PCB Concentrations in Fishes from the Housatonic River, Connecticut, 1984–2014, and in Benthic Insects, 1978–2014

Final Report

Report No. 2016-2

Prepared for the

General Electric Company

by the

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

July 22, 2016

GE Global Operations, Environmental, Health & Safety

159 Plastics Avenue
Pittsfield, MA 01201
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July 29, 2016

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

Mr. Dean Tagliaferro
U.S. Environmental Protection Agency
c/o Avatar Environmental
10 Lyman Street, Suite 2
Pittsfield, MA 01201

Re: Housatonic River, Connecticut
Report on 2014 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, 1984-2014, and in Benthic Insects, 1978-2014*, which was prepared on behalf of the General Electric Company (GE) by the Academy of Natural Sciences of Drexel University. This report presents the results of the Academy's 2014 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.

Please let me know if you have any questions or would like additional copies.

Very truly yours,

Kevin Mooney
Project Manager

Enclosure

cc: Timothy Conway, EPA
Christopher Ferry, ASRC Primus (cover letter and CD of report)

Scott Campbell, Avatar (2 hard copies and CD of report)
John Looney, CT AG
Brian Toal, CDPH
Michael Gorski, MDEP (cover letter and CD of report)
Eva Tor, MDEP (electronic copy of cover letter by e-mail)
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Andrew Silfer, GE (cover letter and CD of report)
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Stuart Messur, Anchor QEA (cover letter and CD of report)
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EXECUTIVE SUMMARY

The Academy of Natural Sciences of Drexel University (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) – now known as the Connecticut Department of Energy and Environmental Protection (CTDEEP) – during 1978–1990 and have been monitored by the Academy since 1992. Data for both groups of organisms have documented a general 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 Smallmouth Bass, Brown Trout, and benthic insects compared to 1992. Concentrations observed in the 1996–2012 studies for fish were roughly similar to those in 1994 and were well below the levels in 1986–1992. For benthic insects, concentrations in the more recent prior years (2001-2012) 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, 2008, 2010, 2012, or 2014, the biennial monitoring program was nevertheless continued in these years, using the same study design as in previous years. The present report details results from the 2014 fish and benthic insect sampling program.

Purpose of Study

The main purpose of the 2014 study was to compare PCB concentrations in Smallmouth Bass, Brown Trout, and benthic insects with levels observed in previous study years, and to compare PCB concentrations in Smallmouth Bass collected at four monitoring stations in 2014.

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).

Taxa Monitored

The taxa sampled for long-term monitoring purposes were the same as in the 2000-2012 studies and included fish and benthic insects. The fish species were Smallmouth Bass (*Micropterus dolomieu*) (collected at West Cornwall, Bulls Bridge, Lake Lillinonah and Lake Zoar) and Brown Trout (*Salmo trutta*) (collected only at West Cornwall). The benthic insect taxa (collected only at West Cornwall) consisted of filter-feeding caddisflies (Hydropsychidae), predatory stoneflies (Perlidae), and predatory dobsonflies (Corydalidae). All fish and benthic invertebrate samples were collected during June, August, September and October 2014 (see Table 1 of the report).

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 (CTPCB) sums the concentrations of all individual congeners (up to 121) quantitated by the analytical method. The Aroclor-based procedure (TPCB) 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, and 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 1992–2014 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 described herein. Station differences were assessed only for Smallmouth Bass as it was 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 (i.e., river age), 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. These statistical assessments have been done by performing pairwise comparisons of covariate-adjusted mean values among stations or among years. The results of these comparisons are presented in the main body of the text.

While these pairwise comparisons are appropriate, their use for testing among-year differences results in a loss of statistical power as additional years are added to the analysis. As discussed in Appendix J to this report, the large number of pairwise comparisons increases the frequency of spurious significant differences and the statistical techniques designed to control that frequency reduce statistical power as well.

An alternate approach to testing significance of temporal trends by pairwise comparisons is presented and discussed in Appendix J. This approach, based on the linear contrast method, involves defining and testing a smaller number of *a priori* comparisons of interest. These comparisons involve contrasting the average data from the three most recent years (the 2010, 2012, and 2014 surveys) with those of different periods which have been shown to have had different mean PCB concentrations. These periods are 1984-1986, a period of intermediate PCB concentrations; 1988-1992, a period of higher PCB concentrations; and 1994-2008, a period of lower PCB concentrations immediately preceding the three most recent years.

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). Data for these purposes are therefore reported without adjusting for the effects of ancillary variables.

Results

Comparison of Fish Results with Previous Years

PCB concentrations in Smallmouth Bass and Brown Trout in 2014 were generally higher than in any of the years between 1994 and 2012, but remained 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). Specifically, for Smallmouth Bass, the results at West Cornwall, Lake Lillinonah, and Lake Zoar all showed increases in PCB concentrations in 2014 from those in 1994-2012. Bulls Bridge, however, did not exhibit this general pattern, and the 2014 concentrations were more comparable to those found between 1994 and 2012. For Brown Trout, the 2014 results likewise showed increases in PCB concentrations from those in 1994-2012. For both species, where increases were seen, the mean wet-weight PCB concentrations were generally in the range of approximately 0.2 mg/kg to 1.0 mg/kg higher than the concentrations reported in the various years from 1994 through 2012. The PCB concentrations at all stations in 2014 were still well below the concentrations observed in 1992 (and most prior years).

Statistical models of PCB concentrations were developed to adjust for differences in lipid concentrations, ages, and, where appropriate, proportions of males and females in samples from different years and stations. These models calculate least squares means (LSMs) of the ln-transformed data, which are the values of ln-transformed concentrations at the mean of all factors (e.g., lipid, age, and sex). These LSMs are transformed back into units of mg/kg to provide adjusted concentrations. Differences between concentrations in recent and earlier years were examined by two techniques, both based on the same statistical models. Multiple pairwise comparisons tested differences among each pair of years, including differences between 2014 and each earlier year. However, these tests have relatively low statistical power. The second technique used linear contrasts to compare concentrations in recent years (2010, 2012, and 2014) with concentrations in three earlier periods, 1984-1986 (intermediate concentrations), 1988-1992 (typically higher concentrations) and 1994-2008 (typically lower concentrations).

For Smallmouth Bass, based on multiple pairwise comparison tests, several significant differences were found between 2014 concentrations and those in earlier years. For data with all stations combined, TPCB concentrations in 2014 were significantly lower than those in 1990, higher than those in 1994 and 2000-2010, and not significantly different from those in any other study year. Similarly, CTPCB concentrations in 2014 were significantly lower than those in 1992, higher than those in 1994 and 2000-2010, and not significantly different from those in any other study year.

When stations were assessed individually, 2014 TPCB concentrations at West Cornwall were significantly lower than those during 1990 and 1992, higher than those in 2000-2004 and 2010, but not significantly different from those in any other study year. At Bulls Bridge, 2014 TPCB concentrations were not significantly different from those in any prior year except that they were significantly lower than those in 1988 and 1990 and higher than those in 2002. At Lake Lillinonah, 2014 concentrations were significantly higher than those in 2000, 2002, and 2010 and were not significantly different from those in any other study year. At Lake Zoar, concentrations were significantly higher than those in 1994 and 2000-2004 and were not significantly different from those in any other study year.

The linear contrasts approach found that both the TPCB and CTPCB concentrations in the three most recent years (2010-2014) were significantly higher than the concentrations in 1994-2008 at West Cornwall and Lake Zoar and with all stations combined, primarily due to the increased concentrations in 2014. It did not find any significant difference at Lake Lillinonah or Bulls Bridge between the most recent years and 1994-2008. This method also found that the TPCB concentrations in 2010-2014 were significantly lower than those in 1984-1986 and 1988-1992 at each station except Lake Zoar (where there was no significant difference between 2010-2014 and those years), and that the CTPCB concentrations in the three recent years were significantly lower than those in 1992 at all stations except Lake Zoar (which saw no significant difference). (Since CTPCB was only calculated from 1992 on, the recent data could not be compared with the 1984-1986 and 1988-1992 periods.)

For Brown Trout at West Cornwall, multiple comparison tests found that 2014 TPCB concentrations were significantly lower than concentrations in 1988-1992, higher than those in

several recent years (1994, 1996, 2000, 2002, 2006, 2010, and 2012), and not significantly different from those in other study years. Similarly, 2014 CTPCB concentrations were significant lower than those in 1992, higher than those in several recent years (1994, 1996, 2000, 2002, 2006, 2010, and 2012), and not significantly different from those in any other year for which CTPCB data exist.

The linear contrasts found that TPCB concentrations in Brown Trout in the most recent period (2010-2014) were significantly lower than those in the two earlier periods (1984-1986 and 1988-1992), but not significantly different from those from 1994-2008. Similarly, CTPCB concentrations in 2010-2014 were significantly lower than 1992 but not significantly different from those in 1994-2008.

Comparison of Fish Results among Stations

Visual inspection of mean TPCB and CTPCB concentrations for Smallmouth Bass in 2014 indicates that wet-weight concentrations appear higher at West Cornwall, Bulls Bridge, and Lake Zoar than at Lake Lillinonah. This pattern is different from previous years in that Lake Zoar exhibited levels comparable to Bulls Bridge and West Cornwall, which have historically proven to have higher concentrations. Similarly, on a lipid-normalized basis, the fish collected in 2014 at West Cornwall, Bulls Bridge, and Lake Zoar had higher TPCB concentration per unit lipid than those from Lake Lillinonah.

Using a statistical model that included data from all years, analysis of covariance revealed statistically significant station differences in mean TPCB and CTPCB concentrations. Pairwise comparisons indicated that TPCB concentrations at West Cornwall were not statistically different from Bulls Bridge and that Lake Lillinonah and Lake Zoar were both significantly different from all other stations. These comparisons also indicated that CTPCB concentrations at West Cornwall and Bulls Bridge did not differ significantly from each other, and Lake Lillinonah and Lake Zoar were both significantly different from all other stations.

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. Eleven of the 40 Smallmouth Bass samples in 2014 (28%) had CTPCB concentrations exceeding the FDA limit (three each from West Cornwall, Bulls Bridge, and Lake Zoar, and two from Lake Lillinonah), and 17 of 40 (42%) had TPCB concentrations exceeding that level (six from West Cornwall, four each from Bulls Bridge and Lake Zoar, and three from Lake Lillinonah). Among Brown Trout, 18 of 30 (60%) had CTPCB concentrations exceeding the limit and 19 of 30 (63%) had TPCB concentrations exceeding the FDA limit.

The percentages of Smallmouth Bass and Brown Trout with concentrations *below* the FDA limit were lower than any year in the study period since 1992 but were substantially higher than most of the percentages observed during 1986–1990 for both species.

Benthic Invertebrate Results

Analysis of benthic insect samples in 2014 showed that PCB concentrations have remained relatively low and generally similar to those in 2000-2012 with some variations. Specifically, 2014 CTPCB concentrations were: (a) for caddisflies and stoneflies, generally comparable to those in 2012 and slightly higher than those in 1998–2010: (b) for dobsonflies, higher than those in 2002 and 2005 and lower than or comparable to those in 1998, 2001, 2008, 2010, and 2012; and (c) for all three taxa, lower than those in 1992–1996. Similarly, TPCB concentrations in 2014 in both filter feeders (caddisflies) and predators (dobsonflies and stoneflies) were generally similar to those in 2000-2012, lower than those in 1992-1998 (except for caddisflies in 1998), and well below those in most of the prior years (1978-1990).

Rank correlation analysis of the entire data series for 1978–2014 revealed a statistically significant temporal trend of decreasing PCB concentrations in both filter feeders and predators since the start of the study.

Summary and Conclusions

Results of the 2014 Academy fish monitoring study show that total PCB concentrations in Smallmouth Bass and Brown Trout in 2014 were generally somewhat higher than those observed during any study year between 1994 and 2012 (with some expected variability), but remained well below the levels observed in 1992 and (where applicable) most prior years.

For Smallmouth Bass, the multiple pairwise comparisons indicate that the 2014 PCB concentrations for all stations combined were higher than those for most recent years, and that the 2014 concentrations for individual stations were higher than those for some recent years but not others at West Cornwall, Lake Lillinonah, and Lake Zoar, but not significantly different from recent years at Bulls Bridge. Similarly, the linear contrasts approach indicates that the PCB concentrations for the 2010-2014 period at West Cornwall and Lake Zoar and for all stations combined were higher than those for the 1994-2008 period (though generally lower than in prior years), but not for Bulls Bridge or Lake Lillinonah, with the recent increases likely driven primarily by the 2014 results.

For Brown Trout, the multiple pairwise comparisons indicate that the 2014 PCB concentrations were higher than those for most, but not all, recent years, although lower than those for 1992 and prior years. However, the results of the linear contrasts approach indicate that the PCB concentrations for the 2010-2014 period were not significantly different from those for the 1994-2008 period.

As noted above and shown in prior Academy reports, the fish monitoring studies conducted over the period from 1994 through 2012 showed that the total PCB concentrations in Smallmouth Bass and Brown Trout were low and generally similar over that period (with variations in some of the analyses). While the 2014 data indicate a slight increase in concentrations at some locations, the data from this one year are insufficient to determine whether that apparent increase simply

represents a one-time anomaly in an overall trend of generally consistent data over the past two decades.

For filter-feeding and predatory benthic insects, the 2014 data show that PCB concentrations have remained low and generally similar to those in 2000-2012; and an analysis of the overall data has continued to show a statistically significant trend of decreasing total PCB concentration over the overall monitoring period (1978–2014).

QUALITY ASSURANCE STATEMENT

Study Number: 236329

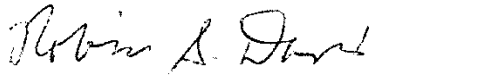
Study Title: PCB Concentrations in Fishes From the Housatonic River, Connecticut, 1984–2014, and in Benthic Invertebrates, 1978–2014.

This study was performed under the general provisions of the Patrick Center's Quality Assurance Implementation Plan (Rev. 1, June 1998). The final report will be reviewed to determine that it is an accurate reflection of the data obtained.

The dates that Quality Assurance activities on this study were completed will be given in the final report.

Data Reviews: Report Review 2/25/2016

Archiving: Raw data and the final report will be filed in the Patrick Center's archives.



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

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INTRODUCTION

The Academy of Natural Sciences of Drexel University (Academy) has conducted biennial fish surveys in the Connecticut portion of the Housatonic River since 1984 (ANSP 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, and 2013). Benthic insects were monitored by the Connecticut Department of Environmental Protection (CTDEP) – now known as the Connecticut Department of Energy and Environmental Protection (CTDEEP) – 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 Smallmouth Bass, Brown Trout, and benthic insects compared to 1992. Concentrations observed in the 1996–2012 studies were roughly similar to those in 1994 and, for fish, were below the levels for 1986–1992. For benthic insects, concentrations in the more recent years (2001, 2002, 2005, 2006, 2008, 2010, and 2012) 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, 2008, 2010, 2012 and 2014, the biennial monitoring program was nevertheless continued in these years, using the same study design as in previous years. The present report details results from the 2014 fish and benthic insect sampling.

The main objectives of the 2014 study were as follows:

- *Measure PCB concentrations in selected Housatonic River fish.* As a continuation of prior studies, the species sampled and analyzed for total PCBs were Smallmouth Bass at West Cornwall, Bulls Bridge, Lake Lillinonah, and Lake Zoar and Brown Trout at West Cornwall (sampling locations are shown in Fig. 1).
- *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 Smallmouth Bass and Brown Trout 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 maximum comparability with previous results, fish samples employed in the monitoring study were collected from the same locations and during the same primary seasonal time period as in prior years. The number of Smallmouth Bass collected at all four stations and the number of Brown Trout collected at West Cornwall were comparable to the numbers collected in all years from 1994 through 2012 (except for 1996, 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 (Smallmouth Bass, Brown Trout, and benthic insects). Supporting information is provided in appendices.

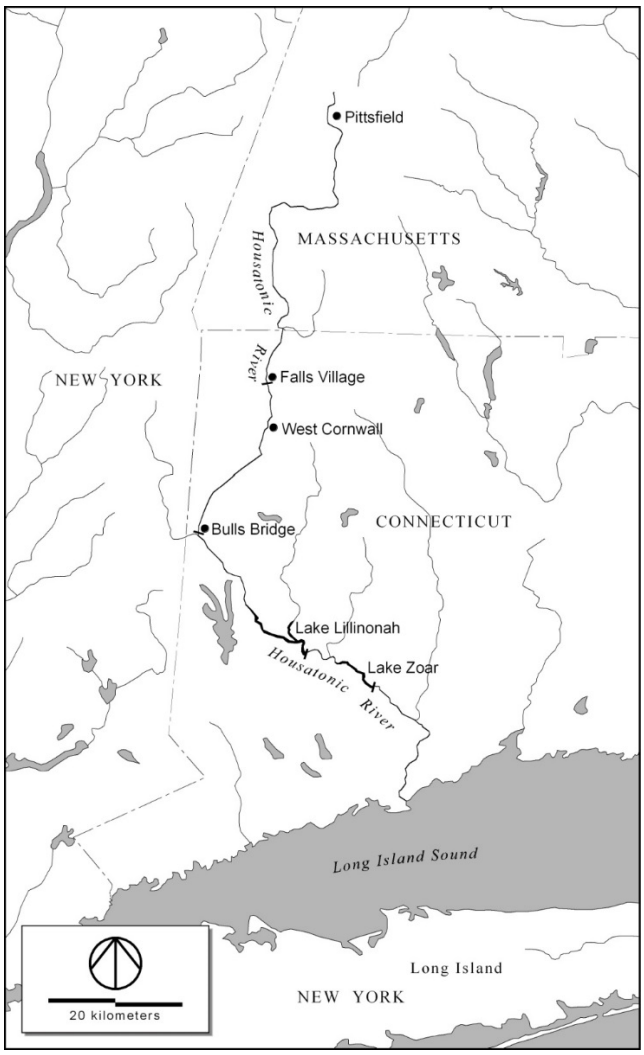


Figure 1. Map of the Housatonic River showing sampling stations for the 2014 fish and benthic insect collections in Connecticut. Smallmouth Bass were collected at West Cornwall, Bulls Bridge, Lake Lillinonah and Lake Zoar. Brown Trout and benthic insects were collected only at West Cornwall. Approximate 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 studies, Brown Trout were collected only at West Cornwall, while Smallmouth Bass were collected at all four stations. Three fish-sampling trips were made by Academy personnel in August and October 2014 to collect fish from all four stations. In addition, during the October visit, fish specimens from West Cornwall collected in September were provided to the Academy by CTDEEP personnel. Table 1 summarizes fish collection dates and techniques for the four sampling stations employed in the monitoring study.

