Fish Contaminant Monitoring Program: Review and Recommendations

Prepared for

Michigan Department of Environmental Quality
Lansing, Michigan
Fish Contaminant Monitoring Program: Review and Recommendations

Prepared for

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## Acronyms and Abbreviations

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<thead>
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<th>Description</th>
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<tr>
<td>DFO</td>
<td>Canadian Department of Fisheries and Oceans</td>
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<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
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<tr>
<td>FCMP</td>
<td>fish contaminant monitoring program</td>
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<tr>
<td>MDCH</td>
<td>Michigan Department of Community Health</td>
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<tr>
<td>MDEQ</td>
<td>Michigan Department of Environmental Quality</td>
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<tr>
<td>MDNR</td>
<td>Michigan Department of Natural Resources</td>
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<tr>
<td>MOE</td>
<td>Ontario Ministry of Environment</td>
</tr>
<tr>
<td>PCB</td>
<td>polychlorinated biphenyl</td>
</tr>
<tr>
<td>PTS</td>
<td>persistent toxic substances</td>
</tr>
<tr>
<td>USGS</td>
<td>U.S. Geological Survey</td>
</tr>
<tr>
<td>YOY</td>
<td>young-of-the-year</td>
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1 Introduction

The presence of persistent toxic substances (PTS) in aquatic ecosystems is one of the most important environmental policy issues currently facing the Great Lakes States. PTS, such as polychlorinated biphenyls (PCBs), DDT, chlordane, and mercury, bioaccumulate to high levels in fish. Contaminated fish are the primary source of these chemicals to most humans and semi-aquatic wildlife, and thus are the cause of widespread fish consumption advisories. Consequently, interpretation of spatial and temporal trends of these chemicals is critical to the planning and assessment of regulatory policies in the Great Lakes region. To that end, several federal, state, and provincial agencies in the United States and Canada have set up fish contaminant monitoring programs (FCMP) to track trends of PTS in the environment.

The Michigan Department of Environmental Quality (MDEQ) retained Exponent to review its FCMP, and specifically that part of its program devoted to tracking trends of PTS. Two factors suggest that such a review would be worthwhile. First, a considerable amount of experience and expertise has been accumulated over the 20 to 30 years that these FCMPs have been in operation. Michigan wanted its FCMP to make use of that experience. In addition, fundamental changes have occurred in our understanding of the processes underlying bioaccumulation of chemicals in fish. Together, these factors have led to ongoing evolution in our understanding of how trends in PTS bioaccumulated by fish and other biota should be assessed, and ultimately, what those trends really mean.

Both factors are best illustrated by a historical perspective. At first, monitoring ecosystem trends of PTS with fish and other biomonitors was, or was thought to be, fairly simple. At most, a few confounding factors were recognized as affecting bioaccumulation—factors intrinsic to the bioaccumulating organism, such as lipid levels, age, and size. Consequently, most FCMPs were designed to control only for fish age or size or both. Early status and trends analyses of the data also assumed a very simple ecosystem. All system components—external loading, abiotic inventories, and biota concentrations—were assumed to be in “steady-state,” or equilibrium, with each other. Accordingly, trends of contaminants in fish or gull eggs were assumed to be tightly coupled to, and reflective of, underlying trends in ecosystem inventories and external loading.1

Initial trends analyses based on PTS concentrations in biota were easy to interpret. These analyses of PTS in fish and other biota tended to show rapid declines of these chemicals during the 1970s and early 1980s (Environment Canada 1991). For a number of reasons, however, analysts in the late 1980s and early 1990s began to report evidence of slowing, cessation, or even reversal of previous declines of PTS in fish and gull eggs (see Baumann and Whittle 1988; Environment Canada 1991; 58 Fed. Reg. 20,806–20,809). Based on the underlying assumption that PTS concentrations in biota are tightly coupled to abiotic inventories and external loading, the supposedly stabilizing concentrations in biota were presented as proof of stable and

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1 Monitoring bioaccumulative PTS in fish and gull eggs was also easier than monitoring PTS in abiotic media such as water, air, and sediments. After bioaccumulation, chemical concentrations in biota are orders of magnitude higher and considerably easier to measure than the low concentrations found in water and air. In addition, biomonitors were assumed to average out spatial and temporal variation found in the abiotic media.

However, there was then, and continues to be, considerable debate over whether any stabilization really occurred (Smith 1995a,b,c, 2000; Stow 1995b; Stow et al. 1995; Pekarik and Weseloh 1998). During this ongoing debate, it has become increasingly clear that analyses of status and trends of PTS in biota were more complicated than previously thought. The relationship between trends of PTS in biota and trends in external loading was not nearly as tightly coupled as initially assumed. For example, there were often distinct differences in the short-term changes of PTS concentrations in different biota from the same lake or river, or worse, differences in the short-term changes in the same biota (Figure 1-1).

In addition, plots of PTS concentrations in biota over time are typically characterized by recurring waves (i.e., short periods of relatively fast decline and concentrations temporarily well below the long-term trend followed by short-periods of slow to no decline and higher than average concentrations) (Figure 1-2). Analyses of these waves demonstrated that they were not random sampling error. Within the same organisms, different PTS concentrations tended to behave synchronously (Smith 1995a,b,c). That is, one chemical such as PCBs tended to rise and fall at the same time and by about the same relative amount as concentrations of other hydrophobic PTS (Smith 1995a,b,c). Because the external source dynamics for each PTS should be unique, the synchronous short-term behavior of different chemicals was not likely caused by, nor was it a reflection of, changes in external loading. In addition, the waves of concentration in biota are generally too large and too short-lived to be caused by changes in external loading or abiotic inventories.

Rather, the concentration waves could be explained by factors affecting bioaccumulation. These factors included food-chain effects (Borgmann and Whittle 1992a; Smith 1995b; Madenjian et al. 1999), weather-related effects on bioaccumulation potential (Smith 1995c), or both (Hebert et al. 1997). Likewise, other empirical analyses demonstrated that spatial trends in PTS concentrations are also better explained by differences in food chain structure and limnological factors than by differences in external loading and abiotic inventories (Rowan and Rasmussen 1992; Rasmussen et al. 1990; Madenjian and Carpenter 1993). Food chain structure and limnological factors are especially important to the bioaccumulation of mercury.

Concurrent with these empirical analyses demonstrating the likely food chain effects on temporal and spatial trends of PTS in biota, the theory of bioaccumulation and biomagnification was also being refined. According to this theory, up to 80 to 90 percent or more of total bioaccumulation of biomagnifying chemicals (i.e., PCBs, mercury, and DDT) comes from food-chain exposure (Thomann 1989; Campfens and Mackay 1997). As food chain structure is constantly changing over time and space, biomagnification theory provided a strong theoretical basis that there should not be tight coupling between short- or long-term trends of PTS concentrations in biota and trends in external loading and abiotic inventories (Smith 1995a,b,c; 1996a,b; Hebert et al 1997; Jackson 1995).

Consequently, instead of tight coupling between biota and external loading, more recent trends analyses have adopted an underlying hypothesis that could be termed “loose coupling” (Huestis
et al. 1996, 1997; Offenberg and Baker 2000). That is, trends of PTS in biota are assumed to be coupled, over the long run and on average, to trends in external loading and abiotic inventories of that PTS. However, these analyses recognize that coupling can be overwhelmed, especially over the short-term, by food-chain dynamics and other factors that affect bioaccumulation (Eby et al. 1997). Consequently, these trend analyses focus on long-term trends and effectively ignore short-term perturbations.

The evolution of trend analyses of biomonitoring data is likely to continue. Mathematical modeling of whole ecosystem fate dynamics of large lakes (Endicott et al. 1990, 1992a,b) suggests that some internal system components, notably the sediments and biota, respond slowly to changes in external loading (U.S. EPA 1993, 1999). When external loading is decreased or increased faster than the sediments and biota can respond, subsequent trends in these media are based on internal fate dynamics and can be independent of levels of external loading for some time. During these periods of non-steady-state, trends in biota concentrations are coupled, albeit loosely, to changes in abiotic inventories, which are dominated by sediment stores. During periods of non-steady state, while sediment inventories are catching up to previous changes in external loading, PTS trends in biota and trends in external loading are effectively decoupled (Endicott et al. 1990, 1992a,b; Smith 2000). It may take a decade or longer for sediment inventories to respond to sharp declines in external loading (Endicott et al. 1990, 1992a,b).

During these periods, trends in biota, even trends as long as several decades, may imply little to nothing about concurrent trends in external loading (see previous references and Figure 1-3 reprinted from Smith 2000), although long-term trends of PTS in fish do presumably track long-term trends in external loading. PCBs in Lake Superior and in Lake Ontario provide an example of the non-steady-state conditions described above (Smith 2000).

Other factors at the whole ecosystem level can affect bioaccumulation. For example, there is a growing realization that ecosystem processes can profoundly affect the relationship between external loading, abiotic inventories, and PTS concentrations of fish. The influence of the ecosystem processes is particularly important for mercury bioaccumulation.

This historical perspective suggests an ongoing evolution in the assumptions underlying biomonitoring of ecosystem trends of PTS. Initially, biomonitoring programs assumed that bioconcentration (e.g., passive transport across the gills) was the primary source of bioaccumulation. Thus, these programs focused on the dynamics of PTS accumulation within the bioaccumulator itself. However, with the development of biomagnification theory and empirical analyses showing the importance of food chain effects, many trends analyses expanded their view to also consider the PTS dynamics of prey stocks (Madenjian et al. 1999; Eby et al. 1997). More inclusive trend analyses of biomonitoring data consider the PTS dynamics of the entire ecosystem (Smith 2000). As the scope and complexity of system linkages that affect bioaccumulation have increased, the assumed coupling between trends of PTS in biota and trends in external loading has weakened, from tight coupling, to weak coupling, to effectively no coupling at all under certain circumstances. The latter circumstances are most pronounced in large aquatic systems over the short-term (i.e., a decade or less).

In short, trend analyses with biomonitoring data are evolving to consider factors at greater spatial scales—from the organism itself to its food chain to the entire ecosystem. These analyses are also evolving to consider factors over greater temporal scales, because of the
increasing response times of whole ecosystems vs. food chains vs. individual fish. Because these changes are ongoing, there is currently no consensus concerning how trends in biomonitored data should be deduced and what they imply. Recent trends analyses of PTS in biota have focused on very short-term (e.g., IJC 2002) or very long-term trends (Offenberg and Baker 2000; Simcik et al. 2000), and they have assumed everything from tight coupling (IJC 2002) to no coupling (Smith 2000) with external loading, over the time period considered. The lack of well-defined methods has produced a panoply of predictions about future declines of PTS in Great Lakes biota.

At one end of the spectrum are analysts who see evidence of slowing and cessation of the rate of decline (see Stow 1995b; Stow et al. 1995; IJC 2002). These analyses suggest that concentrations in biota are reaching a “plateau” or “steady-state” or a “new equilibrium” with external sources, almost always atmospheric loading. According to these analyses, PTS concentrations in the future are predicted to remain constant or decline at very slow, undetectable rates (<5 percent per year). A second group of analysts (Simcik et al. 2000; Offenberg and Baker 2000) posits that rates of decline of PTS in biota are controlled by rates of decline of PTS in the atmosphere. However, because atmospheric levels of PTS are declining at rates of 5 to 40 percent per year, these analyses suggest that biota are close to equilibrium with atmospheric loading, albeit a declining, sometimes rapidly declining equilibrium (Simcik et al. 2000; Offenberg and Baker 2000).

A third view is based on the concurrence between predicted rates of absolute decline of PTS in biota compared to predictions of mathematical models (Endicott et al. 1990, 1992a,b) or to declines observed in abiotic media (Smith 2000). These analyses suggest that PTS concentrations are falling at about 5 to 15 percent per year, as rapidly as if there were no external loading. Finally, a totally empirical analysis (Lamon et al. 1998) predicts that concentrations of PCBs in Lake Michigan salmonids will decline over the next decade at about 5 to 15 percent per year, rates which are described as “very slow.”

It is important to note that very disparate conclusions are often based on inspection of essentially the same data. For example, data on PTS in Great Lakes lake trout, from the U.S. Environmental Protection Agency’s (EPA) lake trout Great Lakes sampling, are the underlying “proof” that declines in PCB concentrations are stopping (58 Fed. Reg. 20,806–20,809; IJC 2002), proceeding at a moderately fast pace in tandem with atmospheric concentrations (Simcik et al. 2000; Offenberg and Baker 2000), and inexorably declining (Endicott et al. 1992a,b; Smith 2000). Similarly, the same large database of information about PCBs in Lake Michigan salmonids is the basis for predictions of near steady-state conditions (Stow et al. 1995) and anticipated declines averaging 10 percent per year (Lamon et al. 1998).

