticiation of all PCB congeners, chlorinated pesticides, and industrial compounds. They are added to samples just before instrumental analysis. A minimum of two internal standards are required, and these include 2,4,6- trichlorobiphenyl (PCB 30) and 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB 204). Recommended concentrations in the 610 ng/mL performance standard (Section 4.4 above) are 8 and 6 ng/mL, respectively. Other internal standards may be used in addition to or in place of the above if they are more appropriate for a particular application.

4.8. Storage of Standards: All standard solutions are to be kept in vials or bottles with Teflon-lined screw caps and stored in a freezer and protected from light. Stock standards should be checked frequently for signs of evaporation, especially just before preparing calibration standards. Stock standards must be replaced after one year, or sooner if problems are apparent.

5. PROCEDURE.

5.1. The extraction and clean-up procedure should follow the appropriate SOP for a given matrix. Although the procedures vary to some degree for different sample matrices, a nonpolar (hexane eluent) and a moderately polar (DCM or DCM/Hex eluent) fraction can be collected for any clean-up procedure. The nonpolar will contain PCBs and 11 chlorinated pesticides and industrial compounds which elute partially or completely in this fraction. The moderately polar fraction will contain the remaining pesticides and industrial compounds.

5.2. Instrument Parameters.

5.2.1. Analysis of samples by high resolution (capillary column) gas chromatography (GC) with an electron capture detector (ECD) is required. It is assumed that GC-ECD analysis will be the method of choice for quantitation because of enhanced sensitivity to organochlorines. An example of the GC instrumental conditions is listed in Table 1. Deviations from these parameters will be acceptable provided instrument performance criteria are met (see Section 5.2.2). If a particular set of congeners is of more interest than others, then the temperature program may be modified to attain better separation in the area of interest.

5.2.2. A calibration standard will be analyzed and the instrument recalibrated with each group of 10-20 samples (depending on project requirements) to monitor resolution, reproducibility, and sensitivity.

5.3. GC Analysis.

5.3.1. Set up GC operating conditions as described in the Section 5.2.1.

5.3.2. The injection is made utilizing an autosampler. A volume of 1.0 µL is used. Manual injection, if necessary, will use at least a 2.0-µL injection. A splitter may be used at the injector to run the sample on both the primary and confirmation column simultaneously.

5.3.3. Samples are analyzed in a set referred to as an analytical sequence. The sequence begins with instrument calibration followed by sample extracts interspersed with

calibration standards. The sequence ends when the set of samples has been injected.

5.3.4. If the sample responses result in poor chromatographic resolution, the extract is diluted and reanalyzed. Additional internal standard may be required in the diluted samples.

5.3.5. If detection is prevented by the presence of interferences further clean-up may be required, such as copper clean-up for sulfur (see SOP P-16-109, Section 7.3; if sample is sediment). Other procedures such as GPC (see SOP P-16-108) or alumina clean-up may be called for.

5.4. Quantification .

5.4.1. Quantification of individual PCBs congeners and pesticides will be congener- or compound-specific and performed using the internal standard method. This method eliminates errors due to variation in the sample injection, and is independent of the final extract volume. The internal standards that will be used are PCB congeners 30 and 204. The internal standard will be added to each sample before GC analysis at a concentration similar to the sample components. Surrogate recoveries will provide a measure of analytical losses and are reported with the congener values for each sample.

5.4.2. Relative response factors relative to the internal standard (RRF) will be generated as required by instrument calibration criteria:

$$RRF = \left(\frac{Mass\,Congener}{Area\,Congener} \right)_{std} \div \left(\frac{Mass\,istd}{Area\,istd} \right)_{std} \quad \textbf{(1)}$$

5.4.3. Congener masses can be calculated from the known total PCB concentration of the calibration standard and the congener composition of the standard (Mullin 1985, see Appendix A). Average RRFs can be determined in one of two ways. (1) Three calibration standards encompassing the expected range of PCB concentrations in the samples can be used to generate RRFs. These standards must encompass a range of at least one and one half orders of magnitude. The internal standard concentrations in each different standard solution must be the same. Sample concentrations that fall outside the range of the calibration standards should be diluted or concentrated as needed and re-run. This method will be sensitive to non-linear responses in the electron capture detector and should only be used over the established linear range of a particular instrument. (2) A single calibration standard can be used to generate RRFs. This method is

also sensitive to non-linear responses of the electron capture detector, and the calibration concentration should be within a factor of five of the concentrations of PCBs in the sample extracts. Sample extracts that fall outside this range should be either diluted or concentrated but only without losing less-concentrated compounds.

5.4.4. Congener concentrations will be calculated from the average RRF, and the internal standard response in the sample, by the following equations:

$$(mass\,congener)_{sample} = (area\,congener)_{sample}\ X\ RRF_{std}\ X \left(\frac{mass\,istd}{area\,istd} \right)_{sample} \quad \textbf{(2)}$$

5.4.5 For PCB analysis, congeners eluting before and including PCB 110 will be quantitated relative to internal standard PCB 30. Congeners eluting after and including PCB 82 will be quantitated relative to internal standard PCB 204.

5.4.6 For pesticide analysis pesticides eluting before and including o,p -DDE will be quantitated relative to internal standard PCB 30. Pesticides eluting after and including Dieldrin will be quantitated relative to internal standard PCB 204.

6. QUALITY CONTROL.

6.1. With each group of 10-20 samples analyzed (depends on project QC requirements), the calibration check standards should be evaluated to determine if the chromatographic system is operating properly. If any changes are made to the system, recalibration of the system must take place.

6.2. The performance of the entire analytical system should be monitored, on the basis of data gathered from analyses of blank, standard and replicate samples at a 5-10% frequency (depending on project QC requirements). Significant peak tailing must be corrected. Tailing problems are generally traceable to active sites on the GC column or to the detector operation.

6.3. A blank, a matrix spike or standard reference material sample, and a duplicate or matrix spike duplicate (if available) must be analyzed at a minimum frequency of 5-10% of samples (depending on project QC requirements), interspersed with each extraction group.

6.4. Limits of detection (LOD) and quantitation (LOQ).

6.4.1. The LOD is defined as the signal that is equal to the sum of the mean noise and 3 standard deviations (σ) of the baseline noise (Keith et al. 1983). The area of the baseline noise over the elution time of each congener shall be determined from injections of a matrix blank that has been spiked with the performance standard to yield a concentration just above the expected LOD (1-5x est. LOD). This procedure is described in the Federal Register (1984). The mean and the standard deviation of the baseline noise for each congener will be determined from injections of seven analyses of the spiked blank. The LOQ is defined as the signal that is equal to the sum of mean noise and 10σ of the baseline noise and is determined in the same manner as the LOD:

$$\text{LOD} = \text{mean noise} + 3\sigma \text{ (expressed as peak areas)} \quad (4)$$

$$\text{LOQ} = \text{mean noise} + 10\sigma \text{ (expressed as peak areas)} \quad (5)$$

6.4.2. LOD and LOQ, expressed as mass of congener injected, can then be determined as shown in section 5.4, Equation 2. Data shall be reported as the calculated value if the concentrations are greater than or equal to the LOQ. Calculated concentrations that are less than LOQ but greater than or equal to the LOD will be reported with the LOQ indicated in parentheses.

6.4.3. The minimum target LOD is 5 pg per analyte injected for water and 25 pg injected for sediment and tissue analysis.

6.5. Precision.

6.5.1. Precision is indicated by the reproducibility of replicate analyses. Precision will be expressed as the relative percent difference (RPD) of duplicate analyses of a split sample:

$$RPD = \frac{(dup1 - dup2)}{ave} X\ 100$$

6.5.2. The average RPD for all congeners must meet established control limits for a given matrix if measured concentrations are $\geq$ 5X the LOD and must be within 2x the control limits if measured concentrations are < 5X the LOD. If these objectives are not met, duplicate samples should be re-extracted and analyzed. If no additional sample is available, these data should be flagged.