Table 1. Summary of fish sampling dates, methods and locations for fish collections on the Housatonic River, Connecticut, in 2014. Symbols: BS = boat electrofishing, WS = walk-along (shore) electrofishing. ** denotes collection of some fish by CTDEEP personnel.

Sampling Location	Sampling Dates in 2014			
	4-6 Aug, 2014	26-28 Aug., 2014	5 Sept., 2014	6-7 Oct., 2014
West Cornwall	-	WS	WS**	WS
Bulls Bridge	BS	-	-	-
Lake Lillinonah	BS	-	-	BS
Lake Zoar	BS	-	-	BS

West Cornwall

Holdover Brown Trout, 2014-stocked Brown Trout, and Smallmouth Bass were collected from several locations at the West Cornwall station and Housatonic River Trout Management Area, including downstream of the covered bridge, the State Park Campground, and "The Elms," on 26, 27 and 28 August 2014 by Academy and CTDEEP personnel using walk-along electrofishing equipment. Additional sampling was conducted on 5 September 2014 by CTDEEP personnel using walk-along electrofishing at Turnip Island and on 6 October 2014 by Academy and CTDEEP personnel using walk-along electrofishing at "The Elms."

Benthic insect samples were collected by Academy personnel on 24 June 2014 within the riffle upstream from the County Road 128 bridge and upstream of Mill Brook at West Cornwall (upstream of the Covered Bridge). This is the same site that was sampled in the 2004, 2006, 2008, 2010, and 2012 studies.

Bulls Bridge

Smallmouth Bass were collected by Academy personnel at Bulls Bridge on 4 August 2014 by boat electrofishing. Boat electrofishing was conducted during daylight 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

Smallmouth Bass were collected by Academy personnel at the Lake Lillinonah station by boat electrofishing on 6 August and again on 7 October 2014. Boat electrofishing was conducted in inlets or coves, around docks, and along rocky ledges and shorelines. Sampling was conducted from about 5.0 km below the State Route 133 bridge to 5.0 km above State Route 133.

Lake Zoar

Smallmouth Bass were collected by Academy personnel at the upper end of Lake Zoar (both banks) by boat electrofishing on 5 August 2014, and again on 7 October 2014. The lower end of the reservoir (both banks) was sampled by boat electrofishing on 5 August 2014. Typical habitat sampled by electrofishing included coves, rock ledges, tree/brush snags, boat docks and bridge pilings. Sampling in the upper end was conducted just downstream of the Shepaug Dam to the State Boat Launch. Sampling for fish in the lower section of the reservoir was conducted from the Eichler Cove Marina to Kettletown State Park and also at both banks of the "Snake Rock" area.

METHODS

Fish Collection and Handling

Brown Trout and Smallmouth Bass were collected by Academy staff, with the assistance of the CTDEEP Western Division Fisheries (West Cornwall only), by walk-along and boat electrofishing. Two Brown Trout from the Burlington fish hatchery were provided by CTDEEP 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 2014 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

All sampling stations except West Cornwall were sampled using a 17-ft electrofishing boat. A Smith-Root model 5.0 GPP electrofisher 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 electrofishing was conducted in the morning and early afternoon. A Robin generator and Coffelt VVP unit operated at AC output fitted in a canoe was provided by CTDEEP and was used for walk-along (tow-barge) electrofishing during daylight hours at West Cornwall.

During boat electrofishing, 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/lake water in a pre-cleaned metal tub (washed with Micro-90® cleaner and rinsed with river/lake 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 boat electrofishing, fish were collected with a walk-along electrofishing unit. While walk-along electrofishing, two operators carried long anode poles connected with a hoop with netters carrying dip-nets. The netters collected the stunned fish and placed them into a tub of river water until they were identified and processed after the sampling effort was completed.

Two hatchery trout were provided by CTDEEP. In 2014, these fish were taken from the Burlington hatchery in October, as in the 2000 and 2010 studies. In 2002, 2004, 2006, 2008 and 2012 hatchery fish were taken from the Burlington hatchery in August. In prior years, hatchery fish were taken in August (1994–1998 studies) and May (1986 and 1988 studies), depending on availability of hatchery fish. In all previous studies, PCB concentrations in hatchery fish have been uniformly low regardless of collection date.

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 a Floy tag inserted into the head of specimens. 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, specimens were placed in freezers until laboratory processing, and sample data were entered into the Fisheries Section database. 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 un-cleaned laboratory surfaces was avoided to minimize chances of contamination.

Benthic Insect Collection and Handling

Benthic insects (Perlid stoneflies, Hydropsychid caddisflies and Corydalid dobsonflies) were collected by rapidly lifting rocks and picking specimens from their surfaces with pre-cleaned forceps within the riffle upstream of the County Road 128 bridge. Benthic 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 Philadelphia laboratories. 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 stocking marks were recorded. During sample preparation, external and internal anomalies, presence of parasites, stomach contents, 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 knives in 10% HCL and bathe plates in 25% HNO_3 for 12 h.
3. Rinse all in acetone and hexane, and then rinse with dichloromethane and air dry.
4. 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 helped in verifying ages of most Brown Trout. CTDEEP 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 2014 included yearling (Survival and Cortland strains) and adult fish (Cortland strain) stocked from the Burlington hatchery in 2014, and a few adult fish stocked in 2013 and 2012.

The majority of the trout collected in 2014 had identifying marks (visible implant elastomer tags) to distinguish when they were stocked. Otoliths were the primary method of determining the year of stocking for fish stocked in 2012. Otolith bands are irregularly formed in the hatchery, but typical banding patterns are evident in fish after stocking. Thus, hatchery fish had a dark central area with irregular banding corresponding to time in the hatchery, with a distal clear area produced after stocking. The holdover fish had one or more annuli, allowing assessment of stocking and hatch year. As in most past studies, holdover trout were distinguished principally by marks (fin clips and/or elastomer dye marks) and length.

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 80% of the Smallmouth Bass. Exact agreement occurred for 67% of Brown Trout solely on the basis of otolith analyses. However, 25 of the 32 trout had marks indicating the stocking year and season. The reader agreement was 100% on the seven trout without marks (stocked without marks or with a regrown clip). A mutually agreed upon determination was reached for discrepancies in age after re-examining the otolith sections.

Analysis of PCBs

The method of PCB analysis was identical to that employed in the 2002-2012 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), with one exception. Congener 178 was not quantitated in the 2002-2010 analyses.

Congener 178 typically occurs as a very small proportion of PCBs in samples, and the exclusion of this congener has essentially no effect on estimates of concentrations of total PCBs. 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 Teflon spoon. Approximately 30 g of Na_2SO_4 (manufactured by J.T. Baker, previously muffled at 450°C for 4 h) 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. 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 1992–2014 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 2014 (Appendix C). In analyses that included all monitoring years, only TPCB was used, while analyses that included only years 1992–2014 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 and Retention

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 males.

Sexes often differ in PCB concentration, presumably because of the loss of PCBs associated with lipid in eggs. Since Brown Trout do not routinely reproduce in the study area, this mechanism is not expected to occur in trout. Furthermore, sex was not recorded for many trout in earlier studies. Therefore, statistical models of PCB concentrations in Brown Trout did not use sex as a factor.

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, and station were incorporated in ANCOVA models as discrete effects for Smallmouth Bass analyses. Only year was incorporated as a discrete effect for Brown Trout analyses as trout were only collected at West Cornwall and sex was not expected to affect PCB concentrations.

River age and lipid content (both ln-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[1] (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. Therefore, following standard statistical practice (e.g., Sokal and Rohlf 1969), TPCB and CTPCB data were ln-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, meaning 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. To avoid overfitting the statistical models, the Akaike Information Criterion (AIC) was used to select among alternative models with different groups of main effects, covariates, and interactions. Use of the AIC is analogous to selecting the model with the highest explanatory power (r^2), except that the AIC also varies with the number of parameters in the model. Thus, inclusion of an effect in a model that requires a large number of parameters but only leads to a small increase in r^2 leads to an increase in the AIC, so the simpler model is preferred (models are selected on the basis of the lowest AIC). The potential for overfitting data increases with the increase in the number of years and total number of specimens in the study, since more complicated models can be built and appear to be supported by the data.

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

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

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) criteria. Thus, any differences detected by these tests represented differences in PCB concentration after accounting for the effects of age, sex, and lipid content.

These pairwise multiple comparisons, in which a separate test is done for each pair of years, have been used throughout the many years of these surveys. This was an appropriate procedure for comparing least-squares means, especially in earlier years when the temporal pattern of concentrations was unclear and no *a priori* hypotheses could be defined. However, as discussed in Appendix J to this report, the use of these pairwise comparisons for testing among-year differences results in a loss of statistical power as additional years are included in the analysis. The large number of pairwise comparisons increases the frequency of spurious significant differences, and the statistical techniques designed to control that frequency themselves reduce statistical power as well.

An alternate approach to testing the significance of temporal trends is presented and discussed in Appendix J. This approach involves defining and testing a smaller number of *a priori* comparisons of interest. These comparisons involve contrasting the average data from the three most recent years (in this case, the 2010, 2012, and 2014 surveys) – used in lieu of only the most recent year given the year-to-year variability – with those of different periods which have been shown to have had different mean PCB concentrations. These periods are 1984-1986, a period of intermediate PCB concentrations; 1988-1992, a period of higher PCB concentrations; and 1994-2008, a period of lower PCB concentrations immediately preceding the three most recent years.[2] This approach uses the statistical method of linear contrasts, as described in Appendix J. Linear contrasts between a single year's data (e.g., the most recent) and other periods were not done, because the amount of year-year variability in concentrations would make it difficult to interpret results of such contrasts.

RESULTS

Summary of the 2014 Monitoring Data for Brown Trout and Smallmouth Bass

Thirty Brown Trout collected at West Cornwall and two Brown Trout from the Burlington Hatchery were analyzed for PCB content (stocking dates are summarized in Appendix D). Of the 30 specimens from West Cornwall, sex could be determined by macroscopic examination for all trout; they consisted of 12 males and 18 females. Forty Smallmouth Bass from four stations were

[2] In future monitoring reports, the appropriate groupings of years will be re-evaluated.

analyzed for PCB content; these included 21 males and 19 females. The (arithmetic) mean and range of CTPCB concentrations and lipid-normalized CTPCB concentrations for the monitoring samples are summarized in Table 3. Hatchery Trout had a geometric mean CTPCB level of 0.004 mg/kg (wet) and were not used in the statistical analyses.

Comparison with Previous Years

Smallmouth Bass and Brown Trout were the primary fish species of interest in the 2014 monitoring study. Comparisons among years were therefore restricted to these two species, excluding hatchery trout. (A tabular comparison of average CTPCB content in all species of fishes collected in 1984–2014, without adjustment for the influence of covariates, can be found in Appendix E.)

Smallmouth Bass

Visual inspection of sample means for Smallmouth Bass suggests that wet-weight TPCB concentrations in 2014 were comparable to the range of concentrations since 1994 at the Bulls Bridge station. However, levels were elevated in comparison to all previous years since 1994 at West Cornwall, Lake Lillinonah, and Lake Zoar (Fig. 2; Table 4). TPCB concentrations in 2014 were lower than observed in 1992 at all stations except Lake Zoar, where concentrations are comparable to those observed in 1992. The wet-weight CTPCB results show a similar pattern (Table 4).

Table 3. Arithmetic means and ranges of congener-based total PCB estimates (mg/kg wet weight) in Brown Trout (all fish and subsets grouped by river age) and Smallmouth Bass collected in 2014. In the "Male/Female" column, the first and second numbers listed for each entry (e.g., 6/4) are the numbers of male and female specimens.

				River Age		CTPCB		CTPCB/LIPID	
Station	# Specimens	Age criteria	Male/Female	Arith Mean	Range	Arith Mean	Range	Arith Mean	Range
Brown Trout									
West Cornwall	30	All	12/18	0.67	0.28-2.35	2.54	0.92-6.68	0.95	0.16-2.12
West Cornwall	22	2014	10/12	0.34	0.28-0.44	1.84	0.92-2.98	0.81	0.16-1.90
West Cornwall	4	2013	1/3	1.07	0.95-1.35	3.64	1.84-4.78	1.11	0.60-1.98
West Cornwall	4	2012	1/3	2.07	1.95-2.35	5.27	1.84-6.68	1.50	1.02-2.12
Smallmouth Bass									
All Stations	40	all	21/19	6.4	3-15	1.48	0.25-5.72	1.33	0.29-4.99
West Cornwall	10	all	6/4	5.2	3-9	1.72	0.71-2.66	1.76	0.51-4.22
Bulls Bridge	10	all	4/6	7.1	3-15	1.25	0.28-2.65	1.34	0.31-3.57
Lake Lillinonah	10	all	6/4	5.4	3-14	1.52	0.25-5.72	0.87	0.29-1.80
Lake Zoar	10	all	5/5	7.8	3-13	1.45	0.25-3.59	1.37	0.42-4.99

The lipid-normalized TPCB data, which removes the variability due to lipid content, indicate that the 2014 concentrations were within range, although at the high end of the range, of the concentrations since 1994 at all stations except Lake Zoar, which exhibited a higher concentration than in all years since 1992 (Fig. 2). Further, all of these datasets (i.e., the wet-weight TPCB and CTPCB data and the lipid-normalized TPCB data) indicate that concentrations during 1994-2014 were lower than those during 1986-1992 (for TPCB) or 1992 (for CTPCB) at West Cornwall, Bulls Bridge and Lake Lillinonah. Lake Zoar has shown comparable mean TPCB and CTPCB

concentrations to the 1992 study; however the 2014 lipid-normalized TPCB is lower than concentrations in 1990 (Fig. 2, Table 4).

Multiple pairwise comparisons of the results of ANCOVA for data with all stations combined detected no statistically significant differences between TPCB concentrations in 2014 and those in 1984-1988, 1992, 1996-1998, and 2012. However, TPCB concentrations in 2014 were significantly higher than concentrations in 1994 and 2000-2010 and significantly lower than concentrations in 1990 (Table 5). For CTPCB, concentrations in 2014 were not significantly different from those in 1996-1998 and 2012 but were significantly higher than those in 1994 and 2000-2010 and lower than those in 1992 (Table 5). (Statistically significant main effects, covariates, and interactions in the ANCOVA models are summarized in Appendix F.) Pairwise comparisons of TPCB data show a trend from higher concentrations in 1988–1992 to lower concentrations in 1994-2010 with an increase in concentrations in 2014. Pairwise comparisons of the CTPCB concentration also show the highest concentration in 1992, followed by lower concentrations in more recent years, with an increase in 2014 comparable to levels in 1996-1998 and 2012.

The results of the analyses of linear contrasts (Appendix J) vary slightly in regards to the above results, most likely because of the grouping of 2014 with 2010 and 2012, given that TPCB and CTPCB concentrations in 2014 were found to be significantly higher than 2010 in the pairwise comparisons. The TPCB concentrations in the three most recent years (2010-2014) were significantly lower than those in 1984-1986 and 1988-1992 and significantly higher than those in 1994-2008. The 2010-2014 CTPCB concentrations were not significantly different from those in 1992 but were significantly higher than those in 1994-2008.