Thus, interpreting trends of chemicals in biota has become something akin to a Rorschach test, where the final trend that is observed depends as much, or more, on the viewer as on the data. Because temporal plots of these data typically have repetitive waves of concentration, analysts can find evidence for any hypothesis, or policy decision, depending upon which data, assumptions, and statistical methods are used. Consequently, trends analyses based on PTS concentrations in biota currently lack scientific rigor.
Recognizing that the underlying science of biomonitoring is evolving and unsettled, MDEQ retained Exponent to review the trends monitoring elements of its FCMP. Specifically, Exponent reviewed literature pertaining to the mechanisms affecting bioaccumulation and detection of trends of chemicals in fish (see Appendix A). Along with a survey of methods employed by MDEQ and other FCMPs, summarized in Appendix B, Exponent reviewed options for biomonitoring with fish, and recommended changes, if warranted, to MDEQ’s program. These recommendations are intended to integrate recent science and accumulated experience, in order to make resulting trends analyses as robust as possible. The following presents the results of Exponent’s review.
2 Review of Confounding Factors Affecting Observed Levels of Bioaccumulation and Detection of Trends

Successfully detecting trends in concentrations of PTS in fish depends on identifying primary sources of variability and controlling for those factors with the sampling design or post-hoc statistical analyses (Bjerkeeng et al. 1998; Utke et al. 1991; Lamon and Stow 1999). To that end, the following presents a review of factors important to bioaccumulation of PTS by fish. In this review, bioconcentration is defined as the increase in chemical concentration in fish relative to the ambient water concentration resulting from uptake of a chemical through the gills. Bioaccumulation is the increase in chemical concentration in fish resulting from bioconcentration and uptake from the diet (e.g., from prey items). Biomagnification is an increase in chemical concentration in fish (i.e., bioaccumulation) that is greater than would be predicted by thermodynamic equilibrium. Biomagnification also implies increasing tissue concentration with increase in trophic level.

This review focuses upon bioaccumulation of chemicals that potentially biomagnify for two reasons. First, biomagnification is generally more complex and variable than bioconcentration. Thus, factors important to bioaccumulation of non-biomagnifying chemicals are a subset of those important to biomagnifying chemicals. Second, the biomagnifying chemicals include PCBs, DDT and its metabolites, toxaphene, and mercury. These chemicals dominate current environmental risks, sampling initiatives by FCMPs, and regulatory action.

The following review is divided into discussion of endogenous factors and exogenous factors that affect bioaccumulation. The first includes the traits specific to the individual fish, such as growth rate, lipid content, size, etc. Most of these factors have historically been recognized as important and are relatively easy to monitor and to control. The exogenous factors include such factors as concentrations in the prey, prey population dynamics, food chain length, and limnological factors (e.g., water quality, temperature, deposition rate) affecting chemical concentrations in water, sediment, and prey as well as the rate of uptake by fish. Compared to the endogenous factors, recognition of the importance of these factors is generally more recent, and attempts to control for these factors are more difficult and less extensive.

Two caveats should be mentioned. Given resource constraints and the voluminous literature on bioaccumulation, this was intended to be a representative rather than exhaustive review of the literature. In addition, given the specific focus upon FCMPs, this review does not generally include information on bioaccumulation by other taxa. Thus, this analysis does not include consideration of ongoing sampling programs and trends analyses of other aquatic or semi-aquatic taxa, such as gull eggs, crustaceans, and molluscan shellfish. Nonetheless, it is recognized that these other databases and literature can be informative. The gull egg sampling program, conducted by the Canadian Wildlife Service, is an especially useful database, with a vigorous literature concerning trends of PTS in the environment (e.g., Smith 1995c; Stow 1995b; Hebert et al. 1997; Pekarik and Weseloh 1998).
2.1 Endogenous Factors Affecting Bioaccumulation and Detection of Trends

The following section considers aspects of the fish that control or affect bioaccumulation of PTS and the final concentrations in tissue. To provide the reader with a basic understanding of how bioaccumulation works, mechanisms thought to be important to bioaccumulation are discussed first. After this, physical factors that potentially reflect these underlying mechanisms are discussed. See Appendix A for a review of some of the recent scientific literature concerning this subject. The following order does not reflect relative importance concerning magnitude of effect on final tissue concentrations of PTS.

2.1.1 Growth Rates/Growth Efficiency

In a simplistic view, the final PTS concentration in a fish is equal to the difference between total chemical ingested and total chemical excreted or metabolized, divided by the total mass of fish. Because excretion and metabolism of biomagnifying chemicals are both very low, this simplistic bioaccumulation equation can be further simplified into setting bioaccumulation equal to the mass of chemical assimilated divided by body mass accreted. Thus, PTS concentrations in fish are a negative function of fish growth rate. Mathematically, many bioaccumulation models treat fish growth as a de facto loss process, in which growth is lumped with excretion and metabolism into a composite “loss” function (e.g., see Thomann and Connolly 1984; Campfens and Mackay 1997). Qualitatively, the effect is called growth dilution, which aptly describes how the mass of assimilated PTS is distributed across an expanding mass of fish.

It is widely recognized in the literature that bioaccumulation is lower in “fast-growing” fish compared to “slow-growing” fish. However, this simple, widely held statement obscures a complicated relationship. For one, it is not growth rate per se that is important, but growth efficiency (Borgmann and Whittle 1991a; Madenjian and Carpenter 1993; Jackson and Schindler 1996; Madenjian et al. 1995, 1998a), which is the ratio of biomass consumed to biomass accreted. Total chemical intake is also related to the mass of prey biomass consumed. Thus, fast-growing fish may, in fact, have higher rates of bioaccumulation if their growth efficiency is low. This may describe the high PTS concentrations found in Lake Ontario lake trout in the late 1970s. In early years of stocking, lake trout had access to huge schools of forage fish, and they responded by growing quickly but inefficiently (Borgmann and Whittle 1991a).

A second subtlety of the relationship between growth efficiency and PTS concentrations is that the relationship depends on whether same size or same age fish are being compared. The widely quoted negative relationship between growth rate and PTS concentrations applies to organisms of the same size. The opposite is true if organisms of the same age are compared. Because they have generally eaten more food, fast growing fish tend to be more contaminated than slow growing fish of the same age (Stow and Carpenter 1994a; Madenjian et al. 1995; Eby et al. 1997). Thus, rainbow trout are slightly more contaminated than lake trout of the same age, but rainbows are considerably less contaminated than similarly sized lake trout (Madenjian et al. 1995).
The effect of growth efficiency on PTS bioaccumulation is a likely mechanism underlying much of the observed variation in bioaccumulation (Jensen et al. 1982). For example, the allometric relationship in which growth efficiency declines as body size decreases is a primary cause of the widespread positive relationship between PTS concentrations and body size (Borgmann and Whittle 1991b; Madenjian et al. 1995). Differential growth efficiency among different taxa is also probably an important mechanism determining differences in bioaccumulation among these taxa. For example, slow-growing lake trout tend to have higher PTS concentrations, at the same size, than fast-growing chinook and coho salmon and rainbow trout (Madenjian et al. 1995). Similarly, the slow-growing lean lake trout tend to have lower concentrations than similarly sized, even more slowly-growing siscowet (Miller et al. 1992; Miller and Schram 1999). The effect of growth efficiency on bioaccumulation may also contribute to the relationship between body lipids and biomagnification. Lipid-rich fish will generally have low growth efficiency because they are accreting energy-dense biomass at a slower rate than less fatty fish of the same size.

Although growth efficiency is recognized as an important factor affecting net bioaccumulation, no FCMPs explicitly consider this factor in analyses of trends, although potential effects of changes in growth efficiency over time are the underlying rationale for the Canadian Department of Fisheries and Oceans’ (DFO) emphasis on fish age (Whittle 2001, pers. comm.). In addition, the potential effects of growth efficiency have been considered in several post-hoc analyses to explain observed trends (e.g., see Borgmann and Whittle 1991a; Eby et al. 1997). Potential effects of changes in growth efficiency have also been considered in predictive models to examine potential effects of management options (e.g., Madenjian and Carpenter 1993; Jackson 1995).

2.1.2 Lipid Concentrations

Lipid concentrations are often assumed to play a pivotal role in the bioaccumulation of hydrophobic organochlorines (Barron 1990). Among different species of fish and other aquatic organisms, there are often strong relationships between organochlorine concentrations and lipid levels (Rasmussen et al. 1990; Rowan and Rasmussen 1992; Bentzen et al. 1999; Kucklick and Baker 1998; Zaraniko et al. 1998). The positive relationship between lipid levels and organochlorine concentrations also tends to occur between species at the same trophic level, between individuals of the same species, and within tissues in a single individual (Voiland et al. 1991; Miller and Amrhein 1995; Amrhein et al. 1999). There are also mechanistic arguments for the effects of lipids on bioaccumulation of contaminant concentrations. The loss rate of hydrophobic chemicals across the gills is generally modeled as an inverse function of the fish’s lipid content (e.g., see Endicott et al. 1990; Campfens and Mackay 1997). However, losses across the gills are a small total flux for chemicals that biomagnify.

Of more importance to the degree of biomagnification is evidence that transport across the intestines is a passive function of the concentration or fugacity (thermodynamic gradient). Primary proponents of this theory (e.g., Gobas et al. 1993; Barber et al. 1991; Barber 2001) suggest that uptake and excretion across the gut wall would both be potentially dependent on concentrations of body lipids. As uptake is a positive function and excretion a negative function of body lipid levels, passive diffusion of organochlorines across the stomach could significantly
affect final bioaccumulation. Passive diffusion of PTS across the gut wall of vertebrates is also supported by a number of different analyses. For example, Gobas et al. (1993) demonstrated that preferential digestion of food, compared to PTS, significantly increased the fugacity gradient of the food bolus as it moved through the digestive tract. The fugacity of food bolus increased as much as four-fold, sufficient to explain observed levels of biomagnification between predator and prey. Other experiments indicate that net absorption efficiency for PCBs and dioxins is an inverse function of body burdens (see detailed reviews of this subject in Barber et al. 1991; Barber 2001). In addition, adding indigestible lipids to the diet of vertebrates, which presumably decreases the fugacity of the food bolus, decreases absorption efficiency of coincidentally consumed hydrophobic chemicals and increases excretion of previously consumed hydrophobic chemicals (Volpenhein et al. 1980; Richter et al. 1982; Geusau et al. 1999).

At the same time, there is also considerable empirical and theoretical evidence that suggests that concentrations of body lipids are not an important factor in bioaccumulation in large fish (e.g., Borgmann and Whittle 1991a; Stow 1995a; Stow et al. 1997). Several analysts, notably Borgmann and Whittle (1991a,b) and Stow et al. (1997), have noted that the relationships between lipid levels and organochlorine concentrations noted among species and trophic levels could be due to size and trophic level effects that tend to covary with lipids. Several lines of evidence support this hypothesis. First, once fish size is entered into statistical models relating fish tissue concentrations of PTS to lipids and other potentially causal factors, lipid often fails to add significantly to the predictive power of many models (Williams et al. 1989; Broman et al. 1992; Stow 1995a; DeVault et al. 1996; Huestis et al. 1996; Lamon and Stow 1999). Second, the relationship between lipids and PTS concentrations within individual fish is sometime weak and/or transitory (Stow et al. 1997). Third, relationships between fish size and PTS concentrations often occur in cases in which lipid cannot be a causal factor. For example, fish species with tight relationships between size, lipid concentrations, and PTS concentrations also often demonstrate similar relationships with mercury and fish size (Figure 2-1). Moreover, those fish with weak to no relationship between size and lipid levels may still show tight relationships with size and hydrophobic PTS concentrations (Amrhein et al. 1999; Miller and Schram 1999). Fifth, bioaccumulation models that make absorption and fecal excretion independent of the predator’s lipid levels work as well, in predicting observed levels, as those that make assimilation and excretion lipid-dependent (e.g., see Borgmann and Whittle 1991a). Alternately, when lipid levels are manipulated in models that do consider lipid effects, the response of biomagnifying chemicals is much less than isometric. For example, doubling lipid levels was predicted to cause less than a 1 percent increase in modeled PCB concentrations in lake trout (Madenjian et al. 1993), while a 50 percent increase in lipid was predicted to produce only a 7 percent increase in DDT concentrations (Borgmann and Whittle 1991a). Sixth, empirical evidence also suggest that absorption efficiency of coho salmon tends to remain more or less constant while its lipid levels varied dramatically over the year (e.g., see Madenjian et al. 1998a vs. Madenjian et al. 2000b). With respect to the latter, lipid levels in some fish may change more rapidly (Madenjian et al. 2000b) than body burdens of PTS are thought to be capable of changing.

Manipulative experiments intending to test the importance of lipid to bioaccumulation have yielded inconclusive results. In experimental treatments with various combinations of high- and low-fat fish eating high- and low-fat diets, absorption efficiency and excretion of PCB
responded, significantly, as predicted in half of the treatments and responded, significantly, contrary to prediction in the other half (Dabrowska et al. 1999). The authors concluded that PTS bioaccumulation was a complex function of body lipids, food lipids, and changes of both over time. Other feeding experiments with redhorse and white sucker found that net bioaccumulation of chlordane increased as predicted with increases in body lipids, although the effect was small. Fish with three times the lipid levels had net bioaccumulation rates that were only 20 to 30 percent higher. Feeding studies with tetrachlorodibenzo-\textit{p}-dioxin and fish suggested that kinetic limitations were more important than equilibrium with body lipids in the rate of absorption (Nichols et al. 1998). Lastly, recent analysis of human digestion suggested that net absorption was an inverse function of body concentrations and temporarily-increased blood lipid levels due to lipid absorption from the food (Schlummer et al. 1998). If true, the latter effect would depend on both the lipid content and lipid digestibility of the food, with opposite effects on net absorption for diets with high levels of food lipids that are poorly absorbed versus high levels of lipids that are easily absorbed.