6.6. Accuracy:

6.6.1. Accuracy indicates the degree to which the analytical measurement reflects the true value of the analyte in the sample:

6.6.2. Accuracy will generally be measured using surrogate spikes and standard reference materials (SRMs). Blank spikes and matrix spikes may also be used periodically to evaulate method performance and matrix effects. A known amount of the surrogate spike is added to every sample and blank prior to extraction. Thus the recovery of every extraction can be estimated by the recovery of the surrogate spike. The recoveries of analytes from SRMs, blank spikes, and matrix spikes represent the actual analytical recovery and can be used to evaluate method performance. SRMs and matrix spikes are also used to evaluate the effect of the sample matrix on analyte recovery. For a given sample set, the average percent recovery of analytes in the SRM, blank, or matrix spike and individual surrogate spike recoveries must be within established control limits for the appropriate sample matrix. If these criteria are not met, then the data from that sample set are flagged. If surrogate spike recoveries do not meet these standards, then that sample must be re-run. If they still fail QA standards, samples should be re-extracted and analyzed. If additional sample is unavailable, then the data will be flagged.

6.7. PCB and Pesticide Identification.

6.7.1. For samples analyzed by GC-ECD, PCB congeners will be identified by retention time relative to the internal standard retention time, as determined in the calibration standard. Peaks must be within 5% of the retention time in the calibration standard to be considered a correct identification. If not, the analyst must recalibrate the instrument and reanalyze the sample. For a given sample matrix, selected analytes found in 5% of the samples may be verified for correct PCB or pesticide identification by GC-MS or by retention time on a second column, depending on the project requirements. The samples chosen for verification should include a range of concentrations.

7. CORRECTIVE ACTIONS.

7.1. Sample response(s) exceed the linear range of the system: see Section 5.3.4.

7.2. Performance standards exceed acceptance criteria: see Section 5.2.2.

7.3. Surrogate recovery exceeds acceptable limits (Section 6.6): sample(s) should be re-extracted and re-analyzed.

7.4. Holding Times: holding times of extracts will be 40 days from time of extraction for PCBs, pesticides, and industrial compounds. It is recognized, however, that required re-analyses resulting from corrective actions as described above may result in holding times being exceeded for individual samples or sample groups or other contingencies may arise that compromise holding times. In these cases, all such violations of holding times must be indicated by flagging the data and by detailing the exceedances in the case narrative accompanying the sample delivery group.

7.5. Presence of interference in elution pattern: see Section 5.3.5.

7.6. Co-elution with an internal standard: see Section 5.4.

8. REFERENCES.

8.1. Keith, L.H. et al. 1983. Principles of environmental analysis. Anal. Chem., 55, 2210-2218.

8.2. Mullin, M.D. 1985. PCB Workshop, USEPA Large lakes Research Station, Grosse, Ile, MI, June.

8.3. Test Methods for Evaluating Solid Waste (SW-846), Revision 1, November 1990, Method 8000A and 8080A.

8.4. USEPA, Quality assurance plan, Green Bay Mass Balance Study. USEPA Great Lakes National Program Office, Chicago, IL, March, 1988.

8.5. Federal Register 1984. Appendix B to Part 139. Definition and procedure for the determination of the method detection limit. Vol. 49, No. 209, October 26.

Table 1. Example GC-ECD conditions for PCB and pesticide analysis[1].

column primary: confirmation:	50 m DB-5, 0.20-mm ID, 0.33-μm film thickness or equivalent[2] 30 m DB-1701, 0.25 mm ID, 0.25μm film thickness or equivalent[2]
carrier gas	hydrogen or helium
carrier linear velocity	~2 ml/min
splitless purge flow	50 to 70 ml/min
splitless purge time	0.7 - 1.0 min
injector temperature	225±25°C
initial temperature; hold time	50°C; 1 min
oven temp.ramp	1st level - 5°C/min to 130°C 2nd level - 0.5 -1°C/min to 260°C 3rd level - 10°C/min to 280°C
final temperature; time	280°C; 10 min
ECD temperature	325±25°C
make-up gas	5% Me/95% Ar
make-up gas flow rate	30 - 40 ml/min

1 These conditions are only a guideline and may be adjusted for specific applications or particular congeners of interest.
2 An equivalent column coating is required.

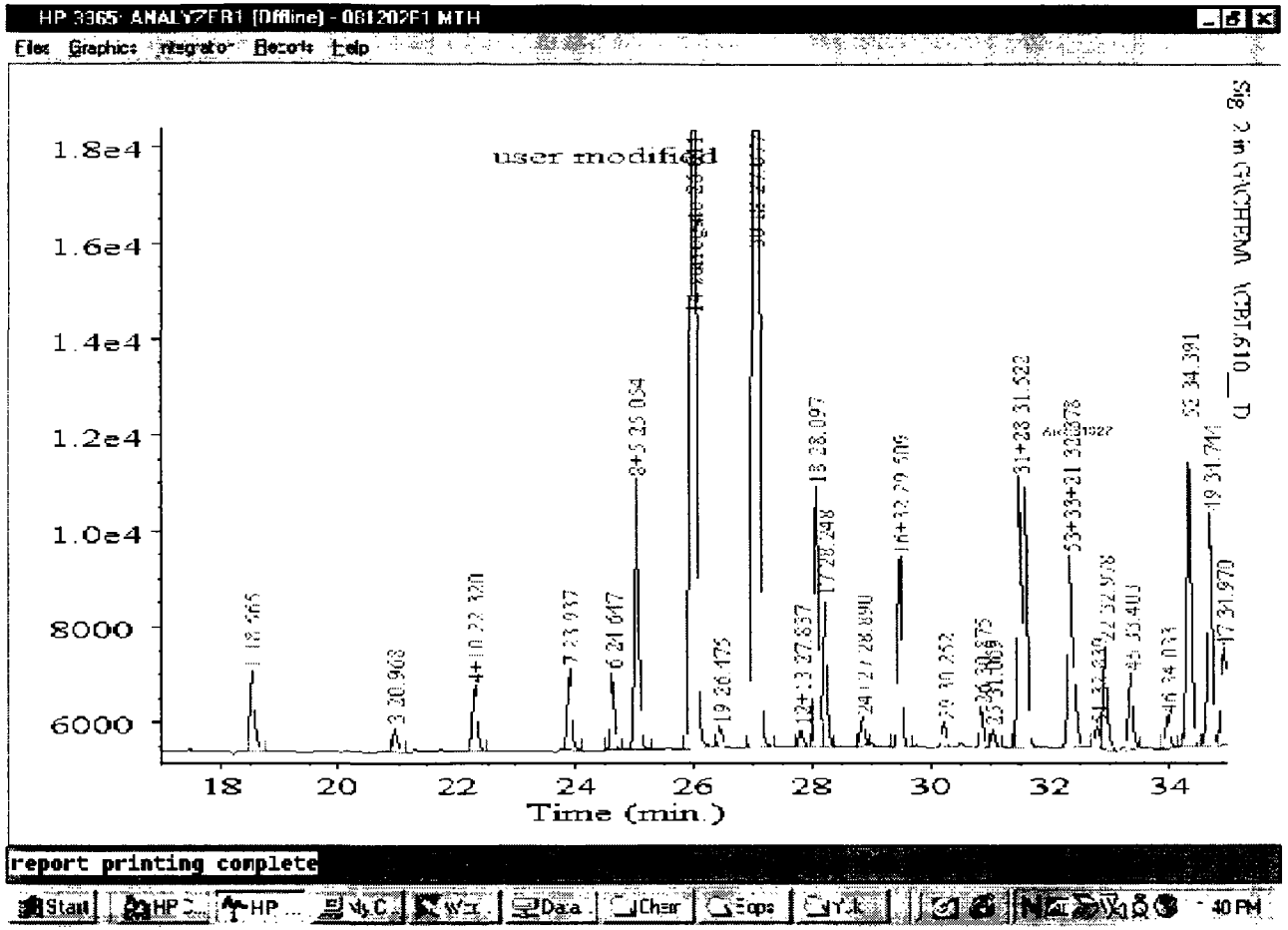

Figure 1. Partial chromatogram of PCB '610' Mixture used as a calibrations standard.