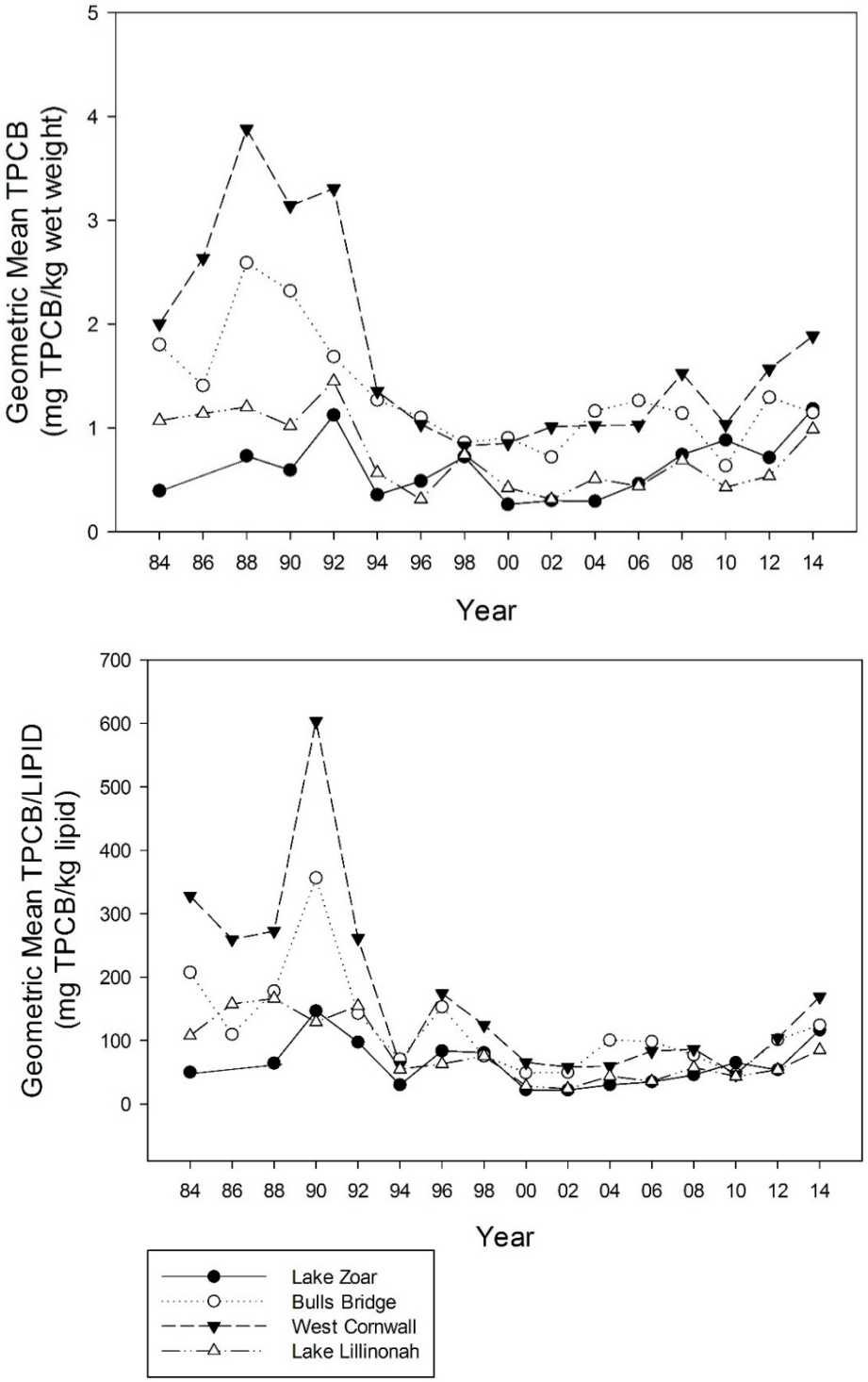


Figure 2. Historical patterns of PCB concentrations in Smallmouth Bass at four sampling stations on the Housatonic River, 1984–2014. Top Panel — Geometric means (unadjusted) of TPCB. Bottom Panel — Geometric means (unadjusted) of lipid-normalized TPCB (TPCB divided by proportion lipid). The pronounced peak in lipid-normalized TPCB in 1990 is due to unusually low lipid levels rather than high TPCB levels (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, 1984–2014. Results presented in units of mg/kg wet weight.

Year	Brown Trout		Smallmouth Bass			
	Cornwall	Hatchery	Cornwall	Bulls Br	Lillinonah	Zoar
CTPCB						
2014	2.21	0.004	1.62	0.93	0.83	0.99
2012	1.48	0.07	1.36	1.05	0.44	0.60
2010	1.13	0.01	0.88	0.50	0.36	0.74
2008	1.53	0.01	1.26	0.88	0.55	0.62
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	1.96	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						
2014	2.54	0.005	1.88	1.15	0.99	1.18
2012	1.74	0.07	1.57	1.29	0.54	0.71
2010	1.32	0.01	1.04	0.63	0.43	0.88
2008	1.82	0.01	1.53	1.14	0.69	0.74
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	0.90	0.42	0.30
1998	1.93	0.12	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	–	3.30	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
1984	2.30	–	2.00	1.80	1.07	0.39

When stations were tested separately for differences among years, there was an overall pattern of decrease after 1992 with a slight increase in the past two survey years (notably in 2014), with some differences in the temporal patterns among stations (Table 6).

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 1984–2014 (left column) and the natural logarithm of CTPCB for 1992–2014 (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	0.3272	efg
1986	0.4066	fg
1988	0.5002	gh
1990	0.6940	h
1992	0.5388	gh
1994	-0.3953	abc
1996	-0.1843	c
1998	-0.0318	cde
2000	-0.7880	a
2002	-0.7599	ab
2004	-0.4089	abc
2006	-0.3624	bc
2008	-0.2543	c
2010	-0.4326	abc
2012	-0.0710	cd
2014	0.1951	def

Station Comparisons

Station	LSM	Group
WC	0.3665	c
BB	0.2033	c
LL	-0.2709	b
Z	-0.6361	a

Summary

Effect	Significance Groups
Years	(94 00 02 04 10) (94 02 04 06 10) (94 96 98 04 06 08 10 12) (98 12 14) (84 98 14) (84 86 14) (84 86 88 92) (88 90 92)
Stations	(Z) (LL) (BB WC)

Ln (CTPCB)

Year Comparisons

Year	LSM	Group
-		
-		
-		
-		
1992	0.4104	f
1994	-0.4211	abcd
1996	-0.1400	cde
1998	0.0487	de
2000	-0.6954	a
2002	-0.6573	a
2004	-0.4920	abc
2006	-0.4853	abc
2008	-0.3984	abcd
2010	-0.5408	ab
2012	-0.1585	bcde
2014	0.1158	e

Station Comparisons

Station	LSM	Group
WC	0.0667	c
BB	-0.0543	c
LL	-0.4424	b
Z	-0.7081	a

Summary

Effect	Significance Groups
Years	(94 00 02 04 06 08 10) (94 04 06 08 10 12) (94 96 04 06 08 12) (94 96 98 08 12) (96 98 12 14) (92)
Stations	(Z) (LL) (BB WC)

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 1984–2014 (excluding 1986 for Lake Zoar) 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.

West Cornwall

Year	LSM	Group
1984	0.9492	fgh
1986	0.9419	efgh
1988	1.2573	gh
1990	1.3822	h
1992	1.2961	h
1994	-0.0475	abcd
1996	0.3214	bcde
1998	0.0354	abcd
2000	-0.3485	a
2002	-0.3470	a
2004	-0.0994	abc
2006	0.1488	abcd
2008	0.1634	abcd
2010	-0.1667	ab
2012	0.4721	cdef
2014	0.6274	defg

Bulls Bridge

Year	LSM	Group
1984	0.7109	def
1986	0.3271	bcd
1988	0.9896	ef
1990	1.1172	f
1992	0.5428	def
1994	0.2634	bcd
1996	0.4374	cde
1998	-0.1747	abc
2000	-0.2321	abc
2002	-0.4647	a
2004	0.1436	abcd
2006	0.3297	bcd
2008	0.1308	abcd
2010	-0.3448	ab
2012	0.2827	bcd
2014	0.3003	bcd

Summary

Station	Significance Groups*
West Cornwall	(94 98 00 02 04 06 08 10) (94 96 98 04 06 08 10) (94 96 98 04 06 08 12) (94 96 98 06 08 12 14) (86 96 12 14) (84 86 12 14) (84 86 88 14) (84 86 88 90 92)
Bulls Bridge	(98 00 02 04 08 10) (86 94 98 00 04 06 08 10 12 14) (86 94 96 98 00 04 06 08 12 14) (84 86 92 94 96 04 06 08 12 14) (84 88 92 96) (84 88 90 92)

*Listed in order of decreasing LSM

Table 6 (continued).

Lake Lillinonah		
Year	LSM	Group
1984	0.2057	de
1986	0.2291	de
1988	0.1583	de
1990	0.0686	cde
1992	0.2871	e
1994	-0.5049	abcde
1996	-0.8957	ab
1998	-0.1513	bcde
2000	-1.0755	a
2002	-1.1825	a
2004	-0.6829	abc
2006	-0.8673	ab
2008	-0.6969	abc
2010	-0.9982	a
2012	-0.5380	abcd
2014	-0.1239	bcde

Lake Zoar		
Year	LSM	Group
1984	-0.5036	bcde
-	-	-
1988	-0.2831	bcde
1990	0.1814	e
1992	-0.0952	de
1994	-1.2066	ab
1996	-0.5321	bcde
1998	-0.1780	cde
2000	-1.5936	a
2002	-1.1490	abc
2004	-1.1753	abc
2006	-1.0018	abcd
2008	-0.7067	abcde
2010	-0.3218	bcde
2012	-0.5368	bcde
2014	-0.0290	de

Summary	
Station	Significance Groups*
Lake Lillinonah	(94 96 00 02 04 06 08 10 12) (94 96 98 04 06 08 12 14) (90 94 98 04 08 12 14) (84 86 88 90 94 98 12 14) (84 86 88 90 92 94 98 14)
Lake Zoar	(94 00 02 04 06 08) (84 88 94 96 02 04 06 08 10 12) (84 88 96 98 02 04 06 08 10 12) (84 88 92 96 98 06 08 10 12 14) (84 88 90 92 96 98 08 10 12 14)
*Listed in order of decreasing LSM	

Visual inspection suggests that wet-weight TPCB concentrations at all stations increased from 2010 to 2014 (Fig. 2), and this apparent pattern was confirmed by ANCOVA at West Cornwall and Lake Lillinonah which detected a significant difference between TPCB concentrations in 2014 and those in 2010.

For the multiple comparison tests, many of the years overlapped in the significant groups, reflecting the relatively low statistical power of the test to demonstrate individual pairwise differences. At West Cornwall, TPCB concentrations in 2014 were not significantly different from those in 1984-1988, 1994-1998, 2006-2008, or 2012, but were significantly lower than those in

1990-1992 and significantly higher than those in 2000-2004 and 2010. At Bulls Bridge, TPCB concentrations in 2014 were not significantly different from those in 1984-1986, 1992-2000, or 2004-2012, but were significantly lower than those in 1988-1990 and significantly higher than those in 2002. At Lake Lillinonah, TPCB concentrations in 2014 were not significantly different from those in any study year during 1984-1988, 1990-1998, 2004-2008, or 2012, but were significantly higher than concentrations in 2000-2002 and 2010. At Lake Zoar, TPCB concentrations in 2014 were not significantly different from those in any study year during 1984-1988, 1990-1992, 1996-1998, or 2006-2012, but were significantly higher than those in 1994 and 2000-2004.

The results of the analyses of linear contrasts (Appendix J) vary slightly in regards to the above results most likely because of the grouping of 2014 with 2010 and 2012, specifically at West Cornwall and Lake Lillinonah, where the multiple comparisons suggested significantly lower values for 2010. At West Cornwall, the average TPCB concentration in the three most recent years (2010-2014) was significantly lower than those in 1984-1986 and 1988-1992 and significantly higher than those in 1994-2008. The 2010-2014 CTPCB concentrations showed similar significance. At Bulls Bridge, concentrations in 2010-2014 were not significantly different from those in 1994-2008 (for both TPCB and CTPCB), but were significantly lower than those in the 1984-1986 and 1988-1992 periods (for TPCB) and those in 1992 (for CTPCB). At Lake Lillinonah, concentrations in 2010-2014 were likewise not significantly different from those in 1994-2008 (for both TPCB and CTPCB), but were significantly lower than those in the 1984-1986 and 1988-1992 periods (for TPCB) and those in 1992 (for CTPCB). At Lake Zoar, TPCB concentrations in 2010-2014 were not significantly different from those in 1984-1986 or 1988-1992, but were significantly higher than those in 1994-2008. CTPCB concentrations at Lake Zoar in 2010-2014 showed similar significance.

Brown Trout

Visual inspection of sample (geometric) means for Brown Trout suggests that mean TPCB and CTPCB concentrations in 2014 were higher than mean concentrations in 1994–2012 but still below 1992 (and 1988-1992 for TPCB) (Table 4; Fig. 3 for TPCB; Appendix G). The lipid-normalized TPCB data show that the 2014 mean concentration was higher than those in 2000-2012, comparable to those in 1996-1998, and lower than those in 1986-1992 (Fig. 3).

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 2014 were significantly lower than those in 1988-1992, significantly higher than those in 1994, 1996, 2000, 2002, 2006, 2010, and 2012, and not significantly different from those in 1984,1986, 1998, 2004, or 2008. Additionally, TPCB concentrations in each study year during 1994–2014 were significantly lower than those in each study year during 1988–1992 (Table 7). Pairwise comparisons of CTPCB concentrations revealed a broadly similar pattern, showing that concentrations in 2014 were significantly lower than in 1992, significantly higher than those in 1994, 1996, 2000, 2002, 2006, 2010, and2012, and

not significantly different from those in 1998, 2004, or2008. Furthermore, CTPCB concentrations in each year from 1994 through 2014 were significantly lower than those in 1992 (Table 7).

The results of the linear contrasts approach (Appendix J) vary to some extent from the results of the pairwise comparisons. The linear contrasts found no significant difference between TPCB concentrations in the most recent years (2010-2014) and those in the 1994-2008 period. This approach also found that TPCB concentrations in the most recent years (2010-2014) were significantly lower than those in both the 1984-1986 and the 1988-1992 periods. Similarly, CTPCB concentrations in the most recent years (2010-2014) were not significantly different from those in 1994-2008 and were significantly lower than those in 1992.

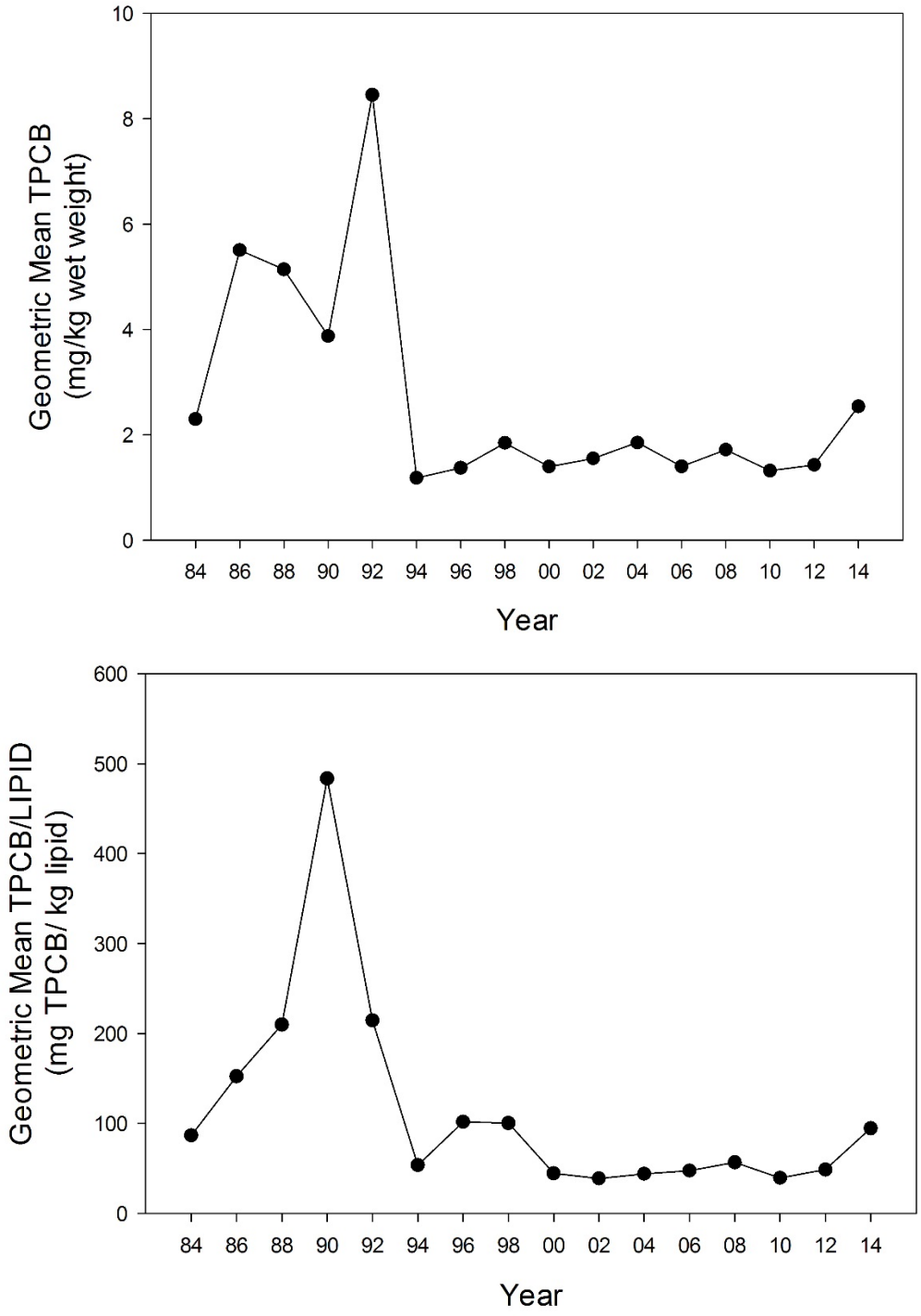


Figure 3. Historical patterns of PCB concentrations in Brown Trout collected from West Cornwall, 1984–2014. Top Panel — Geometric means (unadjusted) of TPCB. Bottom Panel — Geometric means (unadjusted) of lipid-normalized TPCB (TPCB divided by proportion lipid). The pronounced peak in lipid-normalized TPCB in 1990 is due to unusually low lipid levels rather than high TPCB levels (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 1984–2014 (left column) and the natural logarithm of CTPCB for 1992–2014 (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	0.930	cde
1986	1.340	ef
1988	1.483	f
1990	1.681	fg
1992	2.352	g
1994	0.446	abc
1996	0.301	ab
1998	0.610	abcd
2000	0.372	ab
2002	0.204	a
2004	0.708	bcd
2006	0.337	ab
2008	0.618	abcd
2010	0.235	a
2012	0.259	a
2014	0.960	de

Ln(CTPCB)		
Year	LSM	Group
-		
-		
-		
-		
1992	2.0749	e
1994	0.3282	abc
1996	0.2496	abc
1998	0.6009	cd
2000	0.3502	abc
2002	0.2012	ab
2004	0.5469	bcd
2006	0.0822	a
2008	0.4137	abcd
2010	0.0456	a
2012	0.0547	a
2014	0.7965	d

Summary	
Measure	Significance Groups*
Ln(TPCB)	(94 96 98 00 02 06 08 10 12) (94 96 98 00 04 06 08) (84 94 98 04 08) (84 98 04 08 14) (84 86 14) (86 88 90) (90 92)
Ln(CTPCB)	(94 96 00 02 06 08 10 12) (94 96 00 02 04 08) (94 96 98 00 04 08) (98 04 08 14) (92)
*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 5, 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 5, 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. LSMs are presented for both TPCB and CTPCB.