In short, significant causal effects of body lipids on bioaccumulation of hydrophobic PTS are not well established. Some analysts suggest that body lipids are important to both dietary absorption and excretion. Other analysts suggest that body lipids have little to no effect on net bioaccumulation of hydrophobic PTS. Hidden in this debate is an element of consensus. As factors in addition to body lipid levels are recognized to be critical to biomagnification, even proponents of the importance of lipid would agree that other factors are also important. Thus, no current theories of bioaccumulation would suggest that lipids are the only force in bioaccumulation, as is believed true with bioconcentration. (With respect to lipid effects on bioconcentration, Barron [1990] argues that this also is not such a simple relationship.)

Therefore, there is no expectation that concentrations of biomagnifying PTS and lipid levels will vary isometrically, other than by coincidence. This conclusion conflicts with both the theoretical and statistical basis for lipid normalization based on the usual ratio approach. Although generally unstated, the theoretical rationale for lipid normalization is the assumption that PTS concentrations are controlled by lipid levels. This is apparently a vestigial assumption from the bioconcentration literature. However, as suggested by Borgmann and Whittle (1991b), “Lipid levels are not the major factor directly controlling contaminant concentrations although they have some influence on excretion rates. There is, therefore, no advantage to expressing contaminant concentrations on a lipid basis for those contaminants which are accumulated primarily through the food web.”

The statistical basis for lipid normalization using the ratio method is also invalidated once the assumption of an isometric relationship is rejected (Hebert and Keenleyside 1995). Instead, these authors recommend ANCOVA techniques for those cases in which lipids correlate, but not isometrically, with PTS concentrations. It should be noted that the ANCOVA techniques also implicitly assume that there is a causal relationship between lipid concentrations and PTS concentrations, an assumption that may not be consistent with current theory discussed above. Therefore, analysts employing ANCOVA techniques should consider “the consequences if a relationship exists and the observed correlation is spurious” (Stow et al. 1997).

There are several other problems with lipid normalization. There are different methods of extracting and quantifying lipids, which may yield very different results of lipid and lipid-bound
chemicals (Randall et al. 1990, 1997). If lipids and hydrophobic chemical concentrations are extracted with different methods, lipid-normalized concentrations can vary dramatically (Randall et al. 1997), as much as 400 to 500 percent. Moreover, lipid normalization adds the variability of lipid quantitation to the variability of chemical quantitation, such that lipid-normalized values should have reduced precision (Hall 2001, pers. comm.). On the other hand, simultaneous extraction of lipid and hydrophobic chemical could artificially inflate the relationship between the two because concentrations of lipid and hydrophobic chemical will vary with extraction efficiency (Stow et al. 1997).

Notwithstanding the information presented above, it must also be acknowledged that the variance of hydrophobic PTS concentrations in fish can often be reduced, and statistical power of trends analyses increased, by inclusion of lipid as a covariate. This is especially true for carp, for which lipid concentrations are often a better predictor of hydrophobic PTS concentrations that either size or age (MDEQ, unpublished data). It could be argued that lipid normalization is appropriate for detection of trends across time and space. In this utilitarian view, it may not matter if lipids are actually the primary causal factor or a correlate of the actual primary causal factor of total bioaccumulation, as long as the correlation is consistent.

The power, and potential problems, with this utilitarian view is illustrated by recent analysis of toxaphene in Lake Superior smelt (Glassmeyer et al. 1997). Wet weight concentrations of apparent toxaphene in same-sized smelt fell sharply and significantly from 0.41 µg/g to 0.16 µg/g in Lake Superior between 1982 and 1992. On the other hand, lipid-normalized concentrations of toxaphene were statistically stable over the decade: 4.0 µg/g lipid apparent toxaphene in 1982 versus 3.1 µg/g lipid in 1992. These data were analyzed and discussed as lipid-normalized values only and were consistent with some other data. Thus, the conclusion was drawn that toxaphene was not declining in the Lake Superior ecosystem because of local, non-atmospheric sources. In this case, lipid normalization permitted an apparently clearer picture of the trend, or in this case, the lack of such.

However, this clearer pattern afforded by lipid normalization may well have been wrong. No local sources of toxaphene to Lake Superior have been located (Shanks et al. 1999), and external loading likely was declining (James and Hites 2002). Moreover, other fish concentration data, from EPA and MDEQ’s analyses of lake trout (Figure 2-2), suggest that toxaphene concentrations in Lake Superior fish were declining over this period.

In conclusion, the available information suggests that lipid normalization of hydrophobic biomagnifying PTS with the ratio method is inappropriate from empirical, theoretical, and, generally, statistical grounds. Even in those instances in which there is an isometric relationship between chemicals and body lipids, the relationship is potentially, some would suggest probably, not a causal one. However, as lipid concentrations are likely to have some role, albeit probably a limited one, in bioaccumulation, consideration of lipids in trends analyses as a covariate is potentially warranted. Inclusion of lipids as a covariate will often decrease variability and increase statistical power, so these effects should be considered in post-hoc statistical analyses, with the following caveats.

Analysts should be wary of data sets, and resulting trends analyses, in which lipids vary dramatically across time or space. Dramatic changes in lipid concentrations over time and space
signal similarly dramatic changes in growth rates and/or food sources (Borgmann and Whittle 1991a). As these factors can also profoundly affect bioaccumulation, any differences seen or unseen are potentially due to these other factors. Thus, analysts should always be aware that statistically significant relationships between lipids and PTS concentrations are potentially spurious. It also seems advisable that analysts conduct and present trends analyses with and without lipid as a covariate, and be wary of instances where the two methods yield different conclusions.

2.1.3 General Metabolism—Degradation and Absorption of Chemicals

There are significant differences in the rates at which different chemicals are processed by fish, and these can significantly affect bioaccumulation. For example, different congeners of PCBs, dioxins, and furans have different susceptibility to metabolic attack and excretion, which affects the rates at which they are bioaccumulated (Metcalfe and Metcalfe 1997; Morrison et al. 1999). With respect to the dioxins and furans, fish preferentially metabolize less chlorinated congeners (Sijm and Opperhuizen 1988) and congeners with chlorines at sites other than the 2,3,7,8 position (Broman et al. 1992). Hence, final bioaccumulation of these compounds is suppressed.

Although this has not been rigorously studied, there are probably differences among species in the rates at which they absorb, excrete, metabolize, and ultimately bioaccumulate PTS. For example, there appear to be species differences in the net absorption of hydrophobic chemicals. According to measured data, the net absorption efficiency of PCBs is considerably lower for coho and chinook salmon, about 50 percent (Madenjian et al. 1998a), than for lake trout, which is about 80 percent (Madenjian et al. 2000a). The blood of catfish and largemouth bass differ in their affinity for methylmercury, which causes significant differences in the rates at which these two species excrete mercury (Schultz and Newman 1996). Recent evidence also suggests that deepwater sculpin may possess an enhanced ability to degrade certain PCB congeners, which may reduce their body burdens by as much as 10 percent (Stapleton et al. 2001b).

2.1.4 Age and Size (Length and Weight)

With a few exceptions (Olsson and Rutegardh 1986; Larsson et al. 1992b; West and O’Neill 1998), concentrations of PTS increase as fishes age and grow larger. Several mechanisms, acting in concert, apparently cause this nearly ubiquitous phenomenon. As discussed in detail below, a primary mechanism appears to be the reduction in growth efficiency that accompanies increasing fish size. Compared to small fish, larger fish tend to have lower growth efficiencies and growth rates, and contaminant concentrations are an inverse function of growth efficiency. Potential losses of ingested PTS via the gills also becomes progressively less important as the ratio of gill surface to body volume declines with size (Barber et al. 1991). Compared to smaller fish of the same species, larger fish also tend to consume larger, more contaminated prey and to eat at higher trophic levels. Thus, the foods of large fish tend to be more contaminated than the foods of small fish. Larger fish also tend to have higher lipid levels, which may also affect the bioaccumulation potential of hydrophobic PTS.

There is uncertainty whether this effect is entirely due to the size of the fish or whether there are additional effects of age. Most of the causal factors discussed above are related to size, but
some models and theory suggest that there are both size and age-related effects (Borgmann and Whittle 1991a, b; Stow and Carpenter 1994a; Eby et al. 1997). Borgmann and Whittle report some empirical verification for age effects above those associated with fish size. According to their analyses, the slope of concentration vs. size was less steep within an age group than across age groups, presumably because the latter included the effects of size and age.

Several analyses were attempted to determine if age effects are noticeable once fish size is considered. Raw data from DFO’s sampling of Lake Ontario, which contain a range of age classes, were sorted by age and by length. Long-term trends analyses for PCB concentrations were then conducted on 600 to 700 mm fish, the same size that EPA selected for its sampling of lake trout (DeVault et al. 1996). The fish from this virtual sample had an average age of 5.7 years, so the long-term trends were compared to trends of 5 and 6 year-old fish. As can be seen from Figures 2-3, 2-4, and 2-5, long term trends based on similarly-sized fish are the same in terms of slope (ANCOVA, \( p > 0.05 \)) and \( R^2 \) as trends based on same-age fish. Multiple regression with the size-selected fish indicates that age was a significant covariate (\( p < 0.01 \)) in the following equation:

\[
\text{In } PCB = 1.41 - 0.101 \times \text{year} + 0.151 \times \text{age}
\]

The additional variance explained was slight. The \( R^2 \) rose from 0.45 (45 percent of the variance explained) to 0.49 (49 percent of the variance explained) with age added to the regression. These analyses suggest that age has, at most, slight effects beyond those associated with length, although this weak response to age could be due to the moderate variability in growth rates observed in this data set.

A second empirical analysis was suggested by results of modeling, which predict that age should be a better predictor of PCB concentrations than size (Madenjian and Carpenter 1993; Stow and Carpenter 1994a; Eby et al. 1997). Cursory analyses of available data do not support this hypothesis. Based on the percent of variance explained, length is generally as good a predictor of PCB concentrations for data sets with both age and size data. Thus, the importance of age effects, beyond those associated with size effects, remains unresolved.

This debate has more than academic interest for FCMPs because size of fish is so much easier to measure. Consequently, most FCMPs do not routinely measure fish age, even those that explicitly suggest that age is a critical factor. For example, in describing EPA’s sampling of Great Lakes lake trout, DeVault et al. (1996) state “while it is understood that both size and age affect contaminant concentrations,” budgetary constraints preclude aging of fish. Young-of-the-year (YOY) sampling programs also use fish size as a surrogate for age (e.g., Suns et al. 1991). EPA’s monitoring of fall run coho salmon relies on fish behavior—most spawning fish are three years old—to gather a limited age range of fish. Most analyses of bioaccumulation dynamics and trends also rely on fish size as the critical covariate (e.g., see Sorensen et al. 1990; Wente 1997; Lamon and Stow 1999), although this preponderance may be dictated by the greater availability of size data. A few FCMPs, notably DFO, do age fish regularly, while other FCMPs age fish irregularly. DFO researchers (Borgmann and Whittle 1991a,b; Whittle 2001, pers. comm.) maintain that age is the critical covariate. Consequently, DFO monitors fish age, and trends analyses by DFO researches generally consider age, not size, as the critical covariate (Borgmann and Whittle 1991b; Huestis et al. 1996, 1997).
The debate also sometimes has profound impacts on interpretation of data. For example, lean and siscowet lake trout of the same age have about the same PCB concentrations but very different lipid concentrations. Based on this observation, Miller et al. (1992) argue that lipid is not an important factor in bioaccumulation. However, if the comparison is based on fish size, siscowets are considerably richer, than lean lake trout, in both organochlorines and lipids, which would suggest lipids are important to bioaccumulation (Miller and Schramm 1999). Similarly, Eby et al. (1997) argue that changes in the growth rate of bloater could have effectively increased PCB concentrations in more recent sampling, offsetting ongoing declines in ambient ecosystem levels. Their argument is based on the assumption that age effects are important; specifically that older fish will have higher concentrations than younger fish of the same size.

In addition to the potential importance of age, another significant uncertainty is how to statistically control for size and age effects. In post-hoc analyses, regression and ANCOVA techniques are widely used to control for the effect of size/age, but the final results are sometimes sensitive to which covariate, age or size, is used and whether the data are transformed. Regressions of PTS concentrations on fish age are usually developed with log-transformed concentration data (Bache et al. 1972; Borgmann and Whittle 1991b; Miller et al. 1992; Ward and Newman 1999). However, size-concentration relationships have been developed with length and concentration in which neither (Williams et al. 1989; Madenjian et al. 1999), concentration only (MacCrimmon et al. 1982; Ward and Newman 1999), and both are log-transformed (Stow et al. 1997). Regressions of concentration on weight are also not standard.