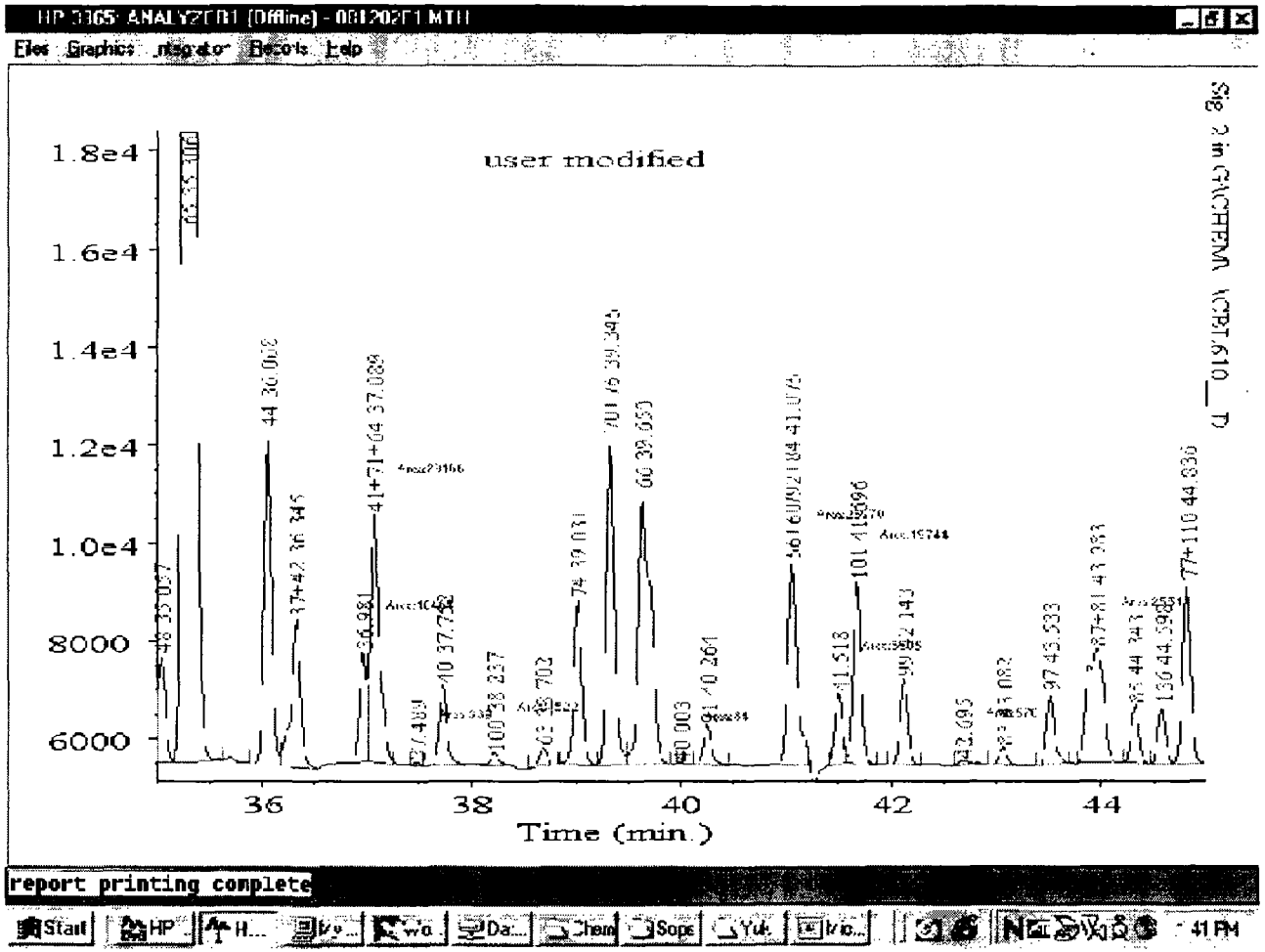

Figure 1 Continued. Partial chromatogram of PCB '610' Mixture used as a calibrations standard.

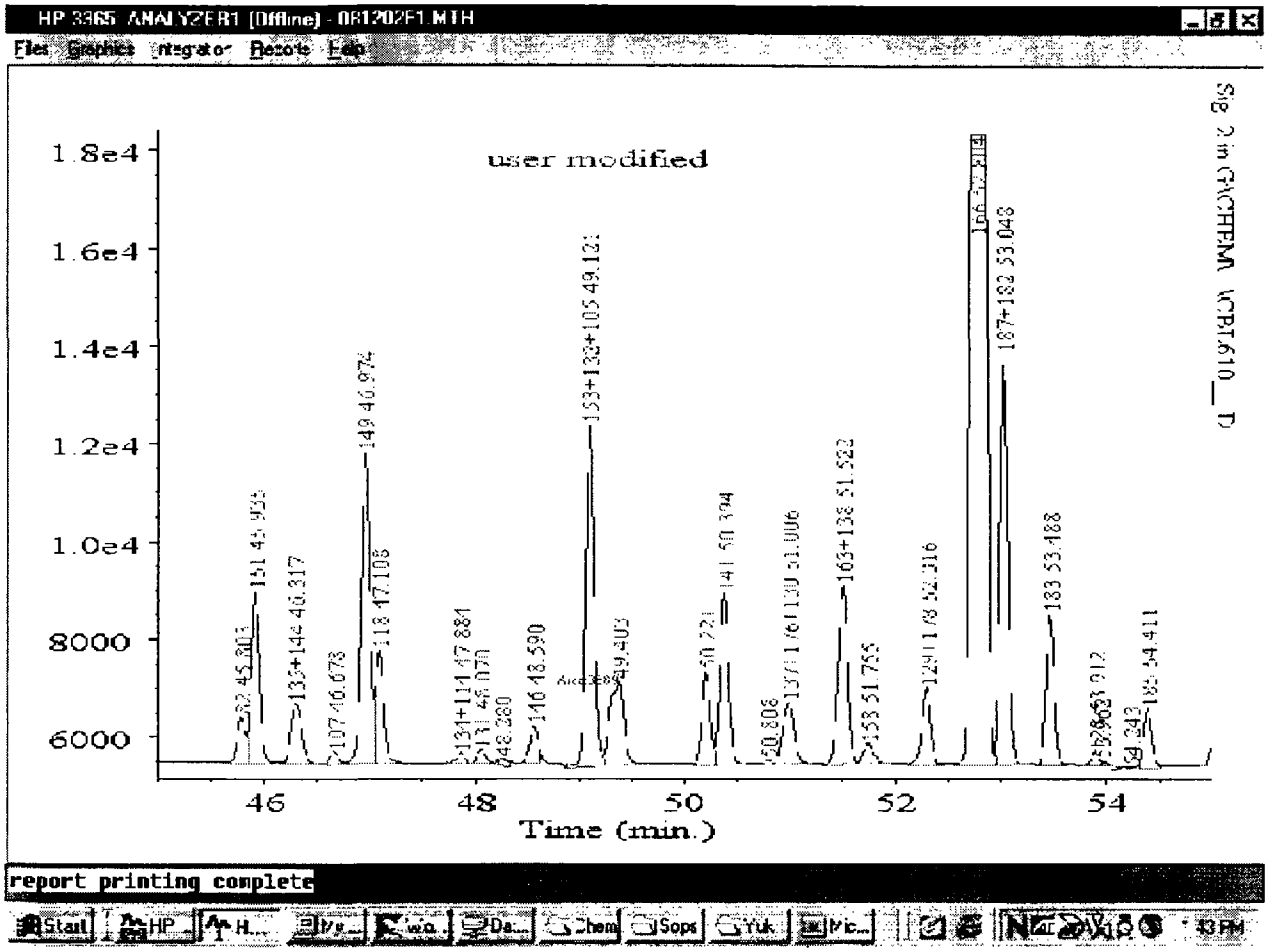

Figure 1 Continued. Partial chromatogram of PCB '610' Mixture used as a calibrations standard.