Year	2014	2012	2010	2008	2006	2004	2002	2000	1998	1996	1994	1992	1990	1988	1986	1984
Smallmouth Bass (TPCB)																
W. Cornwall	1.87	1.60	0.85	1.18	1.16	0.91	0.71	0.71	1.04	1.38	0.95	3.66	3.98	3.52	2.56	2.58
Bulls Bridge	1.35	1.33	0.71	1.14	1.39	1.15	0.63	0.79	0.84	1.55	1.30	1.72	3.06	2.69	1.39	2.04
Lillinonah	0.88	0.58	0.37	0.50	0.42	0.51	0.31	0.34	0.86	0.41	0.60	1.33	1.07	1.17	1.26	1.23
Zoar	0.97	0.58	0.72	0.49	0.37	0.31	0.32	0.20	0.84	0.59	0.30	0.91	1.20	0.75	-	0.60
Smallmouth Bass (CTPCB)																
W. Cornwall	1.74	1.52	0.78	1.06	1.03	0.84	0.80	0.68	1.09	1.35	0.95	2.94	-	-	-	-
Bulls Bridge	1.13	1.10	0.65	0.91	1.17	0.94	0.56	0.88	0.91	1.38	1.01	1.30	-	-	-	-
Lillinonah	0.80	0.50	0.33	0.41	0.35	0.47	0.34	0.45	0.97	0.45	0.62	1.16	-	-	-	-
Zoar	0.92	0.55	0.69	0.45	0.33	0.31	0.39	0.23	0.98	0.65	0.33	0.81	-	-	-	-
Brown Trout (W. Cornwall)																
TPCB	2.61	1.30	1.26	1.86	1.40	2.03	1.23	1.45	1.84	1.35	1.56	10.50	5.37	4.41	3.82	2.54
CTPCB	2.22	1.06	1.05	1.51	1.09	1.73	1.22	1.42	1.82	1.28	1.39	7.96	-	-	-	-

Comparison among Stations

Visual inspection of mean TPCB and CTPCB concentrations for Smallmouth Bass in 2014 indicates that wet-weight concentrations appear highest at West Cornwall, followed by Lake Zoar and Bulls Bridge, with Lake Lillinonah exhibiting the lowest concentrations (Table 4; Fig. 2). This pattern is slightly different from that of previous years. During those years, Smallmouth Bass from the two upstream stations (West Cornwall and Bulls Bridge) generally had higher concentrations than fish from the two downstream stations (Lake Lillinonah and Lake Zoar). In 2014, the concentrations at Bulls Bridge and Lake Zoar were more similar. On a lipid-normalized basis, in 2014, fish from West Cornwall had the highest TPCB concentration per unit lipid, Lake Zoar and Bulls Bridge were roughly comparable, and Lake Lillinonah again exhibited the lowest concentrations (Fig. 2).

Using a statistical model that included data from all years, ANCOVA and pairwise comparisons indicated that TPCB concentrations were significantly different between Lake Zoar and all other stations and between Lake Lillinonah and all other stations. No significant differences were observed between West Cornwall and Bulls Bridge (Table 5). The pairwise comparisons for CTPCB showed similar findings (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 2014. These proportions are not adjusted for differences in age distribution of samples among different years.

Eleven of the 40 Smallmouth Bass (28%) in 2014 had CTPCB concentrations above the FDA limit of 2.0 mg/kg wet weight. Of these, three (a 33.2 cm female, a 38.4 cm male, and a 36.9 cm male) came from Bulls Bridge, two (females of 46.9 cm and 54.9 cm) were caught at Lake Lillinonah, three (a 35.8 cm female, a 29.5 cm male, and a 37.9 cm male) were caught at West Cornwall, and three (a 44.1 cm female, a 48.9 cm male, and a 46.0 cm female) were caught at Lake Zoar.

When TPCB concentrations were analyzed, 17 of the 40 Smallmouth Bass (42%) had TPCB concentrations exceeding the limit. Of these, four (a 31.0 cm male, a 33.2 cm female, a 38.4 cm male, and a 36.9 cm male) were caught at Bulls Bridge, three (a 38.0 cm Male, a 46.9 cm female, and a 54.9 cm female) came from Lake Lillinonah, six (a 41.2 cm male, a 33.5 cm female, a 26.9 cm male, a 35.8 cm female, a 29.5 cm male, and a 37.9 cm male) were caught at West Cornwall, and four (a 40.2 cm male, a 44.1 cm female, a 48.9 cm male, and a 46.0 cm female) were caught at Lake Zoar.

Among Brown Trout, 18 of 30 the fish (60%) had CTPCB concentrations above the FDA limit and 19 of 30 (63%) had TPCB concentrations exceeding that limit. Eleven of the 22 trout stocked in the spring of 2014 had both CTPCB and TPCB concentrations greater than the FDA limit. These trout had river ages between 0.28 and 0.44 years, the average being 0.36 years, and measured 22.5 to 31.3 cm total length. Three of the four trout stocked in 2013 had CTPCB concentrations exceeding the FDA limit and all four of the 2013 stocked trout had TPCB concentrations exceeding the consumption tolerance limit. These trout exceeding CTPCB limits ranged in river age from 0.95 to 1.35 years and measured 34.7, 46.5, and 37.7 cm total length. In addition to the aforementioned individuals, a trout measuring 33.9 cm and 0.95 years was also over the limit for TPCB. All four of the trout stocked in 2012 exceeded FDA limits for both CTPCB and TPCB. These individuals ranged in river age from 1.95 to 2.35 years and measured 42.0, 43.6, 43.0, and 42.6cm total length.

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. The percentage of Brown Trout with TPCB concentrations less than that limit in 2014 was below the percentages found in any year of the study since 1992, but was still more than the percentages found in studies during 1986-1992. CTPCB data exhibited similar patterns for Brown Trout.

The percentages of Smallmouth Bass with TPCB concentrations less than the FDA limit in 2014 were below the percentages in any year since 1992 for West Cornwall and Lake Zoar, and 1990 for Bulls Bridge. These three stations still had higher percentages of bass under the FDA limit in comparison to 1986-1990 (1988-1990 for Lake Zoar). The 2014 percentages at Lake Lillinonah were less than in any other year since 1992, and were roughly similar to those in 1986, 1988, and 1992. CTPCB data for Smallmouth Bass exhibited identical patterns.

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 based on CTPCB (available for years 1992–2014) are presented in parentheses and are given only where different from those based on TPCB.

Year	Brown Trout	Smallmouth Bass			
	WC	WC	BB	LL	Z
2014	37 (40)	40 (70)	60 (70)	70 (80)	60 (70)
2012	50 (57)	70 (90)	90 (100)	90 (100)	90
2010	77	100	100	100	80 (100)
2008	50 (60)	80 (90)	80 (100)	80 (90)	90
2006	90 (93)	90 (100)	80 (100)	100	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

Benthic Invertebrates

Benthic aquatic insect larvae were collected in the general vicinity of West Cornwall in June 2014 and were analyzed for total PCBs and lipids. Three taxonomic groups were sampled: filter-feeding caddisflies (family Hydropsychidae), predatory dobsonflies (family Corydalidae, the aquatic larvae of which 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 and show concentrations in the range of 1.01 to 1.54 mg/kg for CTPCB and 1.11 to 1.92 mg/kg for TPCB.

Historical data on total PCB concentrations in Housatonic River benthic insects are shown in Figure 4 (CTPCB) and Figure 5 (TPCB). The Academy's CTPCB and TPCB data for 1992–2014 are tabulated in Appendix H; TPCB data for years prior to 1992 were provided by CTDEP.

CTPCB concentrations in caddisflies and stoneflies in 2014 were generally comparable to those in 2012 and slightly higher than concentrations in 1998-2010; while for dobsonflies, CTPCB concentrations in 2014 were higher than those in 2002 and 2005, but lower than or comparable to

those in 1998, 2001, 2008, 2010, and 2012 (Appendix H and individual sample data). CTPCB concentrations in all three taxa in 2014 were lower than those in 1992–1996.

Table 10. PCB and lipid levels in aquatic insects collected from the Housatonic River in the vicinity of West Cornwall in June of 2014. 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	Tissue Concentration		Lipid-normalized Concentration	
		CTPCB	TPCB	CTPCB	TPCB
Caddisflies (Hydropsychidae)	0.024	1.05	1.22	43.80	50.97
Dobsonflies (Corydalidae)	0.027	1.54	1.92	58.06	72.50
Stoneflies (Perlidae)	0.030	1.01	1.11	33.85	37.47

The TPCB data allow comparisons with concentrations as early as 1978. After averaging dobsonfly and stonefly concentrations to obtain a single estimate for predators in each year (for consistency with pre-1992 data), TPCB concentrations in both filter feeders and predators in 2014 were generally similar to the corresponding values in 2000–2012, lower than those in 1994–1998 (except for filter feeders in 1998), and well below most of the values in 1978–1992, except for 1985 (Fig. 5).

The historical data series shown in Figure 5 suggests overall 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. 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, indicating statistically significant decreasing trends in both groups of benthic insects since 1978 (Table 11).

Table 11. Results of Kendall's test of rank correlation between TPCB and study year for filter-feeding and predatory insects, 1978–2014. 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.

Insect Group	**Number of Studies**	**Correlation Coefficent (Kendall's τ)**	**p-value**
Filter Feeders	23	-0.533597	0.00036
Predators	23	-0.525692	0.00044

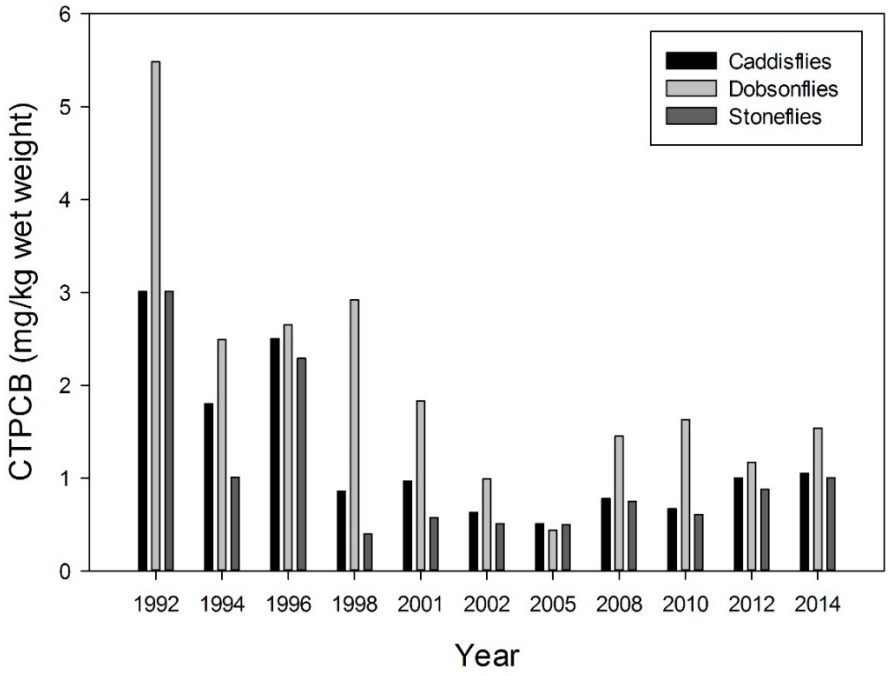


Figure 4. Total congener-based PCB concentrations (CTPCB) in benthic aquatic insects from West Cornwall, 1992–2014. Caddisflies are filter feeders, while dobsonflies and stoneflies are predators. Values are geometric means of two or three composite samples for each group, except in cases where only a single composite sample was analyzed. Plotted values and sample sizes are tabulated in Appendix H.

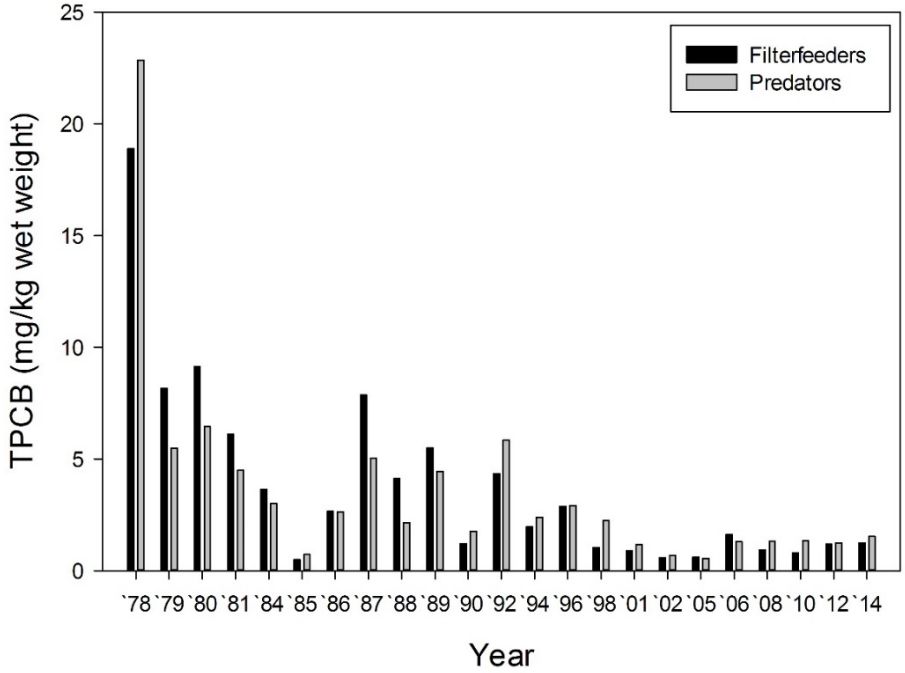


Figure 5. Historical data series of total Aroclor-based PCB concentrations (TPCB) in benthic aquatic insects, 1978-2014. Filter feeders consist of Hydropsychid caddisflies, while predators include both Corydalid dobsonflies and Perlid stoneflies. Values for predators are arithmetic means of separate values for dobsonflies and stoneflies.

Precision, Accuracy, and Detection Limit Analyses

Methods used in 2014 to assess precision, accuracy, and detection limits were the same as in the 2002-2012 studies and are described below. Note: All concentrations are presented on a wet-weight basis.

Detection Limits

Matrix blanks were generated to monitor possible laboratory contamination and to calculate the detection limits for PCBs. (See Appendix I for detection limit calculations.) Each matrix blank, consisting of approximately 30 g of clean Na_2SO_4, was analyzed using the same procedures as the samples. The detection limit was estimated as the blank area plus three times the standard deviation of the average blank peak areas. The method detection is reported on a mass per mass basis (dividing by an average extraction mass of 5.040 ng). The matrix blank-based detection limits for PCBs (see Table I-1 in Appendix I for individual detection limits) ranged from 0.01 ng/g (congener 85) to 16.06 ng/g (congener 3). Based on the matrix blanks, the average detection limit for individual PCB congeners was 0.48 ng/g and that for total PCBs was 34.80 ng/g. The matrix blank run with the extraction set on August 18, 2015 had low surrogate recoveries (22, 22, and 26%).

As discussed further below, the calculation of total PCB concentrations for both TPCB and CTPCB excluded sample results that fell below detection limits.

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. These surrogates were not industrially prepared and therefore are not present in the environment. Average recoveries of congeners 14, 65 and 166 were 94 ± 9%, 88 ± 7% and 91 ± 9% respectively (Appendix I). With relatively low standard deviations, constant recoveries regardless of contaminant concentration, and no known interferences, surrogate congeners are reliable for assessing analyte loss. All reported values for PCB concentration in this study were not corrected for analyte loss.

Duplicate and Triplicate Analyses

Tables I-2 and I-3 show the results from duplicate and triplicate analyses of samples for PCBs. Relative percent differences (RPDs) for duplicates were low, with an average (individual congener totals) RPD value of 15% (Table I-2). Relative standard deviations (RSDs) for triplicates were also low, with an average (individual congener totals) RSD of 13% (Table I-3). In most instances where RPD and RSD values were high, the associated concentration value was very low, increasing the standard error.

Standard Reference Materials

For this study, a National Institute for Standards and Technology (NIST) standard reference material (SRM 1947 Lake Michigan Fish Tissue) was used to evaluate extraction efficiency and

analytical accuracy. 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. Average percent recovery for SRM 1947 was 92% excluding outliers. Outliers (congeners 45, 63, 82, 158, 194 and 206) represent less than 35% of total SRM concentrations. Despite the low and high recoveries for the subset of outlier congeners, the Academy method not only predicts the PCB patterns within the SRMs but estimates the magnitude of most congeners as well (Figure 6).

Method Spikes

Analyte losses for all PCB congeners were determined through method spikes using a 25:18:18 mixture of Aroclors 1232, 1248 and 1262 into a blank matrix (one containing no biological material). The average percent recovery of spiked congeners was 95%. Average recovery excludes outlier data (PCB congeners 3, 29, 85, 131, 158, 129+178, 191 and 209), which represent low concentrations within the PCB standard used (Mullin, 1985). The average % error for method spikes was -5%.