In some cases, a statistical rationale (e.g., maximum $R^2$, normality of the residuals) is provided for the choice of variables and transformations (Wente 1997; Madenjian et al. 1999). GLSFATF (1993) suggests that $R^2$, biological plausibility, and the weight of evidence should determine whether concentrations should be log-transformed, and U.S. EPA (1989a) suggests that the theoretically most valid method should be used. However, neither provides guidance on what theory should be weighted or how biological plausibility should be determined. The modeling of Jensen et al. (1982) suggests that the shape of the relationship between PCB concentrations and fish size will vary across species. This conclusion suggests that different transformations may be applicable to different species, a result consistent with empirical analyses (Madenjian et al. 1999). To further confuse the picture, some data sets cannot be completely normalized regardless of the transformation applied (Kaiser et al. 1996).

Most FCMPs and post-hoc trends analyses control for age/size effects. As discussed above, several trend programs stratify sampling onto limited size and age classes. For example, EPA’s sampling of lake trout concentrates on lake trout from 600 to 700 mm, and the YOY sampling programs also sample a limited age/size class. Given the impracticality of aging fish in the field, DFO samples and analyzes a wide range of lake trout. After aging the already analyzed fish, DFO conducts temporal and spatial trends analyses on the data from four-year-old fish or same age fish (Baumann and Whittle 1988; Borgmann and Whittle 1991b; Huestis et al. 1996, 1997). Other studies have conducted trends analyses with data from a wide range of sizes/ages and corrected for age/size effects with statistical techniques, such as multiple regression (Rasmussen et al. 1990; Borgmann and Whittle 1991b), ANOVA (Stow 1995a), ANCOVA (Paasivirta et al. 1983; Zaranko et al. 1998; Ward and Neumann 1999), or composite models (Wente 1997). Other analysts have subselected the available data to include only a limited size
range of fish (Miller et al. 1992) or used regression to estimate the concentration at a standard length (MacCrimmon et al. 1982; Sorensen et al. 1990; Scheider et al. 1998; Lange et al. 1994).

2.1.5 Gender

Some researches have noted concentration differences between male and female fish of some species, such as walleye (Madenjian et al. 1998b), northern pike (Larsson et al. 1992b), coho salmon (Williams et al. 1989), and largemouth bass (Lange et al. 1994). On the other hand, concentrations in males and females are also sometimes not different (e.g., coho salmon [Norstrom et al. 1978] and herring [Olsson and Rutegardh 1986]). In some cases, differences are due to age/size-related differences in gender. For example, concentration differences between male and female coho salmon disappeared once differences in size were considered (Williams et al. 1989). In contrast, gender-related differences of mercury concentrations observed in largemouth bass were still apparent when size was considered, but virtually disappeared when male and female fish of the same age were compared (Lange et al. 1994).

Several factors alone or in concert could cause such differences in bioaccumulation. For example, spawning losses of hydrophobic PTS could be more significant for egg-laying females. This mechanism is the apparent mechanism underlying the gender differences noted in northern pike. Concentrations of PCBs in mature female pike were considerably lower than in mature males (Larsson et al. 1992b). In addition, contrary to the usual relationship, PCB concentrations in female pike decreased with age, whereas PCB concentrations in male pike probably increased with size. Ovaries contained a significant portion of a female pike’s total burden of PCBs. In contrast, reproductive tissue made up much less of the body weight of a male pike, and tended to be less lipid- and PCB-rich than ovaries. Consequently, spawning presumably causes appreciable losses of PCBs and other hydrophobic organics from female but not male pike.

However, appreciable spawning losses of hydrophobic PTS are unlikely to occur in many species of fish. Niimi (1983) considered the distribution of hydrophobic PTS in gonadal and whole body tissue of five species (rainbow trout, white sucker, white bass, smallmouth bass, and yellow perch). Based on the relative concentrations of PCBs in gonadal versus non-gonadal tissue, Niimi concluded that PCB concentrations would change little after spawning in these species.

In their analysis of differences in PCB concentrations in male and female walleye in the Saginaw River, Madenjian et al. (1998b) proposed three potential causes: differential losses of PTS during spawning, different prey items, and different growth rates. In contrast to northern pike, PCB concentrations in both female and male walleye were predicted to increase slightly after spawning. Madenjian et al. also considered differences in growth rates and prey selection to explain why mature male walleye had about 2.5 times the PCB concentration as mature females. According to their analysis, different growth rates were not sufficient to account for the difference, leaving diet as the most likely cause. The available data were consistent with this last hypothesis. Compared to male walleye caught in the Saginaw River, females were more migratory, and more likely to spend significant amounts of time living, and presumably feeding, in less contaminated Saginaw Bay and Lake Huron. Prey fish in Saginaw Bay had
about one third the mean PCB concentration of those in the Saginaw River, mirroring the differences in female and male walleye.

More recent work suggests that differential growth alone is sufficient to cause differences in PTS concentrations in male and female walleye. In a detailed analysis of walleye from Lakes Huron, Erie, and Ontario, Johnston et al. (2002) found differences in PCB and DDE levels between male and female walleye. Exponent also found differences in concentrations of PCBs and mercury between same-sized male and female walleye (see Section 4.4) that were not likely associated with differential food chain exposure. Exponent also detected differences in PCB and mercury concentrations for male and female carp of the same size.

These examples illustrate the complexity of bioaccumulation with respect to gender. In those relatively uncommon cases in which spawning losses have significant and differential effects on males and females, gender may have a significant effect on PTS concentrations. Probably more common but more variable are cases in which gender-specific behavior (Madenjian et al. 1998b) and growth rates (Lange et al. 1994; Johnston et al. 2002) cause perceptible differences in final bioaccumulation. As illustrated with the Saginaw River walleye, one proximal mechanism of gender-specific bioaccumulation is related to different concentrations in prey taken by females versus males. However, the ultimate mechanism is gender-specific migration patterns. Consequently, more limited gender-specific differences in bioaccumulation would be expected in systems where the lake and river were equally contaminated, and the opposite effect (i.e., higher concentrations in females than in males) might occur in situations with a pristine river and contaminated lake.

Considering the myriad factors that affect bioaccumulation and the different life history strategies pursued by male and female fish, it is likely that gender differences in bioaccumulation are common (Johnston et al. 2002). However, few FCMPs or trends analyses have controlled for gender. The sampling program for the Baltic Sea fish has stratified its sampling to only female fish, but the basis of this decision could not be determined (HELCOM 2002). Madenjian et al. (1998b) and Johnston et al. (2002) also recommend that gender be considered in trends analyses with walleye.

### 2.1.6 Species

An abundance of evidence indicates that bioaccumulation varies from species to species, due to differences across species in the panoply of factors affecting bioaccumulation. Consequently, most FCMPs or trends analyses do not routinely combine data from different species, although this sometimes occurs when the desired species cannot be located (e.g., Skinner 1993; Skinner et al. 1994) or when FCMPs cover very large areas that preclude sampling the same species (Schmidt et al. 1990; U.S. EPA 2000). Suns and Hitchin (1992) investigated the potential for combining data from several species of small fish. Their analyses indicated that species-specific bioaccumulation varied too much to allow concentration data from different species to be combined, even when other covariates such as lipid levels and size were controlled.

Trends analyses with data from multiple species of fish in which species is retained as a covariate are common, as are trends analyses that compare trends among species. These sorts of multi-species analyses have been recommended, as they may be less sensitive to confounding
factors that may affect a single species (Smith 1995a,b; Stow et al. 1995; Eby et al. 1997; Wente 1997; Bentzen et al. 1999).

A second important issue faced by FCMPs involves which species should be monitored. Several authors have addressed factors important in the selection of a biomonitor (e.g., see Norstrom et al. 1978; Sheffy 1980; Uthe et al. 1991). Most FCMPs, which attempt to measure trends, have chosen species that are both abundant and widely distributed (Norstrom et al. 1978; Sheffy 1980; Stahl 1997). The former insures ease of sampling and long-term success in finding fish, and the latter allows analysis of trends from location to location. Larger, older fish at the tops of food chains are generally preferred because these fish tend to have higher PTS concentrations, which are easier to measure (DeVault et al. 1989). However, as top predators are usually game fish, Wisconsin chose carp as its biomonitor because it is not sought by anglers (Sheffy 1980). Long-lived, wide-ranging fish, such as lake trout, walleye, and carp, are preferred for those FCMPs wishing to attenuate changes in concentration over time and space. On the other hand, young, non-migratory fish are preferred for those FCMPs wishing to assess trends over short distances and brief periods (Suns et al. 1991; Suns and Hitchin 1992; Skinner et al. 1994; Sloan 1997). These fish typify the biomonitors of the YOY sampling programs.

Although rationales for the choice of a biomonitoring species are often provided at the beginning of a monitoring period, no retrospective analyses were located in the literature review. With the benefit of hindsight, it seems clear that species differ in their utility as biomonitors. Some species such as spottail shiners and yearling pumpkinseedeed have occasionally been difficult to find (Skinner et al. 1994; Sloan 1997), and chemical concentrations in the small fish are often too low to be measured with conventional methods (Suns et al. 1993; Scheider et al. 1998). For whatever reason (e.g., more variable diet and/or growth rates) coho salmon from Lake Michigan proved to be unreliable biomonitors (Figure 2-6). Concentrations of PTS in coho salmon swing wildly over time. These wide swings convinced many analysts, incorrectly, into predictions of new equilibria and stable states (58 Fed. Reg. 20,806–20,809; Stow et al. 1995) that never occurred (Smith 1995a,b,c; Madenjian et al. 1999; Lamon et al. 1998).

The case of coho salmon illustrates the importance of low variability to effective biomonitoring. Based on the current understanding of factors affecting bioaccumulation, an ideal biomonitor would have a relatively constant diet and constant growth rate over time and space (Utthe et al. 1991). If so, lower trophic-level fish might be expected to have lower variability than upper level predators, since the latter’s food chains should be more complex and unstable. This hypothesis was tested with data presented in Royals et al. (2000), who present data for mercury in a number of fish species from ponds in the Florida Everglades. These data do suggest that there is a relationship between trophic level and the average coefficient of variability for mercury. However, an ideal biomonitor will also have high, easy to measure PTS concentrations. Figure 2-7 plots average mercury concentrations in these fish, illustrating a tight relationship with trophic level. Figure 2-7 illustrates the trade-offs necessary in selection of a biomonitor. That is, high concentrations found in many top predators fish allow easy measurement and easier detection of trends, but they may be accompanied by higher variability.

The choice of which species to use as a biomonitor has generally focused on attributes other than the likely variability of chemical concentrations in that species and the resulting statistical power of trend analyses based on these data. However, the theory and empirical data have both
expanded considerably since most FCMP chose species. Thus, potential variability should also be considered, along with other factors, when selecting a species for biomonitoring.

2.1.7 Tissue Type

Chemicals in fish are measured in a variety of fish tissues, the choice of which can substantially affect concentrations of PTS. In general, FCMPs assess chemical concentrations in whole fish, fillets with skins, and skinless fillets. Occasionally, individual organs, such as the liver or gonads, are also subjected to analyses. The tissue type can have a significant effect on chemical concentrations. For hydrophobic chemicals, concentrations in whole fish are generally greater than those in fillets with skins, which are generally greater than those in skinless fillets (Zabik et al. 1993; U.S. EPA 1995; Amrhein et al. 1999). The distribution of hydrophobic PTS within a fish appears to be largely due to differential amounts of lipid. That is, concentrations of hydrophobic PTS in different tissue types tends to follow the same hierarchy as lipids, and lipid-normalized concentrations are often very similar among tissue types (Niimi 1983; Voiland et al. 1991; U.S. EPA 1995; Miller and Amrhein 1995; Nichols et al. 1998; Amrhein et al. 1999).

Similarly, differences among species in the relative concentrations of hydrophobic PTS in whole fish versus fillets tend to follow differences in the fat distributions among species. Percids, for example, have large fat deposits along their stomach linings where hydrophobic organochlorines tend to concentrate. Consequently, walleye and perch fillets with skins may have as little as one-fourth or one-fifth the hydrophobic PTS concentration as the whole fish (U.S. EPA 1995). In contrast, fat and hydrophobic PTS tend to be more evenly distributed in the salmonids, so losses of hydrophobic PTS with filleting tend to less dramatic (U.S. EPA 1995). Although the ratio tends to vary with the salmonid species (Voiland et al. 1991; Amrhein et al. 1999), salmonid fillets generally have 50 to 70 percent of the concentrations of the whole fish.

Skinning and trimming fillets tends to reduce organochlorine concentrations by another 50 percent in both percids and salmonids (Voiland et al. 1991; Zabik et al. 1993).

As might be expected given methylmercury’s tendency to bind to sulphydryl groups found in muscle, mercury concentrations tend to be higher in muscle-rich tissues. Therefore, mercury concentrations follow opposite trends (Wente 1997) from hydrophobic PTS. That is, concentrations in fillets without skins are greater than concentrations in fillets with skins, which are greater than in whole fish. The effects of tissue type on mercury concentrations do not appear to be as well studied as with hydrophobic PTS. However, analyses (Goldstein and Brigham 1995; Wente 1997) suggest that increases in mercury concentrations, from whole fish to fillet with skins to skinless fillets, can be as high as 60 to 100 percent.