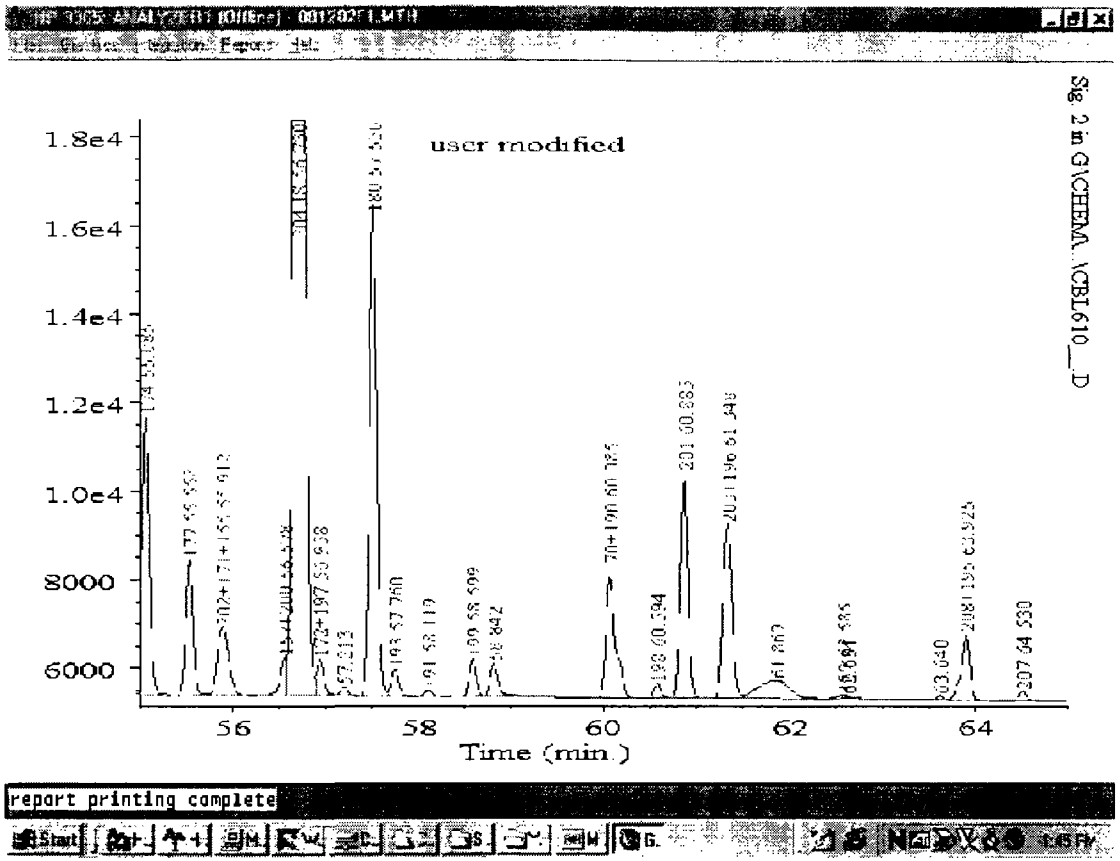

Figure 1 Continued. Partial chromatogram of PCB '610' Mixture used as a calibrations standard.

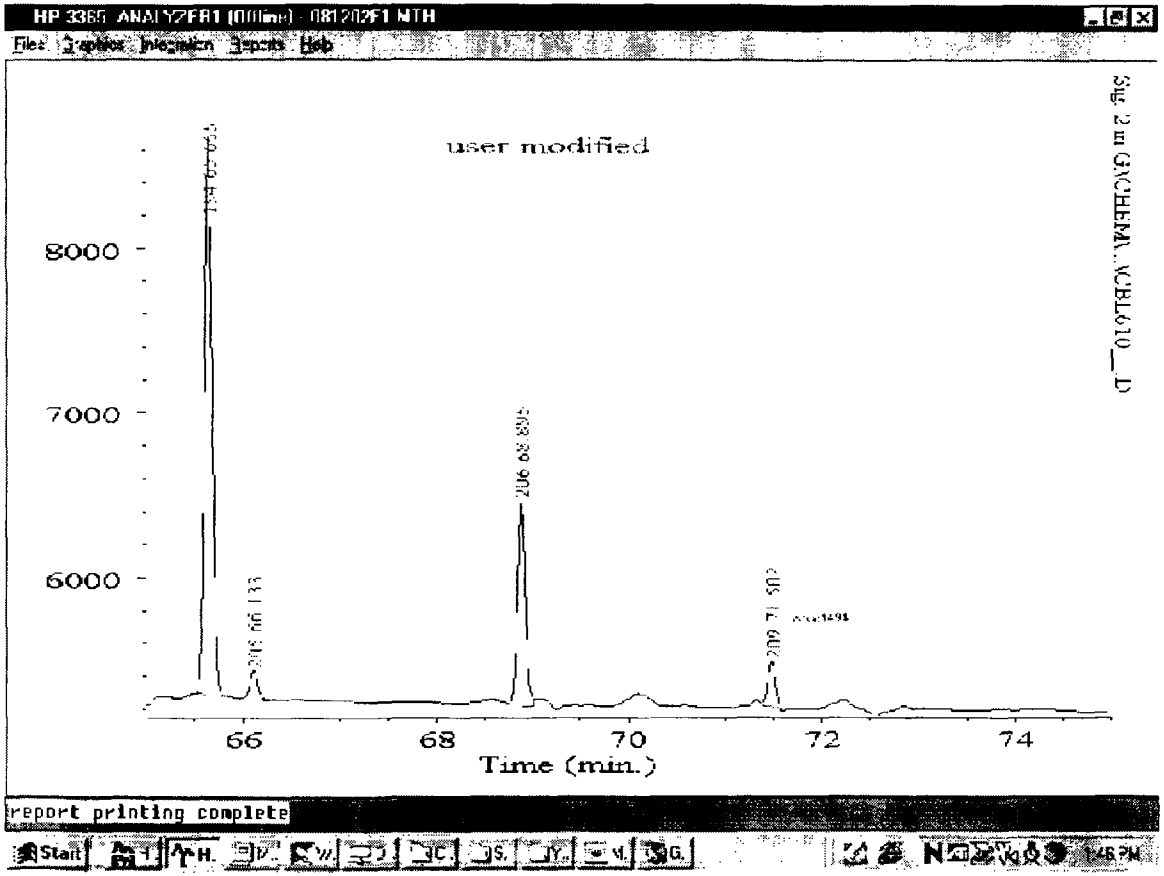

Figure 1 Continued. Partial chromatogram of PCB '610' Mixture used as a calibrations standard.

APPENDIX A.

CONGENER COMPOSITION OF CALIBRATION/PERF. STANDARD FOR PCBs

Mullins, U.S. EPA Large Lakes Research Station, Grosse Ile, MI, should be cited in all publications that use this information as "Mullin, M.D., Workshop, U.S. EPA Large Lakes Research Station, Grosse Ile, MI, June 1985."

A mixed Aroclor standard composed of 250 ng/ml 1232, 180 ng/ml 1248, and 180 ng/ml 1262 will have the congener composition listed on the following pages and varying amounts of individual PCB congeners commonly added to the Aroclor mixture are also listed in italics in units of ng/ml.