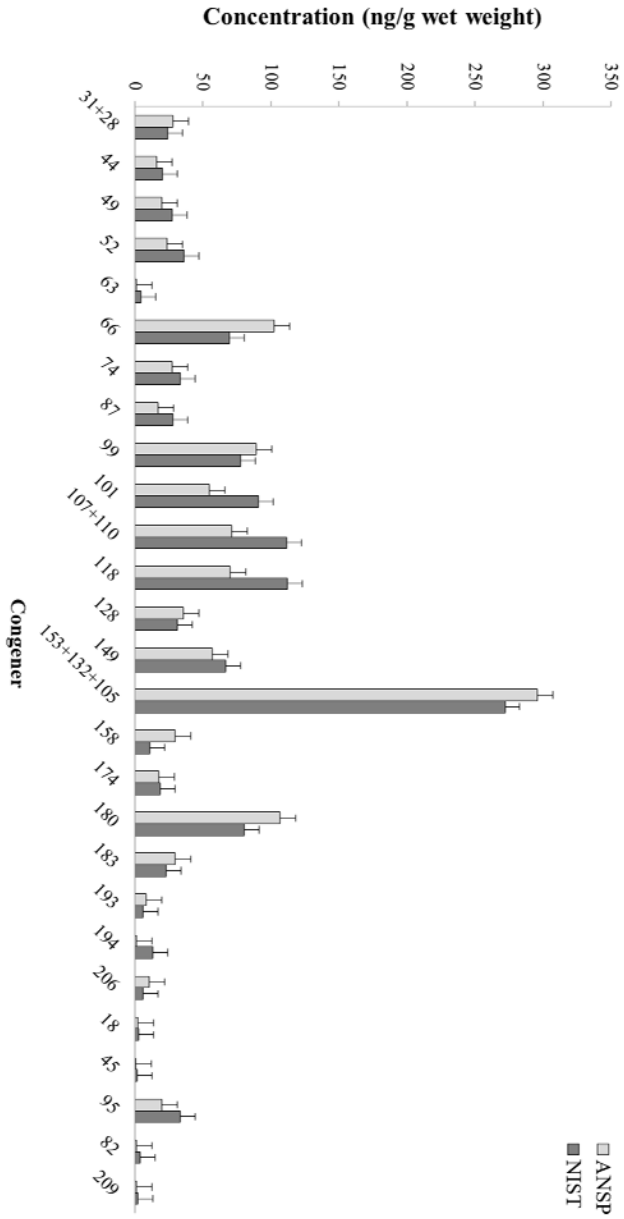
Combining Congeners

In 2014, as in 2010 and 2012, concentrations of PCB congeners 31 and 28 were combined and reported as [31+28], and concentrations of congeners 41 and 71 were combined and reported as [41+71], since individual congeners within these two pairs could not be well resolved chromatographically.

Handling of Non-Quantifiable Congeners

Total concentrations of PCBs, as either TPCB (Aroclor-based) or CTPCB (congener-based), are presented in this report. Concentrations of individual congeners and data qualifiers are not reported here, but were reviewed as part of the quality assurance/quality control (QA/QA) procedure. In that review, results for congeners that were not quantifiable were qualified with one of three qualifiers. Congeners that were not detected (no discernible peak arising from the instrument noise) were denoted as “ND.” Where a peak was found but the resulting concentration fell below the defined detection limit, “BDL” was used in lieu of reporting the concentration. Congener 84 was not analyzed and was denoted as “NA.” All three of these categories of data were excluded from the calculations of total concentrations for both TPCB and CTPCB.

Figure 6. Comparison of Academy (ANSP) and NIST values for SRM 1947-Lake Michigan fish (error bars represent standard error).



DISCUSSION

The results of this study of PCB concentrations in fish and benthic insects in the Connecticut portion of the Housatonic River consist of among-year and among-station comparisons of Smallmouth Bass at four sampling stations (West Cornwall, Bulls Bridge, Lake Lillinonah and Lake Zoar), and among-year comparisons of Brown Trout and benthic insects at a single sampling station (West Cornwall).

Evaluation of Smallmouth Bass Data

For Smallmouth Bass, there was an apparent pattern of overall lower TPCB and CTPCB concentrations during 1994–2014 compared to 1988-1992 (for TPCB) and 1992 (for CTPCB). (CTPCB data are not available for years before 1992.) However, mean TPCB and CTPCB concentrations in 2014 were higher than those in any of the other years between 1994 and 2012 at all stations except Bulls Bridge, showing increases in wet-weight concentrations in the general range of approximately 0.3 to 1.0 mg/kg for TPCB and 0.25 to 0.9 mg/kg for CTPCB compared to those years. These patterns were confirmed statistically for both TPCB and CTPCB using analysis of covariance and pairwise comparisons between years. Though there were some differences in temporal patterns among stations, statistical analyses generally confirmed that the concentrations in 2014 were elevated in comparison to the years between 2000 and 2012, but remained lower than the concentrations in 1992 and, where applicable, prior years.

For data with all stations combined, the adjusted mean TPCB concentrations for 2014 were significantly lower than those in 1990, significantly higher than those in 1994 and 2000-2010, and not significantly different from those in any other study year. The 2014 CTPCB concentrations exhibited a similar pattern in that they were only significantly lower than those in 1992, were not statistically significantly different from those in 1996, 1998, or 2012, and were significantly higher than those in 1994 and 2000-2010. When 2014 TPCB concentrations were grouped with 2010 and 2012 for linear contrasts (Appendix J), the concentrations in the three most recent years were significantly lower than those for the early periods (1984-1986 and 1988-1992) and significantly higher than those in 1994-2008, due primarily to the inclusion of the 2014 results in the most recent period. Use of the linear contrasts approach for CTPCB at all stations combined likewise showed a significant increase in the most recent years compared to 1994-2008, but showed no significant difference from 1992.

When stations were assessed individually, adjusted mean TPCB concentrations in 2014 at West Cornwall were significantly lower than those in 1990-1992 and higher than those in 2000-2004 and 2010, but not significantly different from any other year of the study. At Bulls Bridge, 2014 TPCB concentrations were not significantly different from those in any prior year except that they were significantly lower than those in 1988 and 1990 and higher than those in 2002. At Lake Lillinonah, 2014 TPCB concentrations were significantly higher than those in 2000, 2002, and 2010 and were not significantly different from those in any other study year. TPCB concentrations at Lake Zoar were significantly higher than those in 1994 and 2000-2004 and were not significantly different from those in any other study year. In general, at each station, concentrations in 2014 were elevated in comparison to several years during the 1994-2012 period, mainly 1994, 1998,

2000, 2002, 2004, and 2010, but were generally significantly lower than, or equal to, those in the years during 1988-1992, with a few exceptions at each station.

The results of the linear contrasts approach (Appendix J) reveal patterns between year groups (2010-2014 versus 1984-1986, 1988-1992 and 1994-2008 for TPCB, and between 2010-2014 and 1994-2008, as well as 1992, for CTPCB). That method found that both the TPCB and CTPCB concentrations in the three most recent years (2010-2014) were not significantly different from the concentrations in 1994-2008 at Bulls Bridge or Lake Lillinonah, but were significantly higher than concentrations in those years at West Cornwall and Lake Zoar, again due primarily to the elevated concentrations in 2014. This method also found that both TPCB and CTPCB concentrations in 2010-2014 were significantly lower than those in 1984-1986 and 1988-1992 (1992 only for CTPCB) at each station except Lake Zoar (where there was no significant difference between 2010-2014 and those years).

In terms of spatial distribution, there has been a consistent trend over study years for an upstream-downstream gradient in PCB concentrations, with higher concentrations at West Cornwall and Bulls Bridge and lower concentrations at Lake Lillinonah and Lake Zoar. However, in 2014, Lake Zoar had higher concentrations than Lake Lillinonah and similar to those at Bulls Bridge. Differences between West Cornwall and Bulls Bridge, and between Lake Lillinonah and Lake Zoar, depend on the nature of the comparison (raw concentrations, lipid-normalized, or model adjusted; TPCB or CTPCB). The 2014 TPCB and CTPCB data for Smallmouth Bass indicate higher wet-weight concentrations at West Cornwall, Bulls Bridge, and Lake Zoar than at Lake Lillinonah. Analysis of covariance of data from all years showed that TPCB concentrations were still significantly highest at West Cornwall and Bulls Bridge and that Lake Zoar and Lake Lillinonah were both significantly different from all other stations. Analysis of covariance of data from all years show that CTPCB concentrations were significantly highest at West Cornwall and Bulls Bridge, and that Lake Lillinonah and Lake Zoar had the lowest concentrations and were not significantly different from each other.

Evaluation of Brown Trout Data

For Brown Trout, mean TPCB and CTPCB concentrations in 2014 were higher than those in any year between 1994 and 2012, but well below the levels observed in 1986-1992 (for TPCB) and 1992 (for CTPCB). The increases in mean wet-weight PCB concentrations in 2014 compared to those in the years between 1994 and 2012 were in the general range of approximately 0.6 to 1.3 mg/kg for TPCB and 0.25 to 1.1 mg/kg for CTPCB. This pattern was generally confirmed by analysis of covariance with pairwise comparisons between years. These comparisons showed that TPCB concentrations in 2014 were significantly lower than those in 1988-1992, significantly higher than those in 1994, 1996, 2000, 2002, 2006, 2010, and 2012, and not significantly different from those in 1984, 1986, 1998, 2004, and 2008. Pairwise comparisons of CTPCB concentrations revealed a generally similar pattern, showing that 2014 concentrations were significantly lower than those in 1992, significantly higher than those in 1994, 1996, 2000, 2002, 2006, 2010, and 2012, and not significantly different from those in 1998, 2004, or 2008.

The results of the linear contrast approach (Appendix J) for Brown Trout found that both TPCB and CTPCB concentrations in the three most recent years (2010-2014) were not significantly different from those in the 1994-2008 period, but were significantly lower than those in the 1988-1992 and 1984-1986 periods for TPCB and those in 1992 for CTPCB.

Historical Perspective on Fish Data

Historically, PCB concentrations in fish in the Connecticut portion of the Housatonic River exhibited a pattern of high values for PCB concentrations 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 then a substantial decrease in PCB concentrations in 1994, and that decrease largely persisted in the subsequent 18 years through 2012. The 2014 results indicate a slight general increase in PCB concentrations from those in the years between 1994 and 2010, although the levels are still well below what was observed between 1988 and 1992. Since the 2014 data represent only a single year, it is uncertain whether the apparent increase represents a change in the long-term pattern of PCB dynamics or simply represents a one-time anomaly in an overall trend of generally consistent data over the period from 1994 to date.

Fish Exceeding FDA Fish Consumption Tolerance Limit

A similar 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. From 1994 to 2012, the frequency of Smallmouth Bass with PCB concentrations exceeding the limit was low, generally in the range of 0% to 20%, varying with the station and the type of analysis (i.e., TPCB or CTPCB). The frequency of exceedance increased in 2014, in the range of 20% to 60%, dependent upon station and analysis (CTPCB or TPCB). All stations generally exhibited exceedances comparable to 1992, but much less than the years between 1984 and 1992 for Smallmouth Bass.

Among Brown Trout, nearly all the fish collected from West Cornwall in the years 1986–1992 had PCB concentrations exceeding the FDA limit. In the following years, the percentage of Brown Trout with PCB concentrations exceeding the limit decreased substantially and was generally been in the range of 10% to 50%. In 2014, the percentages were slightly higher than that range; 60% of the Brown Trout had CTPCB concentrations exceeding the limit and 63% had TPCB concentrations above the limit. These levels are still low in comparison to the years between 1986 and 1992.

Evaluation of Benthic Invertebrate Data

Analysis of benthic insect samples in 2014 showed that PCB concentrations have remained relatively low and generally similar to those in 2000-2012 with some variations. Specifically, 2014 CTPCB concentrations were: (a) for caddisflies and stoneflies, generally comparable to those in

2012 and slightly higher than those in 1998–2010: (b) for dobsonflies, higher than those in 2002 and 2005 and lower than or comparable to those in 1998, 2001, 2008, 2010, 2012; and (c) for all three taxa, lower than those in 1992–1996. Similarly, TPCB concentrations in 2014 in both filter feeders (caddisflies) and predators (dobsonflies and stoneflies) were generally similar to those in 2000-2012, lower than those in 1992-1998 (except for caddisflies in 1998), and well below those in most of the prior years (1978-1990).

Rank correlation analysis of the entire data series for 1978–2014 revealed a highly statistically significant temporal trend of decreasing PCB concentrations in both filter feeders and predators. This pattern of PCB concentrations in insects shows substantial decreases from 1978 through the mid-1980s, increases to somewhat higher levels in most years between 1986 and 1992, and then decreases in subsequent years, with some variation among recent years

Summary and Conclusions

In summary, results of the 2014 Academy fish monitoring study show that total PCB concentrations in Smallmouth Bass and Brown Trout were generally somewhat higher than those observed during any study year between 1994 and 2012 (with some expected variability), but remained well below the levels observed in 1992 and (where applicable) most prior years. The data from 2014 are insufficient to determine whether the apparent increase observed in fish concentrations in that year represents a one-time anomaly in an overall trend of generally consistent data over the past two decades.

For filter-feeding and predatory benthic insects, the 2014 data show that PCB concentrations have remained low and generally similar to those in 2000-2012; and an analysis of the overall data has continued to show a statistically significant trend of decreasing total PCB concentrations over the overall monitoring period (1978–2014).

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APPENDICES

APPENDIX A

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-77
Rev. 3 (07/13)

EXTRACTION AND CLEANUP OF FISH TISSUE FOR PCB AND PESTICIDE ANALYSIS

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Procedure No. P-16-77
Rev. 3 (07/13)
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 17 organochlorine pesticides and industrial compounds (only when pesticide analysis is requested).

3.3 Internal Standard

17.5 ng of 2,4,6-trichlorobiphenyl (PCB 30) and 17.5 ng of 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB 204).

3.4 Surrogate Standard

35 ng of 3,5-dichlorobiphenyl (PCB 14), 35 ng of 2,3,5,6-tetrachlorobiphenyl (PCB 65) and 35 ng of 2,3,4,4',5,6-hexachlorobiphenyl (PCB 166).

4.0 APPARATUS

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

For Extraction: Soxhlet extractors (200 ml), Allihn condensers, 250/500-ml round bottom flasks.

For Sample Preparation: 250-ml beakers, stainless steel spatulas, 250 ml Turbovap tubes, 9" Pasteur pipets, 15-ml graduated centrifuge tubes, glass syringe with stainless steel needle, 10-ml volumetric flasks and 12-ml vials with Teflon lined screw caps.

4.2 Glass wool for extraction.

4.3 TurboVap System for sample reduction.

4.4 Sodium Sulfate (pre-baked at 450°C for 4 h).

4.5 Honeywell;Burdick and Jackson Florisil Sep-pak cartridges.

4.6 Instra Analyzed Sulfuric Acid.

4.7 Tekmar Tissuemizer and Waring Pro Blender.

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 are allowed to thaw, filleted and finely ground using a Tekmar Tissuemizer or Waring Pro Blender.

5.2 At the time of analysis, 5 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 Soxhlet with glass wool at the bottom. 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 ~175/350 ml of 1:1 hexane:acetone mixture.

5.4 The sample extract is then transferred from the original 250/500-ml round bottom flask to a clean Turbovap tube. This is done because during extraction, fish and sodium sulfate collect at the bottom of the flask and the new tube is needed for the Turbovap unit. The extract is reduced to approximately 5 ml using a Turbovap evaporator system, exchanged three times with 5-ml aliquots of hexane, and finally evaporated to ~5 ml. In between exchanges, the sample is checked for water. If water is present, it is removed with a Pasteur pipet.

5.5 The sample is then transferred to a graduated centrifuge tube along with three 1-ml rinses of hexane and brought to the 10 ml volume mark. The lipid content of the sample is determined at this point by placing a 1.0-ml aliquot of the extract in a pre-weighed 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 Burdick and Jackson 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 with three additional rinses of hexane and collected into a 10-ml volumetric flask. The volume is adjusted to 10 ml and used for PCB analysis. All deliveries to the sep-pak column are made using a glass Pasteur pipet. If the sample is being analyzed for pesticides, 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 can now be prepared for analysis on the gas chromatograph.

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.

Surrogate Standard:

35 ng of 3,5 dichlorobiphenyl (PCB 14), 35 ng of 2,3,5,6-tetrachlorobiphenyl (PCB 65) and 35 ng of 2,3,4,4',5,6 hexachlorobiphenyl (PCB 166).

Internal Standard:

17.5 ng of 2,4,6 trichlorobiphenyl (PCB 30) and 17.5 ng of 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB 204) are added to the 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. SRMs are used in addition to conventional matrix spikes in this procedure.

8.0 AROCLOR QUANTITATION

Aroclor 1254 is quantitated as the sum of congeners 52, 49, 44, 41+71, 74, 70+76, 95+66, 91, 60+56, 84, 101, 99, 83, 97, 87, 85, 110, 82 divided by 0.5049.

Aroclor 1260 is quantitated as the sum of congeners 129+178, 182+187, 183, 185, 174, 177, 171+202, 172+197, 180, 170+190, 201, 203+196 divided by 0.3811.