Because bioaccumulation potential and PTS concentrations vary across tissue types, FCMPs whose primary concern is detection of chemical trends generally sample whole fish. For example, EPA and DFO monitoring programs sample whole lake trout, and Indiana samples whole creek chubs (Stahl 1997). Although the rationale for this is unstated, analyzing concentrations in whole fish may avoid losses of precision associated with variability in filleting. On the other hand, many FCMPs try to gather information on trends and potential exposure to human consumers, for which fillets and skinless fillets are more appropriate. Those that do sample fillets have tried to standardize preparation of their fillets and skinless fillets. For
example, Ontario Ministry of the Environment (MOE) samples skinless fillets, Michigan samples un-trimmed fillets with and without skins, and New York State samples an untrimmed “standard fillet.” As the filleting/trimming methods may change over time, data from different tissue types should not be combined unless some method is used to translate concentrations in one tissue type to another. For example, some analysts combine whole and fillet data by lipid normalizing, others have applied average conversion factors (e.g., Stow and Carpenter 1994a; Jackson and Schindler 1996). Amrhein et al. (1999) produced species-specific predictive equations for converting fillet and whole fish concentrations for Lake Michigan salmonids because the ratio of hydrophobic PTS differed across species.

Analysts generally do not combine data from tissue types except in cases of extreme data limitation. Consequently, potential problems with combining data from tissue types have not been explored. However, combining data from tissue types with lipid normalization may introduce some error because lipid normalization may not remove all differences between PTS concentrations (Voiland et al. 1991; Amrhein et al. 1999). Combining data from different tissue types with lipid normalization also introduces potential artifacts of lipid normalization on trends analyses (see section above on lipids). Mathematical conversion factors or predictive regressions are not applicable across species and, within a species, may not be applicable to other times, other lakes, other sizes of fish (Voiland et al. 1991; Amrhein et al. 1999), and across different chemicals. There will also be variation in the bioaccumulation potential within a fillet type depending on the level of trimming and cleaning.

In summary, the tissue analyzed can have a significant effect on PTS concentration measurements. Therefore, analysts conducting trends should ensure that tissue type is constant over time and space. If data from different tissues are combined, using conversion factors or normalization, analysts should be cognizant of potential effects on final trends associated with the use of dissimilar tissues.

2.1.8 Recent Migratory Behavior

In the absence of barriers to movement, fish may migrate from relatively uncontaminated areas to very contaminated areas and vice versa. Consequently, PTS concentrations may depend on an individual fish’s recent migratory behavior. The effect of migratory behavior on PTS concentrations in fish has been considered in several analyses. The effects of gender-specific migration patterns on bioaccumulation of PCBs in walleye (Madenjian et al. 1998b) was described previously in Section 2.1.5. Ashley et al. (2000) describe a similar situation in which PCB bioaccumulation depends on the recent migratory behavior of striped bass in the Hudson River. The migration of individual fish from fresh to estuarine to salt water could be determined from the chemistry of their otoliths. Fish that had spent their last growth season in the more contaminated upper Hudson River had much higher PCB concentrations than those that had spent their last growth season in the less contaminated lower parts of the Hudson.

Effects on bioaccumulation caused by different migratory behavior may be important in other systems and species. Recognizing this potential, some FCMPs have tried to control for effects of fish migration. The YOY programs, for example, focus on very young fish specifically because they have not yet migrated. MDEQ considers potential fish movement in its selection
of sampling sites, targeted species and sizes, sampling frequency, and interpretation of data. The factors typically considered are the mobility of species of interest, seasonal movements, and movement related to life stages. For example, MDEQ’s original tributary sites were located above a barrier to fish movement from the Great Lakes.

However, except for these limited cases, the potential effects of this migratory behavior on PTS bioaccumulation are difficult to assay or control for. Madenjian et al. (1998b) relied on fish tagging, which is a very labor intensive, to evaluate migration. Similarly, few aquatic systems have a salinity gradient that can be exploited, as in the study by Ashley et al. (2000), and many sites of interests will not coincide with dams or other barriers to migration. Consequently, recent migratory behavior will likely remain a potentially important but poorly studied confounding factor affecting PTS concentrations in fish.

2.2 Exogenous Factors Affecting Bioaccumulation and Detection of Trends

The following sections review factors external to the fish itself that affect the final concentrations of PTS in tissue. These include a number of factors that are critically important to bioaccumulation, notably food chain effects and, with respect to mercury, limnological factors affecting mercury bioaccumulation. The exogenous factors also include methodological decisions, such as time of sampling, location of sampling, and variability due to analytical methods.

The review of limnological factors indicates that various morphometric and chemical factors can have very large effects on final mercury concentrations in fish. The literature on limnology effects on bioaccumulation of hydrophobic PTS is less extensive, but the limited evidence suggests that these effects are much weaker than effects on mercury bioaccumulation. Because the underlying mechanisms for limnological effects on bioaccumulation are significantly different for the hydrophobic PTS and mercury, these two types of chemicals are discussed separately.

2.2.1 Food Chain Effects—Trophic Level and Prey Concentrations

A wide body of theoretical, experimental, and empirical evidence emphasizes the importance of trophic level and prey concentrations to bioaccumulation of biomagnifying PTS. Current bioaccumulation models, for example, predict that 90 percent or more of more hydrophobic PTS and mercury in predatory fish comes from food chain exposure (Thomann and Connolly 1984; Borgmann and Whittle 1991a; LeBlanc 1995; Abbott et al. 1995; Campfens and Mackay 1997; Barber 2001). Relatively little comes from exposure across the gills. The importance of dietary exposure for biomagnifying PTS is supported by a number of manipulative experiments which have demonstrated biomagnification between trophic levels and the critical effect of food concentrations on bioaccumulation (e.g., LeBlanc 1995; Dabrowska et al. 1999).

This evidence conforms to empirical data from a number of systems for a number of biomagnifying PTS, which suggest that PTS concentrations in fish are largely a function of
concentrations in their diet. Within a species from different lakes, predatory fish from lakes with longer food chains tend to have higher concentrations of biomagnifying PTS than those from lakes with shorter food chains (Rasmussen et al. 1990; Rowan and Rasmussen 1992). This occurs, it is believed, because the forage fish near the top of longer food chains are more contaminated because of higher lipid levels and/or greater potential for biomagnification. Within a lake, year-to-year changes in organochlorine concentrations in lake trout correlate with population dynamics of the forage fish (Borgmann and Whittle 1991b; Smith 1995b).

Organochlorine concentrations in lake trout from both Lake Ontario and Lake Michigan tended to decline dramatically after prey populations crashed, presumably because prey in subsequent years were dominated by young, less contaminated prey. Similarly, bioaccumulation of PCBs by several species of small fish was linked to their feeding habits (Hebert and Haffner 1991; Suns and Hitchin 1992). Disruption of the food web by exotic zooplankton (Bythotrepis cederstroemi) and round goby has been implicated in causing increasing body burdens of PTS in fish (DeVault et al. 1996; Morrison et al. 2000), as has, ironically, the resurgence of native prey species (Whittle et al. 2000). Lastly, the relationship between fish size and mercury concentrations in piscivorous fish is generally steeper and stronger than that observed in omnivores and insectivores (Paasivirta et al. 1983; Wente 1997). These differences could be due to the more stable concentrations of prey for fish feeding at lower trophic levels. In addition to their concentrations of PTS, prey types vary in terms of digestibility, lipid content, and caloric value. These factors can also affect assimilation (Dabrowska et al. 1999) and growth efficiency, which also have potential effects on final bioaccumulation.

The effects of trophic level and food concentration are sometimes considered synonymous, but the former is only important as an indicator of the latter. While it is generally true that PTS concentrations increase with trophic levels, there may be considerable variation in the concentrations of PTS in the prey at any one trophic level and considerable similarity in concentrations in species in contiguous trophic levels. For example, most forage fish consume small crustaceans throughout their lives, but larger, older forage fish may have considerably higher PTS concentrations than smaller fish (Madenjian et al. 1999). Consequently, a predator’s dietary exposure to PTS, and final bioaccumulation, could change appreciably with minimal change in the trophic level of its prey.

The importance of food chain exposure, compared to bioconcentration across the gills, also pertains to some chemicals that do not biomagnify. For example, some dioxins and furans are metabolized and excreted and, thus, do not biomagnify or biomagnify weakly (Broman et al. 1992). Nonetheless, total exposure, and likely final bioaccumulation of dioxins and furans, is dominated by food chain exposure (Broman et al. 1992; Abbott et al. 1995). The importance of food web changes may also apply to other very hydrophobic, metabolizable chemicals such as polycyclic aromatic hydrocarbons (Thomann and Komlos 1999). Much of the discussion above concerning food chain effects also probably pertains to these chemicals.

Differences in PTS concentrations in the diet are capable of causing large-scale changes in bioaccumulation (Borgmann and Whittle 1991a; Abbott et al. 1995). For example, female walleye in the Saginaw River had about one-half the concentrations of male walleye, probably reflecting the higher concentrations in the prey of male fish (Madenjian et al. 1998b). Year-to-year changes in lake trout concentrations attributed to prey stock dynamics are similarly large, showing as much as a 30 to 40 percent change (Borgmann and Whittle 1991b; Smith 1995a,b).
Mercury concentrations in lake trout also varied dramatically across several lakes, depending on the mercury concentrations in smelt, their primary prey (MacCrimmon et al. 1982).

Despite their potentially important effects on final PTS concentrations, no FCMPs surveyed try to assess food chain effects or food chain stability over time or space. Many trends analyses of PTS over time and space also do not consider potential food chain effects (58 Fed. Reg. 20,806–20,809; Stow et al. 1995; Glassmeyer et al. 1997; Lamon et al. 1998). On the other hand, many trends analysts have considered food chain stability over time and space, using a variety of different methods. For example, ratios of predator biomass to primary production were used as a surrogate for food chain length in consideration of trends of PTS concentrations across different lakes (Rasmussen et al. 1990). These authors reasoned that shorter food chains would be more efficient in producing top predators. Correlation between concentrations of different PTS in the same fish has also been used to diagnose food chain effects (Rowan and Rasmussen 1992; Smith 1995a,b,c), with the rationale that external sources would be unlikely to be coordinated. Other authors have considered the correlation between short-term changes in prey stocks and short-term changes in PTS concentrations in predator fish (Borgmann and Whittle 1991b, Smith 1995b) as evidence of food chain stability. The presence or absence of predaceous zooplankton has been used to assess potential differences in food chain length over time (DeVault et al. 1996) and space (Rasmussen et al. 1990). Dissimilar short-term trends of the same PTS in fish and abiotic media has also been used as evidence of food chain effects (Rasmussen et al. 1990; Smith 1995a; DeVault et al. 1996). More direct evidence for food chain instability, and potential effects on PTS bioaccumulation, is provided by observed changes in stomach contents over time (Eby et al. 1997; Madenjian et al. 1999). In addition, delta carbon and delta nitrogen, stable isotopes that biomagnify at each trophic level, have been used to track stability of food webs over time and space (e.g., see Kiriluk et al. 1995, 1999; Hansson et al. 1997; Whittle et al. 2000).

2.2.2 Limnological Factors Affecting Mercury Bioaccumulation

Lake size, lake type, presence/absence of anoxic hypolimnion, and watershed size and land use are lake physical characteristics that are often found to be correlated (positively or negatively) with fish mercury concentration. In a study of remote Canadian Shield lakes, Bodaly et al. (1993) found that fish mercury concentration was inversely related to lake size (surface area) and hypothesized that smaller lakes have higher epilimnetic temperatures that promote the formation of methylmercury. In contrast, Rose et al. (1999) found that mercury concentration in largemouth bass was positively correlated with lake size (surface area) in Massachusetts lakes.

The type of lake is also thought to affect bioaccumulation of mercury. The three primary groups are seepage lakes, drainage lakes, and artificial impoundments (i.e., reservoirs). Numerous studies have shown that reservoirs (impoundments) have elevated fish mercury concentrations (Bodaly et al. 1984; Jackson 1991). Hanten et al. (1998) analyzed data separately for drainage lakes and impoundments in Connecticut. Fish mercury concentrations were 20 percent higher in impoundments than in drainage lakes. Also, the two types of lakes exhibited different relationships between various lake water characteristics and fish mercury concentrations. The type of lake can also influence how water chemistry parameters influence fish mercury concentration. In the upper Michigan peninsula, Grieb et al. (1990) found that seepage and
drainage lakes exhibited different relationships between dissolved organic carbon (DOC) and fish mercury concentrations (i.e., negative correlation for seepage lakes, no correlation for drainage lakes).

While few studies have compared fish mercury concentrations in lakes with and without an anoxic hypolimnion, it is commonly known that total mercury and methylmercury concentrations are higher in anoxic bottom waters than in oxic surface waters of the same lake. Driscoll et al. (1994) found that Adirondack lakes with anoxic hypolimnia had high concentrations of mercury in fish and high concentrations of methylmercury in the water column. Slotton et al. (1995) demonstrated that fall turnover in Davis Creek Reservoir (which contains an anoxic hypolimnion) results in mixing of bottom waters containing methylmercury into surface waters and an increase in methylmercury concentrations in zooplankton, juvenile fish, and adult fish. In addition, simple diffusion of methylmercury from the anoxic hypolimnion into surface water can supply methylmercury to fish during stratification.