PCB Congener	# of Cl	ng/mL
PCB 1	1	43
PCB 3	1	26
PCB 4+10	2	2.8
PCB 7	2	2.2
PCB 6	2	4.2
PCB 8+5	2	50
SURROGATE PCB 14		var
PCB 19	3	1
INTERNAL STD PCB 30		var
PCB 12+13	2	0.92
PCB 18	3	13
PCB 17	3	7.4
PCB 24+27	3	0.87
PCB 16+32	3	13.1
PCB 29	3	0.18
PCB 26	3	2.3
PCB 25	3	1
PCB 31+28	3	38

PCB Congener	# of Cl	ng/mL
PCB 33	3	14
PCB 53	4	2.7
PCB 51	4	0.67
PCB 22	3	11
PCB 45	4	2.7
PCB 46	4	1.4
PCB 52	4	12
PCB 43	4	0.91
PCB 49	4	9
PCB 47	4	5
PCB 48	4	4
SURROGATE PCB 65		var
PCB 44	4	15
PCB 37+42	3	8.8
PCB 41+71	4	9.4
PCB 64	4	6.9
PCB 40	4	3.3
PCB 100	5	0.5
PCB 63	4	0.74
PCB 74	4	8.1
PCB 70+76	4	21
PCB 66	4	22
PCB 95	5	5.2
PCB 91	5	1.4
PCB 56+60	4	18
PCB 92+84	5	4.3
PCB 89	5	0.3
PCB 101	5	4.8
PCB 99	5	2.3
PCB 119	5	0.18
PCB 83	5	0.36
PCB 97	5	1.9

PCB Congener	# of Cl	ng/mL
PCB 81	4	0.32
PCB 87	5	3
PCB 85	5	2.1
PCB 136	6	1.4
PCB 77+110	4	7.1
PCB 82	5	1.3
PCB 151	6	5.7
PCB 135+144	6	2.2
PCB 147+124	5	0.223
PCB 107	5	0.33
PCB 149	6	11
PCB 118	5	3.5
PCB 134	6	0.45
PCB 114	5	0.4
PCB 131	6	0.091
PCB 146	6	1.6
PCB 153+132+105	6	21.6
PCB 141	6	5.2
PCB 137+176	6	1.388
PCB 130	6	0.25
PCB 163+138	6	9.8
PCB 158	6	1.2
PCB 129	6	0.3
PCB 178	7	3.4
SURROGATE PCB 166		var
PCB 175	7	0.6
PCB 187+182	7	15
PCB 183	7	7.7
PCB 128	6	0.47
PCB 167	6	0.11
PCB 185	7	2.2
PCB 174	7	11

PCB Congener	# of Cl	ng/mL
PCB 177	7	5.7
PCB 202+171	7	3.69
PCB 156	6	0.331
PCB 173	7	0.1273
PCB 157+200 obsured by IS	6	2.067
INTERNAL STD PCB 204		var
PCB 172+197	7	2.14
PCB 180	7	24
PCB 193	7	2.4
PCB 191	7	0.45
PCB 199	8	1
PCB 170+190	7	12.1
PCB 198	8	0.67
PCB 201	8	15
PCB 203+196	8	17
PCB 189	7	0.18
PCB 208+195	8	8.0776
PCB 207	9	0.48
PCB 194	8	6.9
PCB 205	8	0.4
PCB 206	9	4.2
PCB 209	10	0.095

APPENDIX B.

CONGENER COMPOSITION OF PERFORMANCE STANDARD FOR PESTICIDES

A mixed pesticide standard composed of various organochlorine pesticides and industrial compounds listed on the following page will have various concentration of ~100 ng/mL. Two fractions, F1 and F2 will be quantified using two calibration standards (chromatograms shown below).

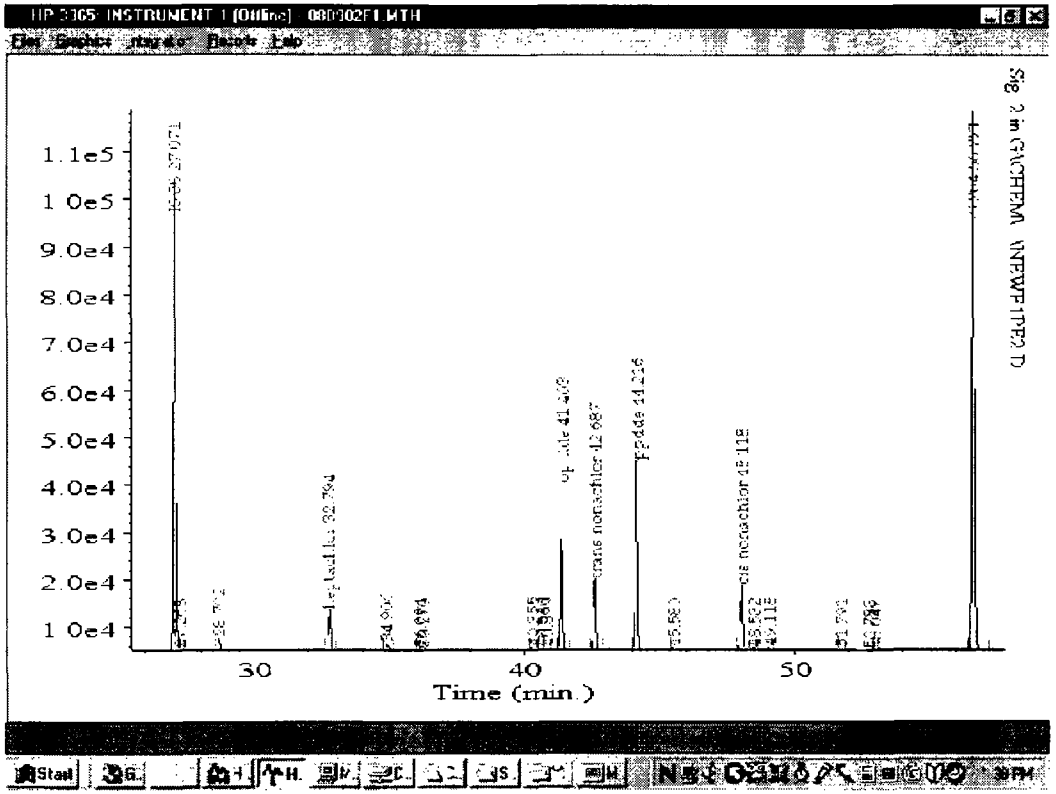

Figure 2. Chromatogram showing elution order of F1 organochlorine pesticides.

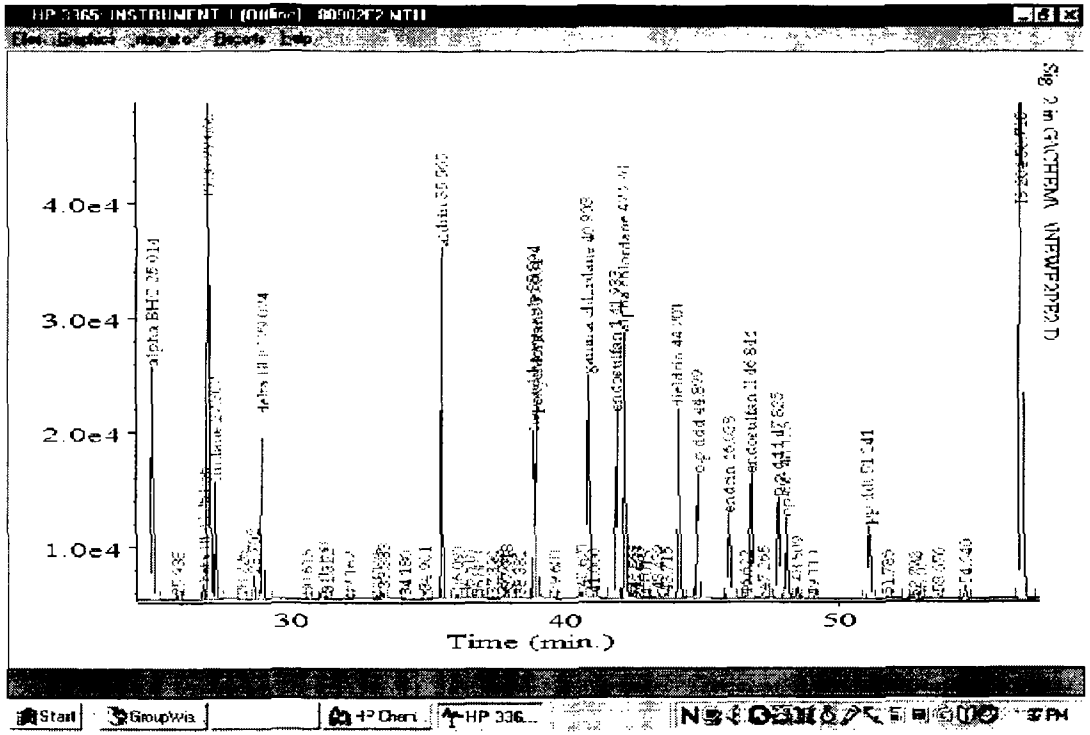

Figure 3. Chromatogram showing elution order of F2 organochlorine pesticides

Compound
BHC (alpha, beta, gamma, delta)
heptachlor
heptachlor epoxide
chlordanes (gamma and alpha)
nonachlors (cis and trans)
dieldrin
DDDs (o,p and p,p)
DDEs (o,p and p,p)
DDTs (o,p and p,p)
oxychlordane