APPENDIX B

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-84
Rev. 6 (7/2013)

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

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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-:m 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 :L is used. Manual injection, if necessary, will use at least a 2.0-:L 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:

$$RRF = [(MassCongener)x(AreaCongener)^{-1}]_{std} \times [(MassIstd)x(AreaIstd)\text{-}1]_{std}$$

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:

$$MassCongener_{sample} = (AreaCongener)_{sample} \times RRF_{std} \times [(MassIstd) \times (AreaIstd)^{-1}]_{sample}$$

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 (Φ) 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 10Φ of the baseline noise and is determined in the same manner as the LOD:

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

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

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:

$$\text{RPD} = (\text{Dup1} - \text{Dup2}) \times (\text{Average})^{-1} \times 100$$

6.5.2. The average RPD for all congeners must meet established control limits for a given matrix if measured concentrations are ∃ 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 B-1. Example GC-ECD conditions for PCB and pesticide analysis[1].

column primary: confirmation:	50 m DB-5, 0.20-mm ID, 0.33-:m film thickness or equivalent[2] 30 m DB-1701, 0.25 mm ID, 0.25:m 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.

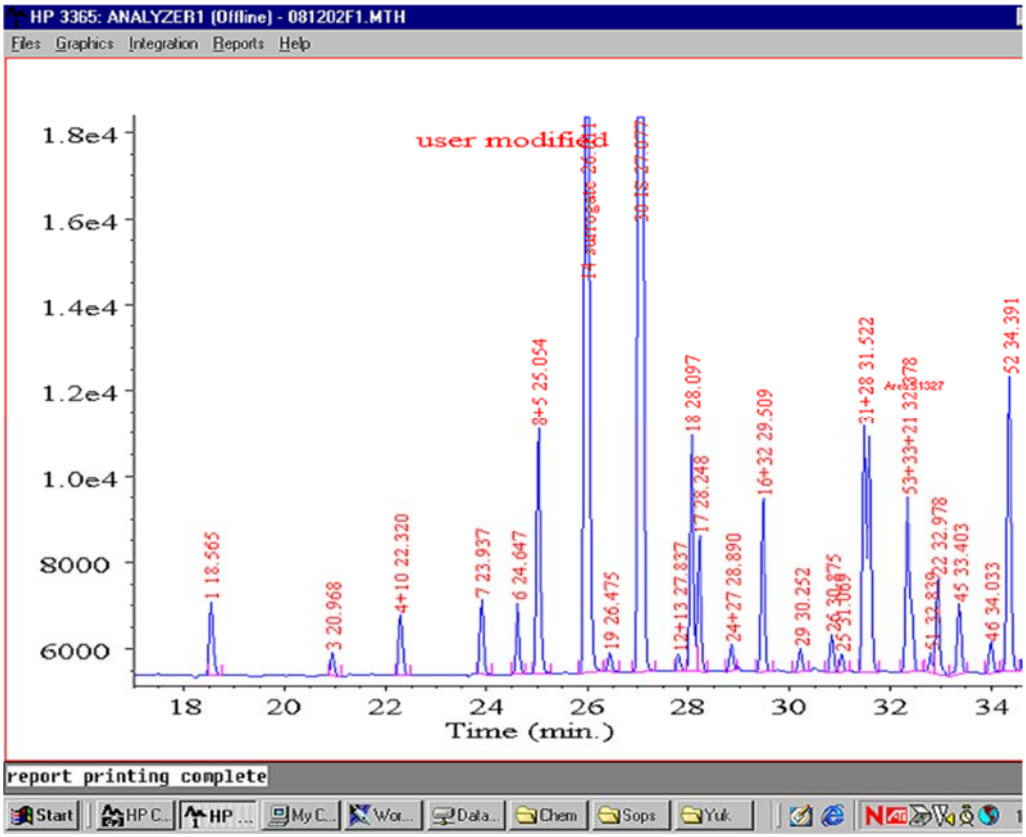

Figure B-1. Partial chromatogram of PCB '610' Mixture used as a calibrations standard.

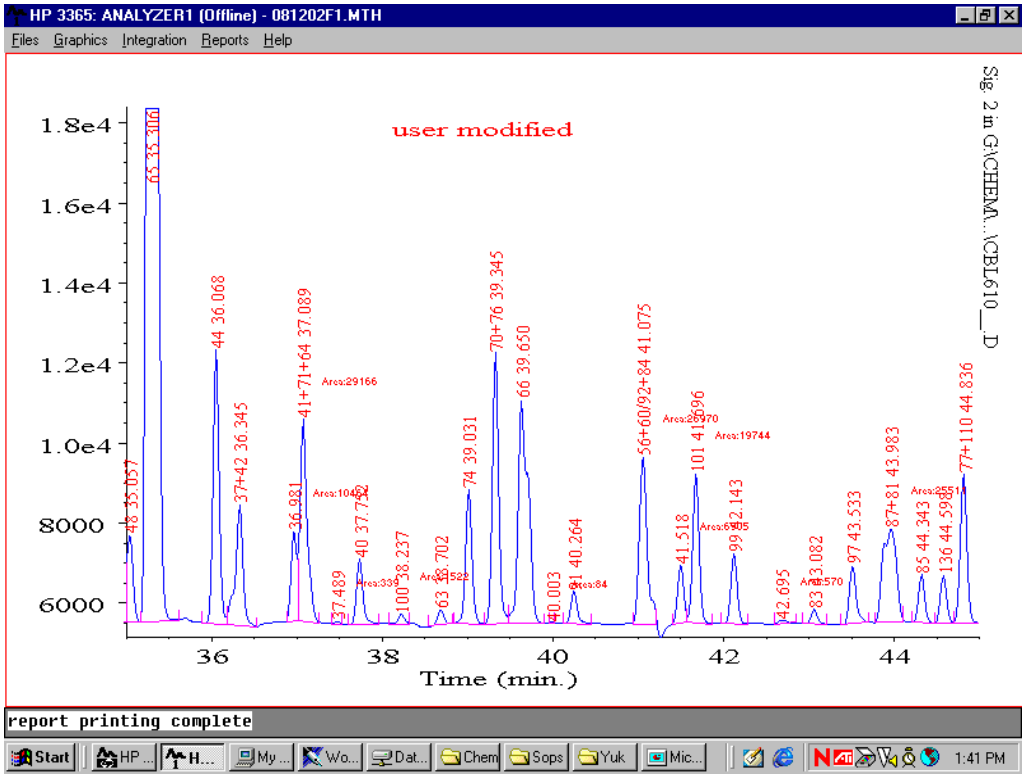

Figure B-1 Continued.

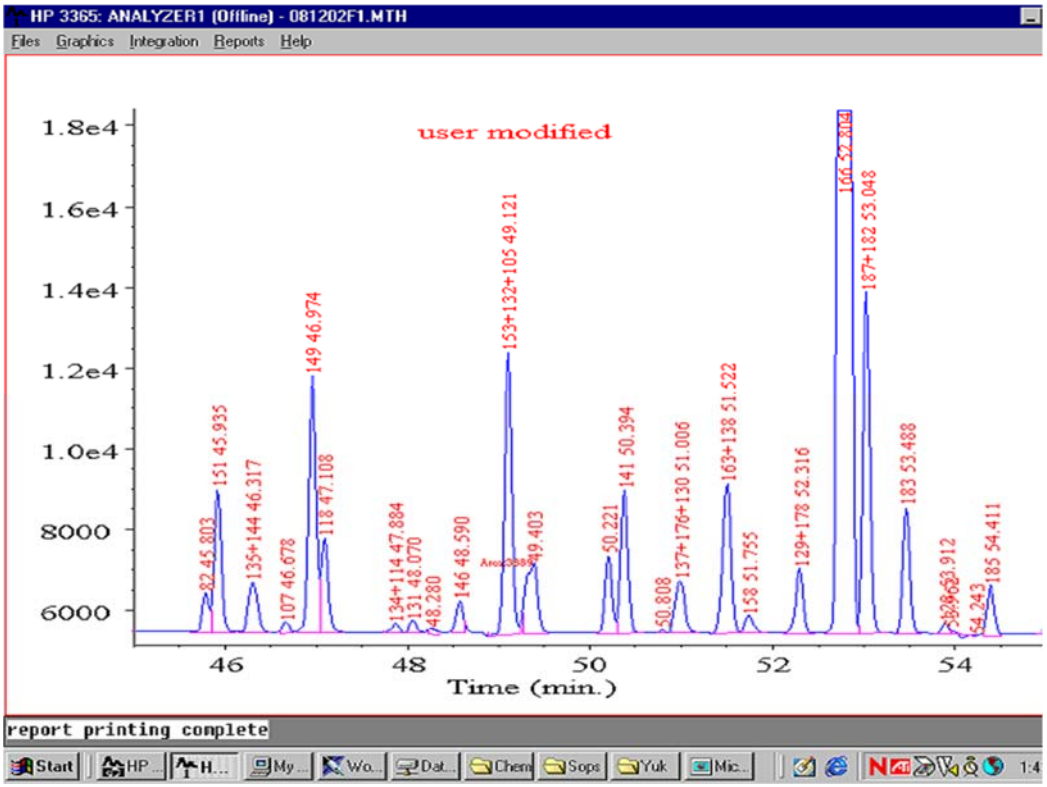

Figure B-1 Continued.

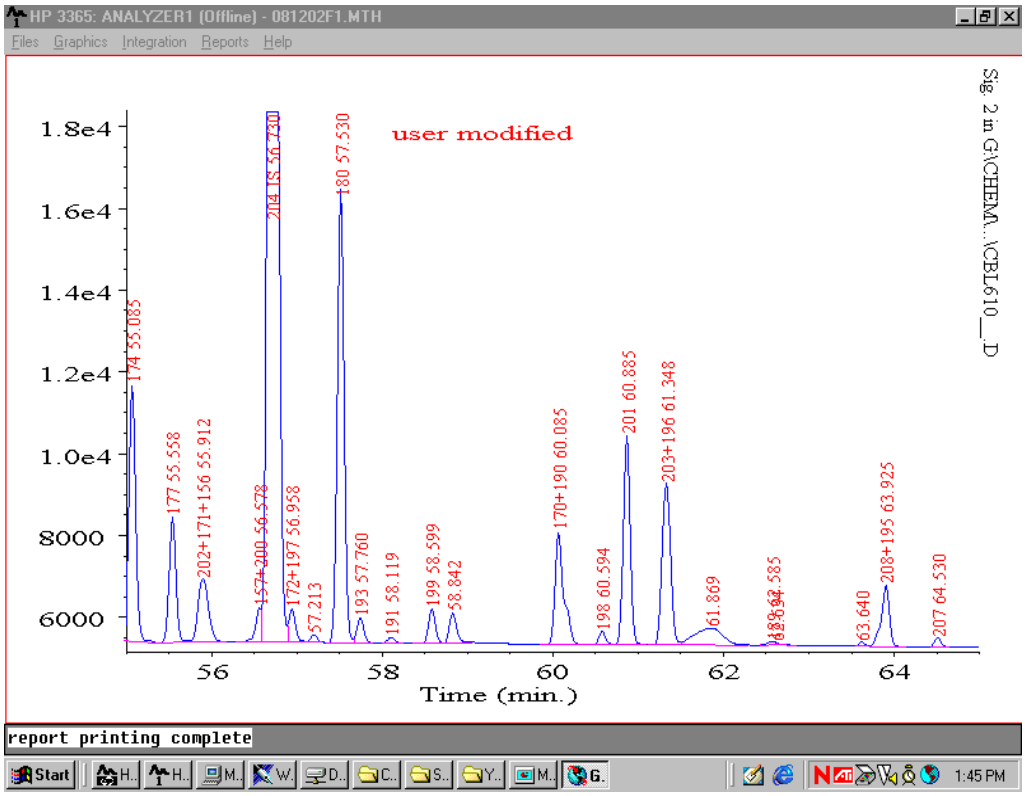

Figure B-1 Continued.

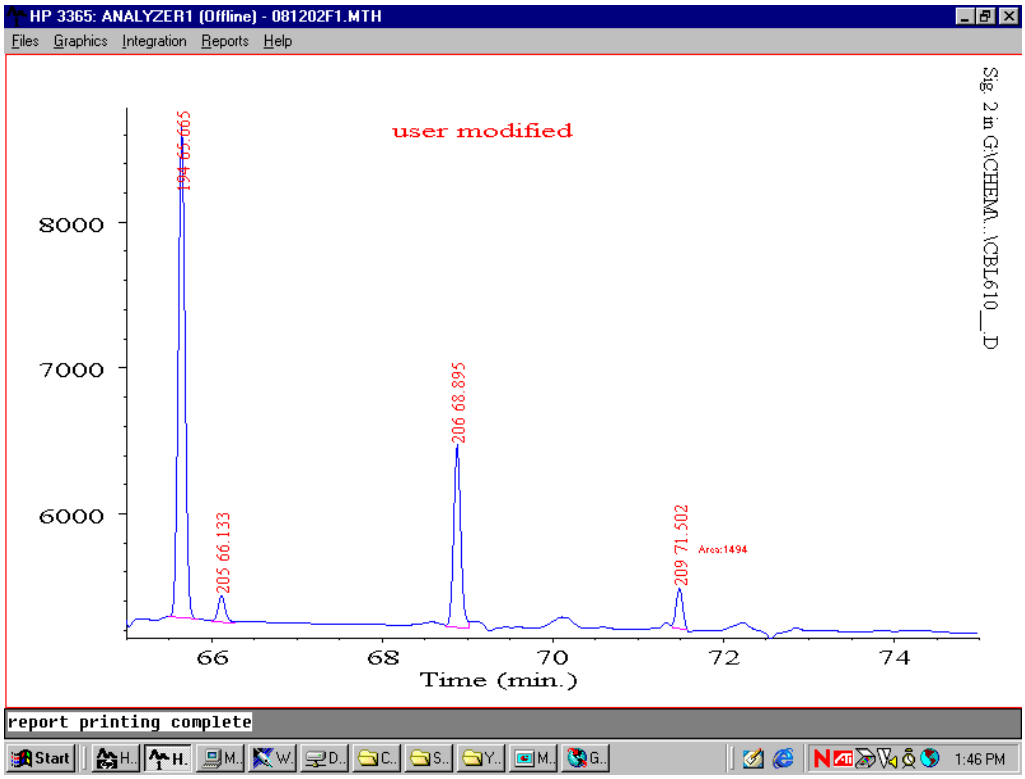

Figure B-1 Continued.

APPENDIX A of P-16-84.

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 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 Congener	# of Cl	ng/mL
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 Congener	# of Cl	ng/mL
PCB 185	7	2.2
PCB 174	7	11
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 of P-16-84.
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).