A number of studies looked at the relationship between watershed size and land use (e.g., wetlands) and found significant correlations with fish mercury concentrations. In Connecticut lakes, Hanten et al. (1998) found that fish mercury concentration was most strongly correlated with watershed area to lake volume ratio (negatively) and retention time (positively) in impoundments. Rose et al. (1999) found that mercury concentration in largemouth bass from Massachusetts lakes was positively correlated with watershed area and wetland area within the watershed. The presence of wetlands in a watershed often indicates higher mercury concentrations in fish. Wetlands produce methylmercury and elevated mercury and methylmercury concentrations are found in the tributaries that drain them. In a study of mercury inputs to a lake in Ontario, Mierle (1990) demonstrated that streams draining wetlands in a watershed contain higher concentrations of total mercury than streams draining non-wetland areas. In Sweden, Sonesten (2001) found that mercury content of roach was greatly influenced by land use in the catchment (i.e., arable vs. forested land). The highest mercury levels in fish were found in boreal forest lakes, whereas lakes surrounded by arable land had lower mercury levels in fish. Westcott and Kalff (1996) found that zooplankton methylmercury concentrations were affected by the supply of mercury from the drainage basin (i.e., concentrations were positively correlated with drainage ratio and percent wetland in the catchment). In contrast, Greenfield et al. (2001) found that watershed traits did not correlate strongly with fish mercury concentration.

Many studies have looked for correlations between lake chemical characteristics and fish mercury concentration. The characteristics that are most often found to influence fish mercury concentration are pH, DOC, and measures of hardness or alkalinity. pH is generally negatively correlated with fish mercury concentration. Cope et al. (1990) found that mean mercury concentrations and burdens in whole perch were negatively correlated with pH in Wisconsin seepage lakes. In a study of Adirondack lakes, Driscoll et al. (1994) found a significant negative regression between fish mercury concentrations and pH. In a study of northern Wisconsin lakes, Greenfield et al. (2001) found that lake pH explained most of the variability in fish mercury concentration and exhibited a negative correlation. Grieb et al. (1990) found a negative correlation between fish mercury and both pH and acid-neutralizing capacity in lakes of the upper Michigan peninsula. Haines et al. (1994) found that mercury concentrations in fish from lakes in two Russian regions were highest in low-pH and colored lakes. Mercury levels in
fish from high-pH lakes were low regardless of color. Hanten et al. (1998) found that for both drainage lakes and artificial impoundments in Connecticut, fish mercury concentrations were inversely related to measures of alkalinity and hardness.

Lange et al. (1993) found that largemouth bass mercury concentrations from Florida lakes were negatively correlated with pH (as well as alkalinity, calcium, chlorophyll $a$, conductivity, magnesium, total hardness, total nitrogen, and total phosphorus). Water pH alone accounted for 41 percent of the variation in fish mercury concentration, based on linear regression. In Massachusetts lakes, Rose et al. (1999) found that mercury concentration in brown bullhead and yellow perch was inversely correlated with pH. In a study of two Ontario lakes with differing pHs, Scheuhammer and Graham (1999) found that pumpkinseed mercury concentration was higher and increased faster with size in the lake with low pH (5.2–5.6) than in the lake with high pH (6.3–6.9). In northern Minnesota lakes, Sorensen et al. (1990) found that fish mercury concentration negatively correlated with pH. Westcott and Kalff (1996), in their study of zooplankton, found that water color and pH explained 73 percent of the variability in zooplankton methylmercury concentrations with water color being more important than pH. Methylmercury concentrations increased with increasing water color and decreased with increasing pH.

In experimentally acidified Little Rock Lake, Wisconsin, Wiener et al. (1990b) showed that mean mercury concentration and body burden in whole age-1 yellow perch from the acid-treated basin were significantly higher than from the reference basin. Wiener et al. (1990a) found that the mean mercury level in walleye from the low-pH lakes (pH range 5.0–6.7) was threefold higher than the level in fish from high-pH lakes (pH range 7.0–8.1) within a given age group. Winfrey and Rudd (1990) concluded that decreased pH enhances methylmercury production in near surface sediments and in the water column, while decreasing volatilization of mercury from the lake water surface. Wren et al. (1991) found that pH (as well as alkalinity, calcium, conductivity, and magnesium) was negatively correlated with mercury concentrations in northern pike but not in walleye. Finally, Sonesten (2001) found that while roach mercury concentration had a negative relationship with dissolved ions (i.e., conductivity, calcium, magnesium, sodium, and potassium) and lake nutrient status (i.e., total phosphorus and nitrogen, fish biomass), pH had little effect on roach mercury concentration in Swedish lakes.

Several studies have found DOC to be positively correlated with fish mercury concentration. In Adirondack lakes, Driscoll et al. (1994) found that when the lake with the highest DOC was eliminated from the analysis, a significant positive regression was found between fish mercury concentrations and DOC. In a study of northern Minnesota lakes, Sorensen et al. (1990) found that fish mercury concentration was positively correlated with total organic carbon (as well as with mercury concentration in water and zooplankton). Wren et al. (1991) found that DOC and iron (variables associated with lake dystrophy) were positively correlated with mercury concentrations in both walleye and northern pike in Ontario lakes.

In summary, the effects of limnological factors on final mercury concentrations are profound. Lakes receiving the same level of loading can have very different mercury concentrations in fish, depending upon the efficiency of mercury capture and retention and, most importantly, on the rate at which methylmercury is produced. A number of FCMPs and trends analyses have
recognized the importance of these factors in assessing temporal and spatial trends of mercury concentrations in fish.

2.2.3 Limnological Factors Affecting Bioaccumulation of Hydrophobic Substances

The literature describing limnological effects on bioaccumulation of hydrophobic PTS is limited. In general, bioavailability of hydrophobic substances for bioconcentration is an inverse function of levels of organic carbon (U.S. EPA 1995; Lyman et al. 1998; Tracey and Hansen 1996). In addition, carbon rich systems may be associated with faster growth rates, which also may reduce PTS bioaccumulation (Larsson et al. 1992a; Jackson 1995). Berglund et al. (2001) found an inverse relationship across lakes between total phosphorus and bioaccumulation of PCBs. However, Rowan and Rasmussen (1992) found only a very weak relationship between primary productivity and bioaccumulation of DDT and PCBs. Moreover, once food chain and trophic level are considered, PCB and DDT concentrations in fish correlate with ambient abiotic concentrations across different lakes despite significant differences in limnology (Rowan and Rasmussen 1992). In contrast to mercury, then, the available data do not support the contention that limnological factors dramatically affect the accumulation of hydrophobic substances, although there may be some effect.

In addition, limnological factors may significantly affect the abiotic concentrations achieved with a constant level of external loading. Given the importance of sediment burial to hydrophobic chemical dynamics, lakes with high rates of sediment deposition will tend to have lower concentrations and faster response times than particle-poor systems. Similarly, lake temperature has impacts on degradation of PTS, such as toxaphene, and volatilization losses for chemicals such as PCBs and toxaphene. Consequently, the limnology of a lake is important in determining ambient abiotic concentrations.

2.2.4 Degree of Steady-State with External Loading

Most PTS tend to adsorb to particles and concentrate in abyssal sediments. Given the slow response time of sediments to changing levels of external loading, many aquatic systems may not be in steady-state with external loading (Endicott et al. 1990, 1992a,b; Gobas et al. 1995; Smith 2000). In contrast, some theory (Endicott et al. 1990, 1992a,b; Gobas et al. 1995) and empirical data (Smith 2000) suggest that the water column responds much more quickly to changes in external loading. Therefore, in a case in which the system is depurating, sediment concentrations may be considerably higher than steady-state concentrations, while the water column will more closely track external loading. Similarly, deep sediments will be more contaminated than surficial sediments so that PTS concentrations in fish could increase significantly after sediment scour even though external loading was falling (Morrison et al. 2000).

The occurrence of non-steady-state and the disparate responses of water column and sediments have several important implications. First, in cases of non-steady-state, bioaccumulation from sediment-based food webs will be more dominant and that from the water column less important than at steady-state (Campfens and Mackay 1997). Second, fish species reliant on the water
column food chain could show different rates of decline than fish foraging on benthic food chains (Hebert and Haffner 1991). Third, during periods of non-steady-state, declines of PTS in fish will be largely independent of changes in external loading (Endicott et al. 1990, 1992a,b; Smith 2000). Thus, extrapolation of short to medium term trends (e.g., over a decade or less) of PTS in fish to trends in PTS loading are tenuous (Smith 2000), because it is difficult or impossible to determine whether the system is at steady-state with external loading. Fourth, FCMPs attempting to monitor the effectiveness of remediation must consider the potential response times of fish and internal sources of chemicals when monitoring and interpreting data. While it is generally true that long-term (i.e., several decades) changes in external loading of PTS will produce similar long-term changes in fish concentrations, short term trends in biota concentrations often do not reflect concurrent short-term trends in external loading. In contrast to the information presented above, Baker and co-workers have presented information suggesting that PCB concentrations in Great Lakes food chains are at steady-state with current atmospheric loading (Kucklick and Baker 1998; Stapleton et al. 2001a). Thus, this issue remains unresolved.

2.2.5 Season of Sampling

Some analysts have noted differences in contaminant concentrations with season (Williams et al. 1989; Slotton et al. 1995; Bjerkeng et al. 1998; Ward and Newman 1999). In some cases, the seasonal effect can be attributed to other factors affecting bioaccumulation. For example, concentrations of PCBs in coho salmon vary significantly at different times of the growing season (Williams et al. 1989). However, this is likely to be an age/size effect, because the relationship between PCBs and size was stable throughout the sampling period. In contrast, seasonal effects on mercury bioaccumulation by largemouth bass were noted even when size of fish was controlled with ANCOVA. In this case, largemouth bass captured in the spring tended to have 25 to 45 percent higher concentrations of mercury than those caught in the summer (Ward and Newman 1999). The underlying mechanism for this seasonal effect was not determined.

Slotton et al. (1995) also found seasonal effects on mercury concentrations in fish. Methylmercury concentrations in zooplankton, juvenile fish, and adult fish increased after fall turnover. The latter was apparently caused by the influx of methylmercury-rich water from the anoxic hypolimnion during fall mixing. In contrast, Cope et al. (1990) used only chemical data collected in the summer for their analysis of fish mercury concentrations in Wisconsin seepage lakes. Their paper suggests that water data should be collected in summer when mercury uptake by fish is most rapid and methylmercury production is greatest.

2.2.6 Analytical Precision, Quantitation and Extraction Method, Analysts, and Lab Effects

Many FCMPs have lasted longer than the lifespan of state-of-the-art analytical methods. Significant changes have been made in the analytical methods used to quantify many of the PTS. Changes in these methods can have significant effects on the observed levels of bioaccumulation. Notable among these changes have been significant changes in methods to assay concentrations of PCBs and toxaphene (Glassmeyer et al. 1997). Quantitation of PCBs
based on single Aroclors® is sometimes not comparable to methods using multiple Aroclors®, and the results of multiple Aroclor® analyses will vary depending upon number of peaks measured (Huestis et al. 1996). Aroclor® measurements are sometimes not compatible with PCB congener methods (Butcher et al. 1997). Use of different analytical techniques also profoundly affects measured concentrations of toxaphene (Whittle et al. 2000). Since different analytical methods for total PCBs and total toxaphene focus upon different congener suites, which have different persistence, trends analyses based on different analytical methods may produce different trends (Whittle et al. 2000, Figure 2-2). A similar effect might be expected with PCB analyses, as the lighter congeners will dissipate more quickly than the more chlorinated congeners. For example, concentrations of Aroclor® 1016 in Hudson River fish declined dramatically following a cessation of inputs, but declines of Aroclor® 1254 were much slower and less dramatic (Sloan et al. 1985). Similarly, analyses of total dioxins and furans will likely yield different temporal trends than analyses of individual congeners (Huestis et al. 1997).

There may also be systematic changes over time in the amount of PTS estimated with the same analytical method. Peaks initially attributed entirely to one chemical are, with experience, sometimes found to be due to several chemicals. Once this is recognized, concentrations of the first chemical decrease, potentially producing a false trend in concentrations. Examples of this are interference with DDE and PCB congeners (Laws 1993), and interference between some PCB congeners, dieldrin, and other coeluting compounds (Hall 2001, pers. comm.).

Finally, there is more or less random variability due to uncontrolled effects of variable performance, different analysts, variable instrument response, etc. Huestis et al. (1996) provide an estimate of the magnitude of these effects. In their analysis, trends of PTS based on historical analyses were compared to trends based on recent analyses of archived tissue using the same analytical technique. There were no systematic differences in the mean concentrations of historical analyses versus recent analyses conducted on archived tissues. This demonstrated that the archived tissue had not lost appreciable PTS. However, the year-to-year variability of concentrations of the archived tissue, which were analyzed over a very short time period, was much lower than the variability of concentrations based on analyses conducted immediately after sampling.

Most FCMPs have quality control methods to minimize the effects of variability due to analytical methods. Some programs, notably DFO, archive fish tissue for later analyses (Huestis et al. 1996, 1997; Whittle et al. 2000). Attempts have been made to translate concentrations made with different methods (Butcher et al. 1997) to produce more reliable trends.