APPENDIX C

Relationship Between TPCB (Aroclor Based) and CTPCB (Congener Based) Measures of Total PCB Concentration

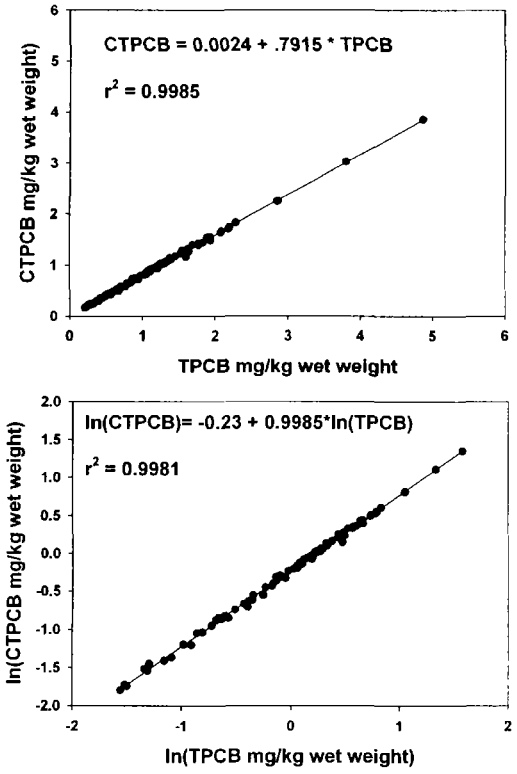

Figure C-1. Relationship between congener-based quantitation of total PCBs and Aroclor-based quantitation for fishes analyzed in the 2006 ANSP Housatonic River study.

As in previous Housatonic River biological monitoring studies, the two methods of quantitating total PCBs were very highly correlated. A scatter plot of CTPCB concentrations versus the corresponding TPCB concentrations for the fish species analyzed (brown trout, smallmouth bass, and northern pike) clearly suggests a linear relationship (Fig. C-1, top). Linear regression analysis of these data produced an intercept (0.0024 mg/kg) that differs negligibly from zero (regression equation: CTPCB = 0.0024 + 0.7915*TPCB, r^2 = 0.999). The slope of this regression implies that CTPCB was about 21% lower than TPCB on average. A regression of ln(CTPCB) versus ln(TPCB) was also performed to stabilize the variance and as an additional test for linearity. The slope of this regression (0.9985; see Fig. C-1, bottom) differs negligibly from 1.0000, indicating a linear relationship.

Relationships between CTPCB and TPCB differed somewhat for the two hatchery brown trout. For these two samples, the CTPCB concentration was similar to or higher than the TPCB concentration. However, these specimens have very low concentrations of both CTPCB and TPCB, so the ratios of the two are not expected to be as precise as those of the other fish.

APPENDIX D

Numbers of Brown Trout from 2006 Analyzed for PCB Content and Their Corresponding Stocking Dates as per Connecticut DEP

Stocking date	Number of individuals	Percent of total
2005 Spring	1	3
2005 Fall	6	20
2006 Spring adults	23	77
Total Housatonic	30	100
Burlington Hatchery	2	–

APPENDIX E

Average CTPCB Concentrations in Fish from the Housatonic River, Connecticut

Results for 1992, 1994, 1996, 1998, 2000, 2002, 2004, and 2006 are based on actual quantified CTPCB values. Results for 1984–1990 were estimated from TPCB data, using regressions between LNCTPCB and LNTPCB that were established with data from 1992 and 1994 (ANSP 1999). C = West Cornwall, B = Bulls Bridge, L = Lake Lillinonah, Z = Lake Zoar, F = Falls Village, HS = Lake Housatonic (only smallmouth bass data presented), H = hatchery.

		Year											
Species	Station	1984	1986	1988	1990	1992	1994	1996	1998	2000	2002	2004	2006
Brown trout	C	2.75	5.27	4.06	4.41	7.25	1.31	2.29	2.29	1.54	1.78	1.64	1.21
Rainbow trout	C	-	-	2.63	-	-	-	-	-	-	-	-	-
Smallmouth bass	C	1.99	2.61	3.77	-	2.78	1.41	1.00	0.78	1.00	1.10	0.94	0.89
Bluegill	B	0.78	-	1.85	-	-	-	-	-	0.49	-	0.27	-
Brown bullhead	B	0.72	1.54	1.68	-	-	-	-	-	0.34	-	0.37	-
Common carp	B	0.95	-	5.17	-	-	-	-	-	-	-	-	-
Largemouth bass	B	1.16	-	2.09	-	-	-	-	-	-	-	0.57	-
Northern pike	B	-	-	-	-	-	-	-	-	-	-	0.45	0.77
Pumpkinseed	B	-	-	0.27	-	-	-	-	-	0.73	-	0.23	-
Redbreast sunfish	B	1.31	-	1.66	-	-	-	-	0.47	-	-	-	-
Yellow bullhead	B	-	-	-	-	-	-	-	-	-	-	0.36	-
Yellow perch	B	1.14	0.72	0.87	0.84	0.56	-	-	0.47	0.27	-	0.36	-
Smallmouth bass	B	1.61	1.34	2.33	2.10	1.35	1.23	0.99	0.95	0.98	0.80	1.05	1.08
Bluegill	L	0.48	-	0.47	0.47	0.45	-	-	-	-	-	0.17	-
Brown bullhead	L	1.99	-	1.42	-	-	-	-	-	-	-	0.28	-
Common carp	L	1.85	-	5.61	-	-	-	-	-	-	-	-	-
Largemouth bass	L	1.13	-	1.15	-	-	-	-	-	-	-	-	-
Northern pike	L	-	-	-	-	-	-	-	-	-	-	-	0.86
Pumpkinseed x redbreast sunfish	L	-	-	0.27	-	-	-	-	-	-	-	-	-
Pumpkinseed	L	-	-	0.03	0.20	0.18	-	-	-	-	-	0.04	-
Redbreast sunfish	L	1.26	-	0.03	0.37	0.47	-	-	0.09	-	-	0.13	-
White catfish	L	4.76	6.27	4.33	-	-	-	-	-	-	-	1.26	-
White perch	L	1.89	1.86	1.53	-	-	-	-	-	-	-	-	-
Yellow bullhead	L	-	-	-	-	-	-	-	-	-	-	0.18	-
Yellow perch	L	0.58	-	0.22	0.35	0.32	-	-	0.11	-	-	0.14	-
Smallmouth bass	L	1.02	1.33	1.20	0.95	1.41	0.51	0.30	0.84	0.51	0.37	0.53	0.35
Bluegill	Z	0.89	-	0.19	0.13	0.25	-	-	-	-	-	0.15	-
Brown bullhead	Z	0.38	-	0.62	-	-	-	-	-	-	-	-	-
Common carp	Z	3.88	-	12.07	-	-	-	-	-	-	-	-	-
American eel	Z	-	-	1.04	2.36	5.30	-	-	-	-	-	-	-
Largemouth bass	Z	0.39	-	1.15	-	-	-	-	-	-	-	-	-
Northern pike	Z	-	-	-	-	-	-	-	-	-	-	-	1.33
Pumpkinseed	Z	-	-	0.11	0.16	0.22	-	-	-	-	-	0.08	-
Redbreast sunfish	Z	0.09	-	0.15	0.20	0.24	-	-	0.71	-	-	-	-
White catfish	Z	2.22	2.55	3.40	-	-	-	-	-	-	-	0.59	-
White perch	Z	0.84	-	1.26	0.87	1.01	-	-	-	-	-	0.51	-
Yellow bullhead	Z	-	-	-	-	-	-	-	-	-	-	0.05	-