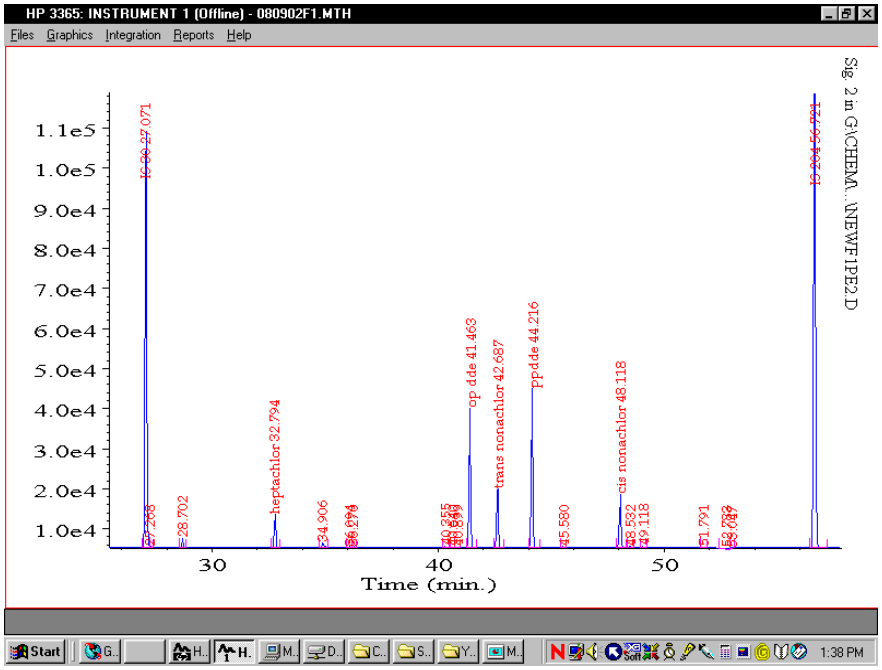

Figure 1. Chromatogram showing elution order of F1 organochlorine pesticides

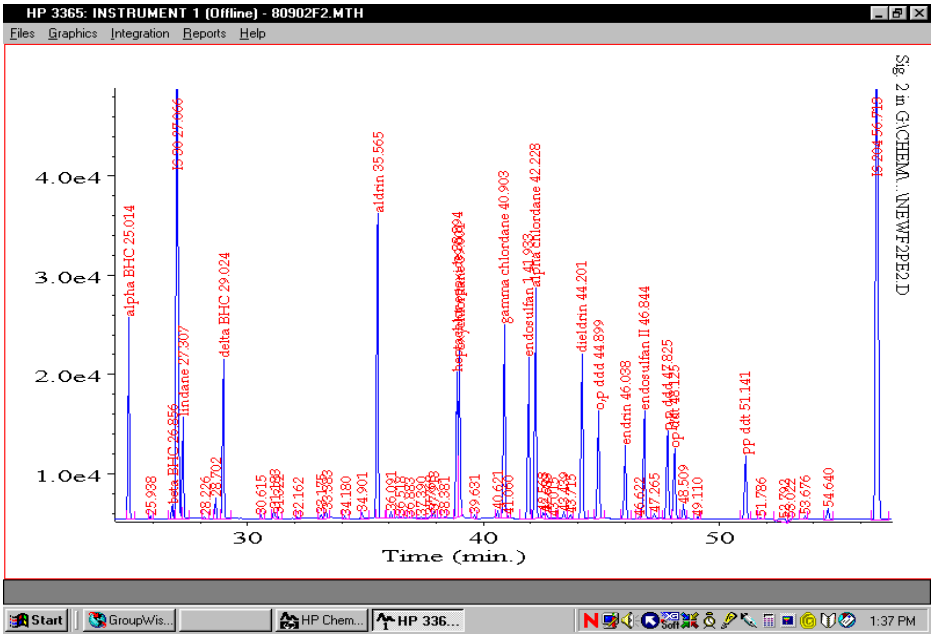

Figure 2. Chromatogram showing elution order of F2 organochlorine pesticides

APPENDIX C

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

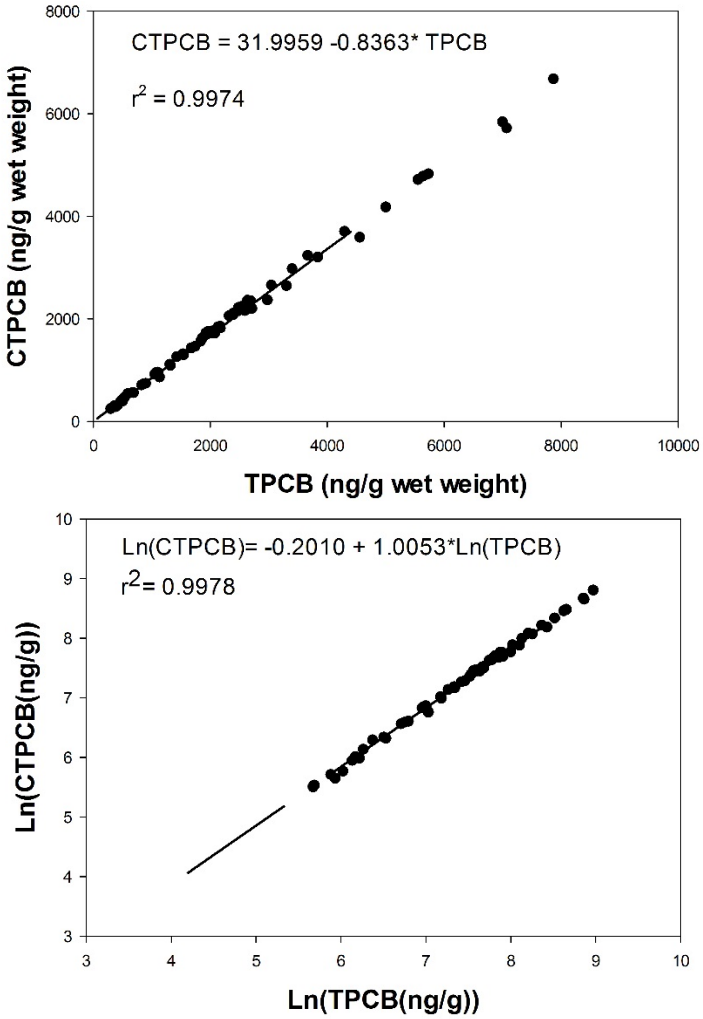

Figure C-1. Relationship between congener-based quantitation of total PCBs and Aroclor-based quantitation for fishes analyzed in the 2014 Academy Housatonic River study.

As in previous Housatonic River biological monitoring studies, the two methods of quantitating total PCBs were very highly correlated. A scatter plot of 2014 CTPCB concentrations versus the corresponding TPCB concentrations for the species analyzed (Brown Trout and Smallmouth Bass) clearly suggests a linear relationship (Fig. C-1, top). Linear regression analysis of all samples produced an intercept (31.9959 ng/g) that differs negligibly from zero, compared to PCB concentrations in this study (regression equation: CTPCB = 31.9959 – 0.8363*TPCB, r^2=0.9974.) The slope of this regression shows that CTPCB was about 84% of TPCB on average. A regression of ln(CTPCB) versus ln(TPCB)

was performed to stabilize the variance and check for linearity. The slope of this regression (Fig. C-1, bottom) does not differ from 1.000, indicating a linear relationship.

APPENDIX D

Numbers of Brown Trout from 2014 Analyzed for PCB Content and Their Corresponding Stocking Dates as per Connecticut DEEP

Table D-1. The number of Brown Trout collected and analyzed for PCB content and their corresponding stocking dates. Information on stocking was provided by CTDEEP. Fish were assigned to groups based on marks on fish and otolith analysis.

Stocking Date	Number of Individuals	Percent of Total
2014 Stock	22	73
2013 Stock	4	13
2012 Stock	4	13
Total Housatonic	30	100
Burlington Hatchery	2	-

APPENDIX E

Average CTPCB Concentrations in Fish from the Housatonic River, Connecticut

Table E-1. Average CTPCB concentrations in all species of fish collected in the Housatonic River, CT. Results for 1992- 2014 are based on actual quantified CTPCB values. Results for 1984-1990 were estimated from TPCB data, using regressions between lnCTPCB and lnTPCB 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.

Species	Station	Mean CTPCB															
		1984	1986	1988	1990	1992	1994	1996	1998	2000	2002	2004	2006	2008	2010	2012	2014
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	1.87	1.26	1.79	2.54
Fallfish	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.78	-
Rainbow trout	C	-	-	2.63	-	-	-	-	-	-	-	-	-	-	-	-	-
Smallmouth bass	C	1.99	2.61	3.77	-	2.78	1.41	1	0.78	1	1.1	0.94	0.89	1.46	0.54	1.41	1.71
Bluegill	B	0.78	-	1.85	-	-	-	-	-	0.49	-	0.27	-	-	0.48	0.37	-
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	1.74	1.48	2.18	-
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	-	0.36	0.39	-	-
Smallmouth bass	B	1.61	1.34	2.33	2.1	1.35	1.23	0.99	0.95	0.98	0.8	1.05	1.08	1.02	0.54	1.11	1.25
Bluegill	L	0.48	-	0.47	0.47	0.45	-	-	-	-	-	0.17	-	-	0.13	0.27	-
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	1.2	1.13	1.52	-
Pumpkinseed x redb	L	-	-	0.27	-	-	-	-	-	-	-	-	-	-	-	-	-
Pumpkinseed	L	-	-	0.03	0.2	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	-	0.12	0.04	-	-
Smallmouth bass	L	1.02	1.33	1.2	0.95	1.41	0.51	0.3	0.84	0.51	0.37	0.53	0.35	0.85	0.48	0.59	1.54
Bluegill	Z	0.89	-	0.19	0.13	0.25	-	-	-	-	-	0.15	-	-	0.16	0.13	-
Brown bullhead	Z	0.38	-	0.62	-	-	-	-	-	-	-	-	-	-	-	-	-
Common carp	Z	3.88	-	12.07	-	-	-	-	-	-	-	-	-	-	-	-	-
American eel	Z	-	-	1.04	2.36	5.3	-	-	-	-	-	-	-	-	-	-	-
Largemouth bass	Z	0.39	-	1.15	-	-	-	-	-	-	-	-	-	-	-	-	-
Northern pike	Z	-	-	-	-	-	-	-	-	-	-	-	1.33	1.49	1.03	3.36	-
Pumpkinseed	Z	-	-	0.11	0.16	0.22	-	-	-	-	-	0.08	-	-	-	-	-
Redbreast sunfish	Z	0.09	-	0.15	0.2	0.24	-	-	0.71	-	-	-	-	-	-	-	-
White catfish	Z	2.22	2.55	3.4	-	-	-	-	-	-	-	0.59	-	-	-	-	-
White perch	Z	0.84	-	1.26	0.87	1.01	-	-	-	-	-	0.51	-	0.49	-	-	-
Yellow bullhead	Z	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-
Yellow perch	Z	0.07	-	0.21	0.24	0.26	-	-	-	-	-	0.17	-	0.16	0.11	-	-
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	0.88	0.98	0.75	1.45
Bluegill	F	-	-	-	-	-	-	-	-	0.68	-	0.41	-	-	1.30	0.72	-
Brown bullhead	F	-	-	-	-	-	-	-	-	0.95	-	0.32	-	-	-	-	-
Northern pike	F	-	-	-	-	-	-	-	-	-	-	10.01	1.06	3.69	6.61	3.30	-
Pumpkinseed	F	-	-	-	-	-	-	-	-	0.21	-	0.27	-	-	-	-	-
Smallmouth bass	F	-	-	-	-	-	-	-	-	-	-	1.01	-	-	-	-	-
Yellow perch	F	-	-	-	-	-	-	-	-	0.36	-	0.49	-	0.43	0.29	-	-
Smallmouth bass	HS	-	-	-	-	-	0.51	-	-	-	-	-	-	-	-	-	-
Brown trout	H	-	-	-	-	-	-	-	0.12	0.03	0.1	0.09	-	0.01	0.01	0.07	0.005

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, all stations)

Response variable: ln(TPCB)
Main effects: year, station
Covariates: none
Interactions: sex*ln(river age), sex*ln(% lipid), station*ln(river age), station*ln(% lipid)
Model r^2: 0.61

Model terms for CTPCB Smallmouth Bass (1992-2014, all stations)

Response variable: ln(CTPCB)
Main effects: year, station
Covariates: ln(%lipid)
Interactions: sex*ln(river age), station*ln(river age)
Model r^2: 0.59

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

Response variable: ln(TPCB)
Main effects: year, sex
Covariates: ln(river age), ln(% lipid)
Interactions: sex*ln(river age)
Model r^2: 0.64

Model terms for TPCB Smallmouth Bass at Bulls Bridge (all years)

Response variable: ln(TPCB)
Main effects: year, sex
Covariates: ln(% lipid)
Interactions: none
Model r^2: 0.53

Model terms for TPCB Smallmouth Bass at Lake Lillinonah (all years)

Response variable: ln(TPCB)
Main effects: year, sex
Covariates: ln(river age), ln(% lipid)
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(river age), ln(% lipid)
Interactions: none
Model r^2: 0.48

Model terms for CTPCB Smallmouth Bass at West Cornwall (1992-2014)

Response variable: ln(CTPCB)
Main effects: year
Covariates: ln(% lipid)
Interactions: sex*ln(river age)
Model r^2: 0.48

Model terms for CTPCB Smallmouth Bass at Bulls Bridge (1992-2014)

Response variable: ln(CTPCB)
Main effects: year, sex
Covariates: ln(% lipid)
Interactions: sex*ln(river age), sex*(% lipid)
Model r^2: 0.46

Model terms for CTPCB Smallmouth Bass at Lake Lillinonah (1992-2014)

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

Model terms for CTPCB Smallmouth Bass at Lake Zoar (1992-2014)

Response variable: ln(CTPCB)
Main effects: year
Covariates: ln(river age), ln(% lipid)
Interactions: none
Model r^2: 0.62

Model terms for TPCB Brown Trout (all years)

Response variable:	ln(TPCB)
Main effects:	year
Covariates:	ln(river age), ln(% lipid)
Interactions:	none
Model r^2:	0.68

Model terms for CTPCB Brown Trout (1992-2014)

Response variable:	ln(CTPCB)
Main effects:	year
Covariates:	ln(river age), ln(% lipid)
Interactions:	none
Model r^2:	0.63

APPENDIX G

Summary of Total PCB Concentrations (mg/kg wet weight) of Fillets of Brown Trout Collected in Academy Surveys of the Housatonic River

Table G-1. Average PCB concentrations (mg/kg wet weight) of Brown Trout filets collected in the Academy surveys of the Housatonic River from 1984-2014 shown as the geometric mean of CTPCB, TPCB, and percent lipid for each river age class at the West Cornwall station. Data for CTPCB are available from 1992-2014.

Year	Hatchery	West Cornwall – Age Class (River 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								
2014	0.004	-	1.63	2.23	4.08	6.25	-	-
2012	0.07	-	0.81	2.15	3.12	3.01	-	-
2010	0.01	-	0.83	1.62	1.92	3.33	-	3.03
2008	0.01	-	-	-	1.93	1.04	-	3.36
2006	0.01	-	1.15	1.01	3.86	-	-	-
2004	0.09	-	1.42	1.83	2.95	-	-	-
2002	0.1	-	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								
2014	0.01	-	1.85	2.57	4.75	7.42	-	-
2012	0.07	-	0.95	2.53	3.70	3.53	-	-
2010	0.01	-	0.97	1.86	2.22	3.92	-	3.80
2008	0.01	-	-	-	2.30	1.94	-	4.09
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	-

Table G-1 (continued).

Year	Hatchery	West Cornwall – Age Class (River 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 Percent Lipid								
2014	8.49	-	2.32	2.63	4.31	3.42	-	-
2012	8.71	-	3.25	4.02	4.32	3.35	-	-
2010	14.22	-	2.98	4.43	4.35	5.81	-	2.29
2008	8.87	-	-	-	2.99	3.26	-	3.61
2006		-	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 PCB Concentration in Benthic Insects from the Housatonic River (1992-2014)

Table H-1. Geometric mean PCB concentrations (mg/kg wet weight) in benthic insects from the Housatonic River, 1992–2014. 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	1.8	2.49	1.01
1996	TPCB	2.69	3.13	2.43
	CTPCB	2.5	2.65	2.29
1998	TPCB	1.05	3.94	0.54
	CTPCB	0.86	2.92	0.4
2001	TPCB	0.9	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	0.6	0.55	0.54
	CTPCB	0.51	0.44	0.5
2006	TPCB	1.61	1.93	0.66
	CTPCB	1.33	1.46	0.64
2008	TPCB	0.94	1.76	0.88
	CTPCB	0.78	1.45	0.75
2010	TPCB	0.8	2.04	0.67
	CTPCB	0.67	1.63	0.61
2012	TPCB	1.18	1.46	1.04
	CTPCB	1.00	1.17	0.88
2014	TPCB	1.22	1.92	1.11
	CTPCB	1.05	1.54	1.01

APPENDIX I

2014 Detection Limit Calculations

Matrix blanks (with extraction reagents) were generated to monitor possible laboratory contamination and to calculate the detection limits for PCBs. Each matrix blank, consisting of glass wool and approximately 30 g of clean Na_2SO_4, was analyzed using the same procedures as the samples. The detection limit was estimated as the blank area plus three times the standard deviation of the average blank peak areas. The method detection is reported on a mass per mass basis (dividing by an average extraction mass of 5.040 ng). The matrix blank-based detection limits for PCBs ranged from 0.01 ng/g (congener 85) to 16.06 ng/g (congener 3). Based on the matrix blanks, the average detection limit for individual PCB congeners was 0.48 ng/g and 34.80 ng/g for total PCBs. The matrix blank run with the extraction set on August 18, 2015 had low surrogate recoveries (22, 22, and 26%).

Table I-1. Summary statistics of the quantitated mass (ng) of all congeners in 14 blank samples and the calculations used to obtain the Minimum Detection Limit for congeners in the 2014 Housatonic River survey. All values in the table are in ng units, except for the Minimum Detection Limit (MDL), which has units of ng/g. The MDL is calculated as the (average + 3*SD)/(average extraction mass g). The average extraction mass is 5.04 g

Congener	Number of Detects	Minimum	Percentiles 0.10	0.90	Maximum	Median	Average	Standard Deviation (SD)	Average + (3*SD)	MDL
1	14	9.72	11.22	23.01	30.73	13.43	16.15	5.99	34.12	6.76
3	14	31.33	35.80	53.46	80.50	44.13	45.89	11.70	81.00	16.06
4+10	14	0.86	0.93	1.59	2.94	1.07	1.23	0.54	2.84	0.56
7	14	0.31	0.34	0.51	0.58	0.37	0.40	0.08	0.64	0.13
6	14	0.74	0.78	1.14	1.18	0.90	0.94	0.14	1.36	0.27
8+5	14	2.09	2.33	4.58	5.74	3.04	3.25	1.04	6.38	1.26
19	14	0.61	0.67	0.98	1.14	0.84	0.84	0.14	1.26	0.25
12+13	14	0.99	1.10	1.60	1.69	1.36	1.35	0.20	1.96	0.39
18	14	0.65	0.70	1.16	1.35	0.93	0.93	0.19	1.51	0.30
17	14	0.67	0.68	0.96	1.06	0.80	0.81	0.11	1.15	0.23
24+27	13	0.37	0.38	0.48	0.49	0.44	0.43	0.04	0.56	0.11
16+32	14	0.73	0.73	1.11	1.15	0.84	0.90	0.15	1.36	0.27
29	14	0.55	0.56	0.81	1.12	0.64	0.68	0.16	1.15	0.23
26	14	0.58	0.63	0.95	1.08	0.74	0.77	0.14	1.19	0.24
25	14	0.47	0.48	0.71	0.73	0.55	0.57	0.09	0.85	0.17
31+28	14	0.66	0.68	1.11	1.24	0.81	0.88	0.19	1.46	0.29
33+21	14	0.59	0.63	1.00	1.10	0.77	0.80	0.15	1.24	0.25
53	14	0.11	0.12	0.19	0.21	0.15	0.15	0.03	0.24	0.05
22	14	0.76	0.81	1.44	1.67	0.95	1.04	0.27	1.86	0.37
45	14	0.40	0.52	0.70	0.88	0.58	0.60	0.11	0.93	0.18
46	12	0.56	0.59	0.87	1.01	0.64	0.69	0.13	1.09	0.22
52	13	0.48	0.50	0.89	0.93	0.65	0.67	0.15	1.12	0.22
49	14	0.44	0.49	0.72	0.81	0.60	0.60	0.10	0.89	0.18
47	13	0.56	0.62	0.86	0.97	0.65	0.69	0.11	1.03	0.20
48	14	0.46	0.47	0.71	0.91	0.65	0.63	0.12	1.00	0.20
44	14	0.43	0.47	0.64	0.74	0.57	0.56	0.08	0.80	0.16
37	14	1.09	1.21	1.65	1.70	1.34	1.38	0.18	1.93	0.38
42	14	0.35	0.35	0.55	0.57	0.46	0.46	0.08	0.71	0.14
41+71	14	0.48	0.53	0.86	0.98	0.60	0.64	0.14	1.07	0.21
40	14	0.41	0.45	0.67	0.82	0.49	0.53	0.11	0.86	0.17
100	14	0.31	0.35	0.60	0.65	0.40	0.45	0.10	0.76	0.15
63	14	0.37	0.43	0.62	0.74	0.44	0.49	0.10	0.78	0.16
74	14	0.44	0.49	0.72	0.94	0.61	0.61	0.13	1.00	0.20
70+76	14	0.63	0.70	1.11	1.38	0.77	0.87	0.21	1.49	0.30
66	14	0.74	0.80	1.21	1.31	0.87	0.94	0.18	1.48	0.29
95	14	0.38	0.45	0.63	0.71	0.57	0.55	0.08	0.81	0.16
91	14	0.35	0.38	0.63	0.82	0.47	0.50	0.12	0.87	0.17
56+60	14	1.02	1.09	1.78	1.79	1.44	1.44	0.26	2.23	0.44
101	14	0.34	0.36	0.43	0.50	0.39	0.40	0.04	0.51	0.10
99	14	0.31	0.34	0.54	0.56	0.39	0.42	0.08	0.66	0.13

Table I-1 Continued.