### 2.2.7 Sampling Location

As originally envisioned and widely interpreted in status and trends analyses, sampling location is a surrogate for local ecosystem concentrations, and thus, not really a confounding factor in the detection of spatial trends of chemicals. However, as has been demonstrated repeatedly, fish from different locations with similar abiotic concentrations can have profoundly different PTS concentrations. For example, see Rowan and Rasmussen (1992), Rasmussen et al. (1990), Madenjian et al. (1999) with respect to hydrophobic PTS, and the section above discussing
limnological factors affecting mercury bioaccumulation. In these cases, the underlying cause of spatial differences was one of the confounding factors described elsewhere, such as food chain effects, limnological effects, differences in growth rates, or others. Thus, observed differences in PTS with different locations should be interpreted with care.

With respect to temporal trends analyses, fish from different locations often have different concentrations. Consequently, failing to control for sampling location is a source of variance that inhibits the statistical power of temporal trends analyses. For this reason, many sampling programs stratify sampling by location.
3 The MDEQ FCMP

MDEQ samples a variety of chemicals in fish across Michigan. There are three elements to the MDEQ program: an FCMP with caged fish, one based on whole-body concentrations, and a third element based on edible portions, usually untrimmed fillets. Each is described below.

3.1 MDEQ Caged Fish FCMP

Since 1986, the Surface Water Quality Division of MDEQ has used caged fish to evaluate whether existing pollution prevention and regulatory and remedial programs are effectively reducing chemical contamination in the aquatic environment. Caged fish are used to identify sources of contaminants and spatial trends in contaminant concentrations. Monitoring focuses on chemicals with high bioaccumulation potential, such as mercury, PCBs, DDTs, and other pesticides (chlorodanes, toxaphene, dieldrin, endrin, mirex, heptachlor, etc.).

MDEQ caged-fish studies are conducted only in inland rivers, lakes, and reservoirs. Specific sampling locations are selected based on a number of factors, such as assessing the efficacy of remediation, requests from other agencies, and requests from the public. For example, Great Lakes tributary mouths are monitored to determine the presence or absence of PTS and the relative magnitude of concentrations compared to other Great Lakes tributaries. In addition, MDEQ has divided the watersheds in the state into different basins, and sampling sites for caged fish are distributed across these basins. In 1999, for example, three watersheds were monitored using caged fish. A total of 20 sites were monitored in these three watersheds.

Fingerling channel catfish, usually 4 to 6 in. in length, are purchased from commercial farms and used as test organisms. Catfish were selected because they are inexpensive, widely available, hardy, native, and widely distributed across the state. The fingerlings are placed in stainless-steel cages and held at a selected site, anchored in the water column above the sediments. Generally, one cage is placed at each site, and each cage contains 32 fish, although this number varies depending upon fish size. Control samples are obtained at the beginning of the test period by randomly selecting a subset of channel catfish and combining them into four composite samples. The remaining fish are held for 28 days, after which they are removed from the cages and, if sufficient tissue is available, divided into 4 composite samples of whole fish. Each composite sample must have at least 40 g, but 100 g per composite is preferable because it permits duplicate analyses. The number of fish per composite depends on the size and number of fish surviving the 28-day test. In general, the remaining fish are divided into the four composite samples. Each composite usually contains from 5 to 8 fish, but composites based on a few as 3 fish may occur if mortality is high.

Because the catfish are not fed and the cages are anchored above the bottom sediments, the fish are assumed to eat very little over the 28-day exposure period. In most cases, caged fish lose weight and lipids. Because bioaccumulation via the food chain is minimal, these experiments should be primarily measuring bioconcentration (e.g., uptake across the gills). However, in
infrequent cases such as when the cages are colonized by aquatic insect larvae, the fish gain weight and lipids. Food chain bioaccumulation could be significant in these cases.

Net uptake of each contaminant is calculated as the difference between the concentrations in the control samples and the concentrations in the test samples. To account for differences in lipid content across sites, concentrations of organic parameters are normalized by dividing the contaminant concentration by the lipid concentration. Mercury concentrations are evaluated as wet weights. Temporal trends for these data are generally based on perusal of data. Differences among stations (i.e., spatial trends) are determined with t-tests and ANOVA. A limitation of these analyses is the assumption that a 28-day period is representative of the annual condition of the site.

3.2 MDEQ Edible Portion

The primary objective of the edible-portion monitoring program is to develop sport fish consumption advisories and commercial fishing restrictions. Data collected from the edible portion FCMP are also used to evaluate environmental quality (303(d) list, Remedial Action Plan/Lakewide Management Plan impaired uses, etc.). In a few cases, the data are also used to assess temporal or spatial trends of chemicals in fish. Although data on chemical concentrations in fish have been collected since the late 1960s, the electronic database contains only data collected since 1980.

Sampling sites on the Great Lakes are selected based on MDCH sport fish consumption advisories and Michigan Department of Natural Resources (MDNR) collection practices. Sampling sites for inland rivers are selected based on proximity to barriers to fish movement (i.e., dams), sources of contaminants, and the ability to obtain samples. Sampling is also often targeted toward sites where there are known or suspected sources of PTS, public access, or sites that are popular with anglers. In most cases, no repeat sampling at a site occurs if concentrations are low and no new sources are suspected. However, repeated sampling is conducted if more samples will allow better characterization of the need for an advisory or to assess the effects of regulatory action or remediation. Sites with advisories are also resampled periodically to determine if concentrations have declined enough to change the advisory.

As these data are used primarily to advise fish consumers, a variety of fish species and sizes are collected. Generally, a predator and a bottom feeder are collected from each site, but more than 20 different species of fish are collected across Michigan. Fish of differing size are caught and analyzed as individual fish, or sometimes as composites of similar-sized fish. In general, different species are processed as either skin-on or skin-off fillet, based on surveys of cleaning methods employed by the typical angler. Most predators are processed as fillets with skins, while most bottom feeders are processed as skinned fillets.

Generally, mercury, PCBs, DDTs, and other pesticides (chlordanes, toxaphene, dieldrin, endrin, mirex, heptachlor, etc.) are analyzed. Only mercury is measured in some remote inland lakes or reservoirs with no known source of chlorinated organics (other than atmospheric deposition). Dioxins and furans are also periodically monitored at some stations. About 80 percent of the
edible portion samples are collected by other agencies, specifically the MDNR Fish Division, tribal organizations, and other non-MDEQ agencies.

Although these data are primarily used to issue advisories, they are occasionally used for trends analyses by MDEQ. When data adhere to the underlying assumptions of ANCOVA, this statistical test will sometimes be used to evaluate spatial differences between fish from different water bodies. ANCOVA is used because of the dependence of contaminant concentrations on length, which is especially important for trends analyses with mercury. Alternately, regression is used to estimate concentrations at a “standard length,” and then changes are tracked in these “standardized” concentrations over time.

3.3 MDEQ Whole Fish

The near-term goal of the FCMP whole-fish monitoring program is to identify spatial differences and temporal trends in the quality of Michigan’s surface waters. The ultimate goal is to evaluate whether existing pollution prevention and regulatory and remedial programs are effectively reducing chemical contamination in the aquatic environment.

The Michigan whole fish monitoring program has been in existence since 1990. Species and locations were selected to complement and avoid duplication with the EPA/U.S. Geological Survey (USGS) Great Lakes whole-fish trend monitoring program. A total of 26 locations within the Great Lakes, its connecting channels, rivers, and inland lakes and reservoirs are sampled every two to five years. A consistent, limited size of larger, adult fish of lake trout, walleye, carp, and largemouth bass are targeted. However, fish of the targeted size are sometimes not available, and the available fish are analyzed. Chemical concentrations in individual, whole fish are analyzed. As with other elements of Michigan’s FCMP, the whole fish monitoring also focuses on chemicals with high bioaccumulation potential: mercury, PCBs, DDTs, chlordanes, toxaphene, dieldrin, endrin, mirex, and heptachlor.

This FCMP considers potential impacts of fish movement and migratory behavior. Factors such as the mobility of species of interest, seasonal movements of fish, and movements related to life stages are considered in determining sample locations, targeted species and sizes, and sampling frequency. MDEQ considered potential effects of fish migration in the selection of fixed sampling stations. For example, original tributary sites were located above a barrier to fish movement from the Great Lakes. Temporal trends are assessed with regression techniques. The data are transformed using natural logs of wet weight. Samples are evaluated to confirm that the sizes of the fish do not change over time. If sizes have changed significantly over time, a correction factor is applied or no conclusions about contaminant changes are made from those samples. If significant changes in organic concentrations are detected, the data are lipid-normalized and the change is evaluated again. Spatial trends analyses are limited to general conclusions based on review of graphs (e.g., mercury trends to be higher in inland lake walleye than Great Lakes walleye).

Potential problems and concerns raised by MDEQ about its whole fish FCMP program include the following:
Collecting similar sized fish at some sites, especially smaller lakes and rivers, is sometimes difficult. The resulting variability in fish sizes makes observed trends, or lack thereof, difficult to interpret.

Some fish species, notably carp and walleye, may have migrated long distances to the site at which they are collected. This could potentially influence trends analyses.

Because MDEQ relies on other agencies to collect fish, no effort is made to control the date of collection from year to year. The potential influence of season of collection is unknown.

Growth rates between sexes and between sites have not been evaluated. MDEQ is concerned whether these factors can significantly affect trends analyses.

MDEQ is concerned about the effect of fish movement on its analyses. For example, tagging studies indicate that walleye move freely between fixed stations in the St. Clair River, Lake St. Clair, Detroit River, and Lake Erie. MDEQ believes that carp also move freely across these stations. Nonetheless, there are often differences in temporal trends for a chemical in the same species from different sites.

3.4 Estimation of Power of Current Sampling (Whole Fish Sampling)

The power of MDEQ’s current whole fish sampling program was estimated using data from ten combinations of location and species. The following location-species combinations were selected: carp from Detroit River at Grassy Island, Kalamazoo River at Lake Allegan, Lake Erie at Brest Bay, Muskegon River at Croton Pond, and St. Joseph River above Berrien Springs Dam; largemouth bass from Gull Lake and Gun Lake; walleye from Lake Gogebic and Lake Huron at Saginaw Bay; and lake trout from Lake Michigan at Grand Traverse Bay. These were chosen by MDEQ as representative of its FCMP. Most of these location-species combinations were sampled about every other year from 1990 through 2000, providing 5 years of monitoring data. For each location-species, we estimated power to detect temporal trends for mercury, PCB, and lipid-standardized PCBs.

3.4.1 Approach/Methods

The intent of power analyses is to estimate the adequacy of available data to detect temporal trends in concentrations in fish tissue. Power is the probability of detecting a significant trend or a significant correlation based on the actual data for each location and species. A temporal trend is defined here as a significant slope obtained in linear regression of the natural log (ln) of concentration vs. time. The ln transformation was used to satisfy the homogeneity of variance and normality assumptions of the linear regression method. This transformation also conforms with the underlying mechanisms of PTS decline, which is likely to be a first order process (see
Section 4). A linear trend in log space is equivalent to an exponential trend in linear space (i.e., an annual fixed reduction in \( \ln \) concentration is equivalent to a fixed percentage reduction of concentration per year in linear space).

The power level is a function of the amount of available data, the variability in that data, and the strength of the underlying trend for each data set. Because it is a probability, the value of power ranges from 0 to 1. 1 – power is the type-2 (false negative) error rate or the probability of concluding no significant trend exists where one actually does. Power levels of 50 percent or lower mean that one has a 50 percent or higher chance of falsely concluding that no trend exists when one really does exist. To keep the false negative error rate low (20 percent or less) the power level must be high (80 percent or higher).

In general, the power to detect a trend increases as the magnitude of the trend increases, but for a given trend level, the power decreases with the variability of the data. If the individual concentrations for a given year are highly variable, then the trend must be greater in order to detect it. The statistical power is also expected to be related positively to the amount of data and the length of time considered. However, the data sets considered in this analysis contained relatively constant amounts of data sampled over a relatively constant time frame, and thus these factors were not addressed. To compare between the different location-species combinations, both the strength of the trend and the power to detect the trend are reported.

Power analyses were conducted for both the actual sampling data (i.e., for the actual trends observed) and for simulations constructed from the actual data sets that were designed to evaluate alternate trends. Two different techniques were used to conduct these different power analyses.

### 3.4.1.1 Method for Power Analyses of Actual Data

Power analyses were conducted using the correlations actually observed in the sampling data. Estimation of the statistical power of each combination of location–species was done using a method designed for calculating the level of power for a correlation estimate (Zar 1974). A simple linear regression model provides an estimate of the relationship (or correlation) between the independent and dependent variables. The square root of the \( R^2 \) value of a fitted regression model is the magnitude of the Pearson correlation coefficient. Using the Pearson correlation coefficient from each location-species regression model, the power is calculated using Fisher’s \( z \)-transformation and the appropriate critical value for the correlation from a table. This method is explained in detail in Appendix C.

### 3.4.1.2 Method for Power Analyses of Simulated Trend Data

Another potentially valuable power calculation is that of the probability of detecting a range of trends for each combination of location–species. This calculation provides a means of evaluating the current level of sampling. This analysis allows comparisons of sampling power across combinations of location-species or across trend levels for a specific location-species. Simulation methods were used to calculate the power to detect temporal trends for trends with 100 percent power down to low power levels.
Each year sampled was represented by a normal distribution to characterize the ln-transformed concentrations. The standard deviation of each annual distribution was the average of the standard deviations of annual data monitored for that location-species. The annual means for each distribution of ln-transformed concentrations were altered to fit the trend level being evaluated. For example, to calculate the sampling power of a 10 percent per year decline, the mean of the distribution for the initial year was left unchanged and the means of distributions for subsequent years were adjusted to fit a 10 percent per year decline (the year two mean was set equal to 90 percent of the year one mean, etc.).