Species	Station	Year											
		1984	1986	1988	1990	1992	1994	1996	1998	2000	2002	2004	2006
Yellow perch	Z	0.07	-	0.21	0.24	0.26	-	-	-	-	-	0.17	-
Smallmouth bass	Z	0.45	-	0.84	0.59	1.13	0.43	0.48	0.87	0.32	0.36	0.28	0.58
Bluegill	F	-	-	-	-	-	-	-	-	0.68	-	0.41	-
Brown bullhead	F	-	-	-	-	-	-	-	-	0.95	-	0.32	-
Northern pike	F	-	-	-	-	-	-	-	-	-	-	10.01	1.27
Pumpkinseed	F	-	-	-	-	-	-	-	-	0.21	-	0.27	-
Smallmouth bass	F	-	-	-	-	-	-	-	-	-	-	1.01	-
Yellow perch	F	-	-	-	-	-	-	-	-	0.36	-	0.49	-
Smallmouth bass	HS	-	-	-	-	-	0.51	-	-	-	-	-	-
Brown trout	H	-	-	-	-	-	-	-	0.12	0.03	0.10	0.09	0.01

APPENDIX F

Summary of ANCOVA Models Used in Statistical Analyses of the Text, Showing All Statistically Significant Terms Retained

Model terms for TPCB smallmouth bass (all years except 1986, all stations)

Response variable:	ln(TPCB)
Main effects:	year, station
Covariates:	ln(river age), ln(% lipid)
Interactions:	year × station, station × sex, station × ln(% lipid), year × ln(% lipid)
Model R^2:	0.72

Model terms for CTPCB smallmouth bass (1992–2006, all stations)

Response variable:	ln(CTPCB)
Main effects:	year, station, sex
Covariates:	ln(river age), ln(% lipid)
Interactions:	year × station, station × ln(% lipid)
Model R^2:	0.71

Model terms for TPCB smallmouth bass at West Cornwall (all years)

Response variable:	ln(TPCB)
Main effects:	year
Covariates:	ln(% lipid)
Interactions:	year × ln(% lipid), sex × ln(% lipid), year × ln(river age), sex × ln(river age)
Model R^2:	0.79

Model terms for TPCB smallmouth bass at Bulls Bridge (all years)

Response variable:	ln(TPCB)
Main effects:	year
Covariates:	ln(% lipid), ln(river age)
Interactions:	year × ln(% lipid), sex × ln(river age)
Model R^2:	0.69

Model terms for TPCB smallmouth bass at Lake Lillinonah (all years)

Response variable:	ln(TPCB)
Main effects:	year, sex
Covariates:	ln(river age)
Interactions:	(none)
Model R^2:	0.54

Model terms for TPCB smallmouth bass at Lake Zoar (all years except 1986)

Response variable:	ln(TPCB)
Main effects:	year
Covariates:	ln(% lipid), ln(river age)
Interactions:	(none)
Model R^2:	0.47

Model terms for TPCB brown trout at West Cornwall (all years)

Response variable:	ln(TPCB)
Main effects:	year
Covariates:	ln(% lipid), ln(river age)
Interactions:	year × ln(river age), year × ln(% lipid)
Model R^2:	0.75

Model terms for CTPCB brown trout at West Cornwall (1992–2006)

Response variable:	ln(CTPCB)
Main effects:	year
Covariates:	ln(% lipid), ln(river age)
Interactions:	year × ln(river age)
Model R^2:	0.79

Model terms for CTPCB smallmouth bass at West Cornwall (all years)

Response variable:	ln(CTPCB)
Main effects:	year, sex
Covariates:	(none)
Interactions:	(none)
Model R^2:	0.54

Model terms for CTPCB smallmouth bass at Bulls Bridge (all years)

Response variable:	ln(CTPCB)
Main effects:	year, sex
Covariates:	(none)
Interactions:	sex × ln(river age)
Model R^2:	0.34

Model terms for CTPCB smallmouth bass at Lake Lillinonah (all years)

Response variable:	ln(CTPCB)
Main effects:	year
Covariates:	ln(river age)
Interactions:	year × ln(% lipid)
Model R^2:	0.63

Model terms for CTPCB smallmouth bass at Lake Zoar (all years except 1986)

Response variable:	ln(CTPCB)
Main effects:	year
Covariates:	ln(% lipid), ln(river age)
Interactions:	(none)
Model R^2:	0.64

APPENDIX G

Summary of total PCB concentrations (mg/kg wet weight) of fillets of brown trout collected in Academy surveys of the Housatonic River.

Year	Hatchery	West Cornwall – Age Class (years)						
		< 0.20	0.20–0.33	0.34–0.99	1.00–1.99	2.00–2.99	3.00–3.99	> 3.99
Geometric Mean of CTPCB								
2006	0.01	–	1.15	1.01	3.86	–	–	–
2004	0.09	–	1.42	1.83	2.95	–	–	–
2002	0.10	–	1.13	1.33	1.92	1.08	3.38	3.06
2000	0.03	1.39	1.28	–	2.72	2.35	3.46	–
1998	0.12	–	1.27	1.68	3.31	4.09	11.13	–
1996	0.04	0.12	1.54	1.84	2.82	–	4.77	6.89
1994	0.04	–	1.07	0.81	–	3.88	–	–
1992	–	3.32	6.88	6.73	10.77	9.65	–	–
Geometric Mean of TPCB								
2006	0.01	–	1.65	1.19	2.87	–	–	–
2004	0.09	–	1.63	2.01	4.25	–	–	–
2002	0.10	–	1.10	1.29	1.86	1.04	3.32	3.00
2000	0.04	1.38	1.29	–	2.73	3.31	3.10	–
1998	0.12	–	1.28	1.64	3.22	4.18	11.16	–
1996	0.03	0.11	1.65	2.00	3.13	–	5.15	7.93
1994	0.04	–	1.18	0.84	–	5.01	–	–
1992	–	4.18	8.72	8.69	14.03	12.54	–	–
1990	–	–	–	4.93	6.84	7.83	6.23	–
1989	0.03	–	–	–	–	–	–	–
1988	0.06	–	3.75	4.42	7.06	5.22	10.40	5.74
1987	0.03	–	–	–	–	–	–	–
1986	–	–	3.30	–	5.16	7.34	8.55	16.17
1984	–	–	1.37	–	6.89	4.97	7.56	–
Percent Lipid								
2006	9.53	–	4.19	3.50	3.42	–	–	–
2004	8.89	–	4.76	4.00	4.67	–	–	–
2002	7.85	–	3.51	2.74	5.32	4.67	4.07	4.88
2000	5.69	4.00	2.57	–	4.84	3.42	5.51	–
1998	2.47	–	2.04	1.87	3.88	1.21	5.29	–
1996	3.54	2.25	1.78	1.00	2.15	–	1.08	1.03
1994	5.87	–	2.74	1.79	–	2.33	–	–
1992	–	3.99	3.99	2.69	6.29	4.60	–	–
1990	–	–	–	1.19	1.83	0.56	1.68	–
1989	3.60	–	–	–	–	–	–	–
1988	1.82	–	1.88	1.32	4.32	4.37	4.64	3.60
1987	0.40	–	–	–	–	–	–	–
1986	–	–	4.04	–	3.83	3.67	3.70	4.35
1984	–	–	2.81	–	3.30	2.85	3.35	–

APPENDIX H

Geometric mean total PCB concentrations (mg/kg wet weight) in benthic insects from the Housatonic River, 1992–2006. Both Aroclor-based and congener-based estimates of total PCBs are shown (TPCB and CTPCB, respectively). Caddisflies are filter feeders, while dobsonflies and stoneflies are predators.