Congener	Number of Detects	Minimum	Percentiles 0.10	0.90	Maximum	Median	Average	Standard Deviation (SD)	Average + (3*SD)	MDL
83	14	0.22	0.25	0.37	0.42	0.30	0.31	0.05	0.47	0.09
97	14	0.31	0.32	0.50	0.50	0.41	0.41	0.07	0.62	0.12
87	14	0.22	0.22	0.36	0.55	0.27	0.29	0.09	0.55	0.11
85	13	0.02	0.03	0.04	0.04	0.03	0.03	0.01	0.05	0.01
136	14	0.34	0.34	0.43	0.57	0.37	0.39	0.06	0.57	0.11
77+110	14	0.31	0.34	0.52	0.58	0.41	0.42	0.08	0.65	0.13
82	14	0.24	0.27	0.36	0.36	0.31	0.31	0.04	0.43	0.09
151	14	0.34	0.35	0.61	0.70	0.46	0.47	0.11	0.79	0.16
135+144	14	0.34	0.35	0.50	0.51	0.42	0.42	0.06	0.61	0.12
107	13	0.24	0.25	0.33	0.33	0.30	0.29	0.03	0.38	0.08
149	14	0.40	0.41	0.54	0.64	0.44	0.47	0.07	0.67	0.13
118	14	0.31	0.32	0.41	0.45	0.37	0.37	0.04	0.49	0.10
131	13	0.20	0.21	0.26	0.27	0.23	0.23	0.02	0.30	0.06
146	13	0.49	0.54	0.69	0.77	0.58	0.61	0.08	0.85	0.17
153+132+	14	0.73	0.89	4.14	5.00	1.05	1.88	1.44	6.20	1.23
141	14	0.34	0.37	0.55	0.56	0.44	0.45	0.07	0.68	0.13
137+176	14	0.31	0.33	0.44	0.46	0.39	0.39	0.05	0.53	0.11
163+138	13	0.37	0.38	0.55	0.60	0.44	0.46	0.07	0.67	0.13
158	13	0.51	0.58	2.93	3.10	0.92	1.34	0.93	4.14	0.82
129+178	12	0.45	0.49	0.66	0.82	0.58	0.59	0.10	0.89	0.18
187+182	14	0.73	0.76	1.12	1.44	0.85	0.90	0.19	1.48	0.29
183	14	0.41	0.42	0.56	0.66	0.51	0.51	0.07	0.71	0.14
128	14	0.27	0.29	0.53	0.56	0.36	0.38	0.09	0.65	0.13
185	14	0.27	0.28	0.40	0.42	0.34	0.34	0.05	0.48	0.10
174	14	0.29	0.30	0.44	0.46	0.35	0.36	0.06	0.53	0.11
177	14	0.35	0.37	0.61	0.63	0.42	0.46	0.10	0.75	0.15
202+171	14	0.30	0.31	0.47	0.54	0.35	0.37	0.07	0.59	0.12
157+200	14	0.51	0.53	0.79	0.92	0.64	0.65	0.11	1.00	0.20
172+197	14	0.40	0.41	0.61	0.66	0.55	0.52	0.08	0.77	0.15
180	14	0.29	0.32	0.46	0.56	0.39	0.39	0.07	0.61	0.12
193	14	0.57	0.59	0.84	0.88	0.73	0.72	0.10	1.01	0.20
191	14	0.44	0.49	0.72	0.84	0.58	0.59	0.11	0.92	0.18
199	14	0.19	0.20	0.31	0.32	0.22	0.24	0.04	0.37	0.07
170+190	14	0.38	0.41	0.57	0.75	0.46	0.49	0.10	0.77	0.15
201	14	0.39	0.40	0.58	0.78	0.53	0.52	0.10	0.83	0.16
203+196	14	0.41	0.43	0.60	0.63	0.51	0.51	0.07	0.71	0.14
208+195	14	0.51	0.51	0.87	1.28	0.61	0.68	0.21	1.30	0.26
207	14	0.26	0.30	0.47	0.49	0.36	0.37	0.07	0.59	0.12
194	13	0.17	0.19	0.23	0.25	0.21	0.21	0.02	0.28	0.06
205	14	0.20	0.21	0.31	0.34	0.26	0.26	0.04	0.39	0.08
206	14	0.26	0.30	0.46	0.48	0.33	0.35	0.07	0.56	0.11
209	14	0.11	0.12	0.24	0.31	0.16	0.18	0.06	0.36	0.07
Total (ng)		63.36	71.17	114.18	155.43	86.34	92.15	24.00	164.15	34.80
									Average MDL	0.48

Table I-2. Results from duplicate analyses of 2014 Housatonic River samples for PCBs. RPDs (relative percent differences) for duplicates were low, with an average (individual congener totals) RPD value of 15%.

ID	Mass Extracted (g wet)	% Lipid (wet wt.)	Surrogates (% recovery) 14	65	166	Total PCBs (ng/g wet)	Arochlor 1254 (ng/g wet)	Arochlor 1260 (ng/g wet)	Arochlor 1254+1260 (ng/g wet)
02583	5.047	2.27	106	91	85	933.89	225.96	843.42	1069.38
02583dup	5.046	1.50	101	88	89	909.16	226.60	818.16	1044.76
Average			103.86	89.66	87.03	921.53	226.28	830.79	1057.07
RPD			5	4	4	3	0	3	2
02587	5.027	2.12	87	87	97	1955.67	367.19	1949.70	2316.89
02587dup	5.033	1.90	103	84	102	2125.50	411.56	2112.55	2524.12
Average			94.88	85.29	99.20	2040.58	389.38	2031.13	2420.50
RPD			16	3	5	8	11	8	9
02597	5.075	1.92	89	97	85	2295.77	421.50	2249.20	2670.71
02597dup	5.015	2.43	93	87	91	2066.84	379.98	2021.03	2401.01
Average			91.04	92.07	87.97	2181.31	400.74	2135.12	2535.86
RPD			5	11	6	10	10	11	11
02592	5.045	2.96	97	82	81	1736.81	344.18	1608.00	1952.18
02592 dup	5.018	3.10	86	82	86	1846.05	364.51	1708.11	2072.62
Average			91.50	82.24	83.56	1791.43	354.35	1658.05	2012.40
RPD			12	1	6	6	6	6	6
02610	5.015	0.56	93	91	92	247.35	46.27	243.96	290.24
02610dup	5.022	0.83	102	99	100	263.16	50.10	259.44	309.54
Average			97.86	95.25	96.18	255.26	48.19	251.70	299.89
RPD			9	9	8	6	8	6	6
02616	5.029	1.01	103	96	89	319.54	42.96	370.08	413.03
02616dup	5.025	0.92	96	89	80	427.00	57.55	486.39	543.94
Average			99.33	92.66	84.41	373.27	50.25	428.23	478.48
RPD			7	8	10	29	29	27	27
02624	5.038	1.49	96	94	92	2158.69	323.27	2264.72	2587.99
02624dup	5.045	1.78	103	93	87	2053.27	307.34	2134.74	2442.08
Average			99.28	93.57	89.68	2105.98	315.30	2199.73	2515.04
RPD			7	2	6	5	5	6	6
02632	5.043	0.93	97	90	97	1088.43	168.11	1147.23	1315.34
02632dup	5.029	0.97	94	95	93	1108.87	163.92	1178.70	1342.62
Average			95.40	92.51	94.99	1098.65	166.01	1162.97	1328.98
RPD			2	5	5	2	3	3	2
01596	5.030	2.10	77	69	71	891.12	159.50	876.41	1035.90
01596dup	5.032	2.00	105	88	86	1206.61	212.67	1181.13	1393.80
Average			91.26	78.66	78.60	1048.86	186.08	1028.77	1214.85
RPD			31	25	18	30	29	30	29
01598	5.047	2.44	96	87	100	1267.22	153.01	1438.82	1591.83
01598dup	5.070	2.52	97	79	84	1242.86	149.00	1435.61	1584.61
Average			96.82	83.20	91.70	1255.04	151.00	1437.21	1588.22
RPD			1	9	18	2	3	0	0
01601	5.057	3.61	96	85	93	1117.89	161.77	1071.69	1233.47
01601dup	5.091	3.08	93	82	95	1145.50	159.68	1098.45	1258.13
Average			94.84	83.58	94.03	1131.70	160.73	1085.07	1245.80
RPD			3	5	2	2	1	2	2
								Average RPD	15

Table I-3. Results from triplicate analyses of 2014 Housatonic River samples for PCBs. RSDs (relative standard deviations) for triplicates were low, with an average (individual congener totals) RSD value of 13%.

ID	Mass Extracted (g wet)	% Lipid (wet wt.)	Surrogates (% recovery) 14	65	166	Total PCBs (ng/g wet)	Arochlor 1254 (ng/g wet)	Arochlor 1260 (ng/g wet)	Arochlor 1254+1260 (ng/g
02606	5.029	1.05	98	87	95	696.17	103.82	714.52	818.35
02606dup	5.043	1.12	103	84	79	733.74	115.13	758.63	873.76
02606trip	5.064	0.76	89	90	96	637.78	100.68	655.99	756.67
avg			96.72	86.97	90.24	689.23	106.54	709.71	816.26
stdev			6.87	3.17	9.43	48.35	7.60	51.49	58.57
RSD			7	4	10	7	7	7	7
02551	5.020	9.46	100	90	87	3.36	2.26	3.00	5.26
02551dup	5.058	9.03	92	92	92	1.78	1.78		1.78
02551trip	5.027	8.11	98	92	91	2.45	1.89	1.42	3.32
avg			96.41	91.52	90.03	2.53	1.98	2.21	3.45
stdev			4.18	0.99	2.28	0.79	0.25	1.12	1.75
RSD			4	1	3	31	13		51
								Average RSD	13

APPENDIX J

Linear Contrasts of TPCB and CTPCB Concentrations Among Groups of Years

Introduction and Methods

Throughout the many years of surveys since 1984, including the most recent year, statistical comparisons among different years have been based on pairwise comparisons of least squares means concentrations, i.e., a separate test has been done for each pair of years. This was an appropriate procedure, especially in earlier years when the temporal pattern of concentrations was unclear and no *a priori* hypotheses could be defined. Furthermore, the exact statistical models for adjusting concentrations for differences in sex, lipid, and fish age changed with each additional year's data, since the additional data provided greater resolution of these covariate effects. However, this approach is less appropriate at this point in the monitoring program, since the patterns of earlier years have been established. Because of the amount of earlier data, covariate models do not change greatly with the addition of each additional year's data. There is thus a major drawback to use of pairwise comparisons among years, since statistical power is lost with increasing numbers of years of data. Statistical power is the ability to reject a null hypothesis when that hypothesis is false. For example, statistical power often decreases with decreasing sample size, smaller deviation of the true value from that posited by the null hypothesis, and higher replicate variation among samples. For a given data set, statistical power is related to the probability of rejecting the null hypothesis when it is true (false positives). For example, using a less stringent p-value to determine statistical significance increases statistical power (it's easier to find a significant difference), but also increases the probability of finding significance when there is no real effect. Thus, test procedures balance the desire for greater statistical power and lower probability of false positives. In the case of pairwise comparisons, statistical power is lost by the need to adjust the level of significance to reduce the frequency of false positives.

Pairwise comparisons involve a large number of separate tests, n*(n-1) tests for n years of data. As a result, there is a high probability of finding some proportion of tests to be significant even if there is no real difference. For example, with an alpha level of 0.05, one significant result would be expected for every 20 tests done, if tests were independent. Pairwise-comparison tests, including the HSD test used for the Housatonic PCB data, are designed to control for this potential error. One result of this correction is that the statistical power of comparisons decreases with the number of tests done – i.e., as the number of tests increases, the difference between pair members has to be greater to be demonstrated as significantly different. For the Housatonic PCB data, there are data for 18 different years for TPCB and 12 different years for CTPCB, so the loss of power may be substantial.

An alternate approach to testing the significance of temporal trends is based on defining and testing a much smaller number of statistical hypotheses involving the comparison of the recent years' data with data from selected groups of previous years. This alternate

approach provides greater statistical power, since it focuses on a limited number of statistical questions. Moreover, it can be limited to those questions about the relationships between the most recent years of data and certain groups of prior years, rather than making comparisons among individual years within earlier groups of years, which are no longer of primary interest in assessing long-term trends in PCB concentrations.

The alternate approach uses the statistical method of linear contrasts. Tests are performed on linear combinations of yearly data (for example, the average of a group of years is a linear combination, with each year given equal weight). As with the earlier method, tests are performed on least squares means.

For the Housatonic PCB data, previous studies showed a pattern of moderate concentrations from 1984-1986, higher concentrations in 1998-1992, and lower concentrations from 1994 to 2012. Based on this pattern, the linear contrasts approach has been used to compare the average of the three most recent years (in this case, the 2010, 2012, and 2014 surveys) – which was used in lieu of only the most recent year due to year-to-year variability – with the following groups of years:

1) The immediately preceding period of lower concentrations (1994-2008);
2) The period of higher concentrations (1988-1992); and
3) The earlier period of intermediate concentrations (1984-1986).

These contrasts were done for TPCB concentrations for Smallmouth Bass for each of the four stations and for Brown Trout from West Cornwall. CTPCB was not calculated until 1992, so the last two contrasts could not be done for CTPCB (although the recent years' concentrations were compared to those from 1992). There were no Smallmouth Bass from Lake Zoar in 1986, so the contrasts for TPCB at Lake Zoar exclude that year from the comparison. (In future monitoring reports, the appropriate groupings of years will be re-evaluated.)

The contrasts were performed using Statistica software.

Results

Smallmouth Bass

With all stations combined, concentrations of TPCB in Smallmouth Bass in the three most recent years (2010-2014) were significantly higher than concentrations in the 1994-2008 period and significantly lower than those in the earlier periods (1984-1986 and 1998-1992). When stations were examined individually, TPCB concentrations in the three most recent years (2010-2014) were significantly higher than those in the 1994-2008 period at West Cornwall and Lake Zoar, but not at Bulls Bridge or Lake Lillinonah (Table J-1). Concentrations in the recent years were significantly lower than concentrations in 1984-1986 and 1988-1992 at all stations except Lake Zoar, where there was no significant difference.

Concentrations of CTPCB in Smallmouth Bass in the three most recent years, when all stations were grouped, were significantly higher than those in 1994-2008 and were not significantly different from concentrations in 1992. Examining stations independently showed that CTPCB concentrations in the three most recent years were significantly higher than those in 1994-2008 at West Cornwall and Lake Zoar, but were not significantly different from the data for those years at Bulls Bridge and Lake Lillinonah, and were significantly lower than 1992 concentrations at all stations except for Lake Zoar, which exhibited no significant difference (Table J-1).

Table J-1. Results of Smallmouth Bass linear contrasts of recent years (2010-2014) with other year groups representing periods of intermediate concentrations (1984-1986), high concentrations (1988-1992 for TPCB or 1992 for CTPCB), and low concentrations (1994-2008). Significance was at p=0.05.

Comparison Group		Station				
		WC	BB	LL	Z	All Stations
TPCB	1984-1986	<0.00001	<0.00001	<0.00001	ns	<0.00001
	1988-1992	<0.00001	<0.00001	<0.00001	ns	<0.00001
	1994-2008	0.000726	ns	ns	<0.00001	<0.00001
CTPCB	1992	<0.00001	0.027558	<0.00001	ns	ns
	1994-2008	<0.00001	ns	ns	<0.00001	<0.00001

Brown Trout

In Brown Trout, TPCB concentrations in the three most recent years (2010-2014) were not significantly different from those in 1994-2008, but were significantly lower than concentrations in 1984-1986 and 1988-1994 (Table J-2).

Similarly, CTPCB concentrations in Brown Trout in the recent years were not significantly different from those in 1994-2008, but were significantly lower than those in 1992 (Table J-2).

Table J-2. Results of Brown Trout linear contrasts of recent years (2014, 2012, 2010) with other year groups representing periods of intermediate concentrations (1984-1986), high concentrations (1988-1992 for TPCB or 1992 for CTPCB), and the preceding period of low concentration (1994-2008). Significance was at p=0.05.

Comparison Group		WC
TPCB	**1984-1986**	<0.00001
	1988-1992	<0.00001
	1994-2008	ns
CTPCB	**1992**	<0.00001
	1994-2008	ns