Year	PCB Measure	Caddisflies (Hydropsychidae)	Dobsonflies (Corydalidae)	Stoneflies (Perlidae)
1992	TPCB	3.94	7.45	3.71
	CTPCB	3.01	5.48	3.01
1994	TPCB	1.92	2.93	1.09
	CTPCB	1.80	2.49	1.01
1996	TPCB	2.69	3.13	2.43
	CTPCB	2.50	2.65	2.29
1998	TPCB	1.05	3.94	0.54
	CTPCB	0.86	2.92	0.40
2001	TPCB	0.90	1.81	0.53
	CTPCB	0.97	1.83	0.57
2002	TPCB	0.58	0.94	0.46
	CTPCB	0.63	0.99	0.51
2005	TPCB	0.60	0.55	0.54
	CTPCB	0.51	0.44	0.50
2006	TPCB	1.61	1.93	0.66
	CTPCB	1.33	1.46	0.64

APPENDIX I

40 CFR 136, Appendix B: Protocol for Detection Limit Calculations.

Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11

Definition

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific, and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

(a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

(b) The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

(c) That region of the standard curve where there is a significant change in sensitivity, i.e., a break in the slope of the standard curve.

(d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the initial estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) Prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S 2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} X_i\right)^2}{n}\right] \qquad S = \left(S^2\right)^{\frac{1}{2}}$$

where:

Xi; i=1 to n, are the analytical results in the final method reporting units obtained from the n sample aliquots and Σ refers to the sum of the X values from i=l to n.

6. (a) Compute the MDL as follows:

MDL = t(n-1,1-α=0.99) (S)

where:

MDL = the method detection limit

t(n-1,1-α=.99) = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence interval estimates for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (χ^2 /df).

LCL = 0.64 MDL

UCL = 2.20 MDL

where: LCL and UCL are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike the matrix at this calculated MDL and proceed through the procedure starting with Step 4.

(b) If this is the second or later iteration of the MDL calculation, use S2 from the current MDL calculation and S2 from the previous MDL calculation to compute the F-ratio. The F-ratio is calculated by substituting the larger S2 into the numerator S2A and the other into the denominator S2B. The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows: if S2A/S2B<3.05, then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{\frac{1}{2}}$$

if S2A/S2B>3.05, respike at the most recent calculated MDL and process the samples through the procedure starting with Step 4. If the most recent calculated MDL does not permit qualitative identification when samples are spiked at that level, report the MDL as a concentration between the current and previous MDL which permits qualitative identification.

(c) Use the Spooled as calculated in 7b to compute The final MDL according to the following equation:

MDL=2.681 (Spooled)

where 2.681 is equal to t(12,1-α=.99).

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from precentiles of the chi squared over degrees of freedom distribution.

LCL=0.72 MDL

UCL=1.65 MDL

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

Tables of Students' t Values at the 99 Percent Confidence Level

Number of replicates	Degrees of freedom (n-1)	tcn-1,.99)
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
00	00	2.326

Reporting

The analytical method used must be specifically identified by number or title ald the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL and indicate if the MDL procedure was iterated. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level of analyte in the sample was below the determined MDL or exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

[49 FR 43430, Oct. 26, 1984; 50 FR 694, 696, Jan. 4, 1985, as amended at 51 FR 23703, June 30, 1986]

[Document accessed via EPA website http://www.epa.gov/epahome/cfr40.htm on 20 June 2005]

APPENDIX J

Summary of Supplemental Fish Sampling Effort

In addition to the two primary fish species (brown trout and smallmouth bass) that are part of the long-term monitoring program, supplemental samples of northern pike were collected from Falls Village, Bulls Bridge, Lake Lillinonah, and Lake Zoar in 2006 at the request of CTDEP. Northern pike were collected from Bulls Bridge, Lake Lillinonah, and Lake Zoar at the same time as the primary species (see Table 1 of the report); collections from Falls Village occurred on 10 August 2006. Collection methods included boat electroshocking and angling. Five northern pike were collected from each of the four sampling locations. Methods of specimen handling, sample preparation, and sample analyses were identical to those for the primary species (see Methods section of the report). Ages of northern pike were estimated using otoliths, but annuli were not as clearly differentiated as in the primary species, and there appeared to be greater variation in the length-at-age. As a result, reader agreement was relatively low, and ages therefore are not presented in this Appendix.

Results for the northern pike samples are summarized in Table J-1. CTPCB concentrations ranged from 0.35 to 3.02 mg/kg wet weight, while TPCB concentrations ranged from 0.42 to 3.80 mg/kg wet weight. There was no clear pattern of downstream decrease in CTPCB or TPCB concentrations. For example, the mean CTPCB concentrations in the northern pike collected in 2006 were 1.27 mg/kg at Falls Village, 0.77 mg/kg at Bulls Bridge, 0.86 mg/kg at Lake Lillinonah, and 1.33 mg/kg at Lake Zoar.

One of the 20 samples (5%) (from Lake Zoar) had a CTPCB concentration (3.02 mg/kg wet weight) greater than the U.S. Food and Drug Administration (FDA) fish consumption tolerance limit of 2.0 mg/kg wet weight, while two samples (10%), which included that same fish plus one other (from Falls Village), had TPCB concentrations (3.80 and 2.08 mg/kg wet weight) greater than the FDA limit. Unlike the situation in 2004, when one northern pike sample had an anomalously high PCB concentration, no such anomalies were found in the 2006 data.

Table J-1. Total PCB concentrations in northern pike from supplemental samples in 2006. CTPCB and TPCB concentrations (mg/kg wet weight) are from individual fillets. Station codes: FV = Falls Village, BB = Bulls Bridge, LL = Lake Lillinonah, LZ = Lake Zoar. Northern pike ages are considered unreliable and are not included.

Species	Station	Month	Sex	Fisheries ID	Total Length (cm)		Total Weight (g)	Lipid (%)	TPCB (mg/kg)	CTPCB (mg/kg)
					Field	Lab				
Northern pike	FV	August	M	F-1756	82.0	79.9	2939	0.66	1.63	1.31
Northern pike	FV	August	M	F-1758	87.2	86.0	4050	0.66	2.08	1.64
Northern pike	FV	August	F	F-1771	76.0	73.7	2500	0.66	0.70	0.54
Northern pike	FV	August	F	F-1774	91.2	89.5	4300	0.59	1.78	1.39
Northern pike	FV	August	F	F-1767	82.0	81.3	3300	0.75	1.94	1.48
Northern pike	B	August	F	F-1763	88.0	87.2	4250	1.06	1.53	1.22
Northern pike	B	August	F	F-1761	69.7	67.7	2050	0.50	0.53	0.42
Northern pike	B	August	M	F-1770	67.7	66.4	1820	0.93	1.03	0.81
Northern pike	B	August	F	F-1766	72.5	71.3	2400	0.94	0.79	0.64
Northern pike	B	October	F	F-1762	77.4	74.4	2400	0.40	0.91	0.75
Northern pike	L	August	M	F-1759	76.5	75.5	2950	1.07	0.87	0.70
Northern pike	L	August	F	F-1757	79.0	78.0	3412	1.61	0.42	0.35
Northern pike	L	August	M	F-1769	69.8	69.1	2000	0.72	1.32	1.04
Northern pike	L	August	F	F-1768	98.3	96.3	5800	2.36	1.83	1.43
Northern pike	L	August	M	F-1760	86.5	86.5	4800	1.50	0.98	0.79
Northern pike	Z	August	F	F-1772	83.6	81.3	3600	1.13	0.88	0.70
Northern pike	Z	August	M	F-1764	84.3	81.0	3600	1.17	3.80	3.02
Northern pike	Z	August	M	F-1765	76.4	75.5	3000	1.32	1.05	0.84
Northern pike	Z	August	F	F-1775	98.3	94.6	5800	1.19	1.57	1.25
Northern pike	Z	August	F	F-1773	90.8	89.0	4600	1.43	1.08	0.84