Additionally, to more accurately reflect the variance found in the actual data sets, the mean for each annual distribution was adjusted to reflect the imperfect fit of the actual yearly means to the estimated trend. The adjustment to each year’s mean was based on the differences between the annual means of the original data and predicted average concentration for each monitored year. Specifically, the adjustment distribution was defined with a mean of zero and the standard deviation of the differences between annual measured mean ln-concentrations and annual predicted ln-concentrations. In generating a final simulated sample, an adjustment value was randomly selected from the adjustment distribution for each year monitored and added to the mean of each year’s distribution. The net effect of the adjustment can be thought of as moving each year’s normal distribution slightly up or down from the overall trend line of interest. After this, the normal distributions for each year were randomly sampled at the same rate as the actual data, and a linear regression model fit to the generated sample concentrations over time. This process is repeated 1,000 times for each rate of change, and the power to detect the trend was calculated as the proportion of significant trends based on the regression models.

3.4.2 Results

3.4.2.1 Results of power analyses of actual data

Table 3-1 summarizes the current power and trend levels in mercury, PCB, and lipid-standardized PCB concentrations for the 10 location-species evaluated. The power of current sampling is sufficient to identify temporal trends of 10 percent or more change per year in mercury, PCB, and lipid-standardized PCB concentrations at all location-species evaluated. Gun Lake has a high level of power for a weaker trend (4.7 percent for mercury) but not equivalently high power for PCB concentrations. Lake Michigan lake trout and Lake Gogebic walleye are the only location-species with high power for all three compounds, 100 percent power for trends ranging from 7.5 to 21 percent change per year. 100 percent power means there is virtually no chance of concluding a trend exists when in fact it does not. Lake Michigan lake trout show an increasing trend in both wet-weight and lipid-standardized PCBs (7.5 and 11 percent, respectively) whereas Lake Gogebic walleye show a decreasing trend in both PCB measures (−13 and −21 percent, respectively). Mercury for each site follows the same pattern of increasing concentrations at Lake Michigan (12 percent) and decreasing at Lake Gogebic (−11 percent).

Kalamazoo River and Muskegon River carp have high power for PCB concentrations, both wet weight and lipid-standardized, but have no detectable trend for mercury. These two location-species have consistent decreasing trends for both PCB measures with the lipid-standardized
PCB trend slightly steeper. Detroit River carp, Lake Erie carp, and Gun Lake largemouth bass have high power only for mercury, 99, 98, and 98 percent, respectively. The power levels for PCB concentrations at these three location-species are low (3 to 60 percent) as the trend levels are not detectable. Lake Huron walleye show a similar pattern of higher power for mercury (72 percent) and non-detectable trend levels for PCBs. Gull Lake largemouth bass and St. Joseph River carp have low trend levels and therefore low power levels (4 to 66 percent).

Locations with low power need to be evaluated to determine whether the trend levels need to be detected and therefore more sampling would be recommended, or whether the trend levels are so low that the current ability to detect stronger trend levels is adequate. Given the strength of the trends present in the data available, Lake Michigan lake trout and Lake Gogebic walleye could be sampled less so as to lower the power levels from 100 percent. Gull Lake largemouth bass and St. Joseph River carp require additional monitoring to detect the trends present in the data. All other location-species with high power for one compound had low power for another; therefore, an increase in sampling would be required to detect the trends present in all three compounds. Most of the trend levels not detectable were 5 percent change per year or less. If it is unnecessary to detect a trend this small, then additional sampling is not warranted.

3.4.2.2 Results for Power Analyses of Simulated Trend Data

Power level calculations for the range of detectable slopes for each site similarly have higher power levels for mercury than for PCB concentrations. Table 3-2 presents the power levels for the range of trends for each location-species evaluated. All five locations where carp were measured show mercury to be less variable than PCB, and thus smaller trends are detectable for a given power level. Lipid-standardized PCB concentrations are generally less variable than the wet-weight concentrations, although for Lake Erie, Kalamazoo River, and Muskegon River, the variability of the two PCB concentrations are about the same. Largemouth bass from Gull and Gun Lakes also have higher power for mercury concentrations than PCB concentrations, but the wet-weight PCB concentrations have higher power levels than the lipid-standardized concentrations. Walleye from Lake Gogebic and Lake Huron do not show similar patterns of higher power for PCBs and lower power for mercury. Lake Michigan lake trout and Lake Huron walleye are the only locations evaluated that have lower power levels for mercury than for PCB concentrations.

Locations can also be compared by contrasting their power levels for a consistent trend level. For a trend of 10 percent change per year, four location-species have power levels greater than 80 percent for all three compounds evaluated, specifically Lake Michigan lake trout, Kalamazoo River carp, Gull Lake largemouth bass, and Lake Huron walleye. If trends in PCB concentrations could be evaluated using either wet-weight or lipid-standardized values, then only Lake Erie and Muskegon River carp need additional sampling in order to increase the power levels to 80 percent or higher for a 10 percent per year trend in PCB concentrations. PCB power levels ranged from 61 to 77 percent for these location-species. Detroit River and St. Joseph River carp have lower power levels for wet-weight PCB concentrations (67 percent for both) but greater than 80 percent power for lipid-standardized concentrations (87 and 91 percent, respectively). Similarly, Gun Lake largemouth bass and Lake Gogebic walleye have low power levels for lipid-standardized PCB concentrations (76 and 73 percent, respectively) but high power levels for wet-weight (86 and 94 percent, respectively). Because wet weight
concentrations are preferred for evaluating trends, Detroit River and St. Joseph River, in addition to Lake Erie and Muskegon River, need additional sampling to achieve 80 percent or higher power for a 10 percent per year trend. Appendix D provides tables and plots of the power levels for the full range of detectable trends for each location-species.

Several of the data sets had power lower than the minimum desired level of 80 percent. Many instances of low power are largely due to the problem of detecting an underlying trend that is very small. The potential adequacy of sampling in these cases must be interpreted in terms of the ultimate goals of the FCMP, which is to assess efficacy of regulation. Very, very slow declines of PTS are likely to be viewed as a failure of regulation. For example, a 1 percent per year decline of concentrations would require almost 70 years for concentrations to fall by half. Failure to detect such a low rate of decline is, therefore, inconsequential to the FCMP. In these cases, increased sampling to improve the statistical power would not be cost-effective. Power can be improved by a higher density of samples, e.g., more samples per year, or by sampling over a longer time period. Because short-term trends of PTS in fish are unreliable indicators of long-term trends (see discussions in Sections 1 and 4.14), Exponent believes that it would be better to increase the time period as opposed to the density per year for those data sets having power below 80 percent. Given all these constraints, the current sampling density of 10 fish per year from each site seems appropriate.
4 Options and Recommendations

This section considers various options for revising the MDEQ FCMP. Each question pertains to a specific aspect of the FCMP and was developed in conjunction with a review of the literature, surveys of other FCMPs, and consultation with MDEQ. The responses are based on literature review and data analysis presented in previous sections. In some cases, additional analysis and examples are provided here. Each response concludes with Exponent’s recommendation for the MDEQ FCMP.

At MDEQ’s request, Exponent used an example data set to demonstrate how to estimate trends incorporating these recommendations. The example data set included mercury and PCB concentrations for walleye from South Manistique Lake and Upper Peninsula Inland lakes. The results of this analysis are provided in Appendix E.

4.1 Should MDEQ adopt randomized site selection for some monitoring?

With a few exceptions, most FCMPs, including all three of MDEQ’s FCMP elements, sample fish at fixed stations. Fixed stations offer some logistical and statistical advantages. For analyses of temporal trends, fixed stations increase statistical power by reducing variance due to spatial heterogeneity. Fixed stations also are generally simpler in terms of logistics—no complicated analyses need be conducted to determine sampling location and sampling becomes easier as samplers become familiar with a site. In addition, there are some trends analyses (e.g., pre- and post-remediation sampling, trends of chemicals in a hot spot, identification of hot spots) for which fixed stations are necessary. Sampling for fish consumption advisories is also typically geared to problematic areas, so contaminant data gathered for advisories and used secondarily for trend analyses are also generally collected at fixed stations.

However, sampling at fixed stations reduces the applicability of the results at those fixed stations. For example, if the source of contamination at a site is primarily a localized historical source, then the declines of PTS in fish at that site will be a function of site-specific factors (assuming the site is large and/or the fish are relatively immobile). Observed trends from this site will be less applicable to other aquatic systems. Sampling at fixed stations can also be problematic if the response of local fish to changes in exposure is somehow unique or abnormal. Thus, for example, long-term declines of PCBs in Lake Michigan lake trout varied across Lake Michigan as a result of localized behavior (Madenjian et al. 1999). The prey selected by trout at one site apparently shifted to a more contaminated prey, which may have caused a temporary cessation of decline noted in the data from that fixed station (58 Fed. Reg. 20,806–20,809). Prey selection remained more constant at another site, and PCB concentrations continued to decline. Similarly, the rate of depuration of PTS should be a site-specific function of the limnology of the aquatic system and local loading history. Thus, the declines of PTS should vary across different systems and, likely, at different locations in the same aquatic system. Fixed stations also become problematic if the target species become locally rare or are
extirpated for some reason. Lastly, migration of fish from areas with different ambient levels of PTS complicates interpretation of data from fixed stations.

Random site selection offers solutions to each of the problems noted above. For example, trends observed in fish concentrations from randomly selected sites should be indicative of overall rates of decline. These trends should be applicable over the geographic and limnologic range of aquatic systems sampled. This sort of trends analysis is especially warranted in situations where the primary source of chemicals is a widely distributed source, such as atmospheric loading. Similarly, trends observed in fish from randomly selected sites are not unduly affected by unique conditions at any one site, and intermittent disappearance of fish at a small number of randomly selected sites is not as problematic as loss of data from a long-term fixed site. On the other hand, randomized site selection will likely reduce statistical power by including the variability contained across sites. In the case of the situation described above for Lake Michigan lake trout, randomized site selection would likely have averaged out the response of the two sites and obscured the information contained in both sites. Randomized site selection also addresses potential concerns about the effects of fish migration among fixed sites. With randomized site selection, different sites will be sampled each year. For these samples, potential effects of migration on temporal trends at any one site are no longer relevant. Data from the randomized sampling sites can be used to detect spatial trends. However, these spatial trends are likely to apply over large areas, corresponding to different levels of atmospheric deposition and/or different alkalinity regions of the state. Such areas are generally larger than the home ranges of most of the fish.

As the brief discussion above suggests, many factors should be considered when deciding whether to sample at fixed vs. randomly selected locations. However, the primary determinant appears to be one of geographic scale. Fixed sampling sites appear to be better for answering questions about trends over small spatial scales. This sampling is best for determining spatial and temporal trends that will be applicable to small areas and for chemical trends arising from localized sources. Randomized site selection appears more suited to trends analyses concerned with large spatial scales. This sampling would be better for questions concerning trends across many lakes or large parts of the state and for widely dispersed pollution sources.

A review of the Michigan FCMP suggests that it intends to address both local and widespread contamination, arising from both local and dispersed sources. Much of the sampling of edible tissue for fish consumption advisories and before-after assessments of remediation concern local sources and/or localized concentrations. Trends analyses focused on this type of analysis are best addressed with sampling at fixed sites. Advisories based on mercury, on the other hand, are primarily based on dispersed sources and limnological factors such as lake alkalinity, that vary across geographic regions. Thus, questions addressing the efficacy of ongoing regulation of mercury and long-term trends of mercury contamination in fish are better addressed by randomized site selection.

Several FCMPs, including the Indiana program and EPA’s national study, have begun to use randomized sampling designs, and randomized sampling designs were recommended for

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2 Sources in this case refers to both historical sources—localized sediment hot spots—as well as active sources such as discharge pipes and contaminated terrestrial sites.
Minnesota’s FCMP (Shierholz et al. 1996). Generally, these sampling designs are stratified in
certain ways to achieve a balance between logistics and the statistical demands for randomness.
Thus, for example, EPA sampling of lakes allocates a specific number of sites within lake
categories, and Indiana’s sampling stratifies its samples by watershed and drainage area. The
stratified sample recommended by Shierholz et al. (1996) for Minnesota includes a number of
strata (geography and lake size) that made sure samples were routinely taken in large,
recreationally important lakes and that samples were spread across the state.

Exponent recommends a combination of fixed stations and randomized design, depending on
the chemical of concern and the habitat. Based on the above analyses, a randomized site design
would seem better adapted to gauging trends of mercury in inland lakes and rivers.
Atmospheric loading is likely to be the primary source of mercury to these systems, and
randomized design would seem better adapted to determining trends across many inland ponds,
lakes, and streams. In contrast, high levels of organochlorines appear to be a more localized
problem, resulting largely from past localized sources. Such locations should continue to be
monitored with fixed sites.

The delineation between localized and dispersed sources with respect to mercury and
organochlorines is admittedly simplistic. Dispersed sources are likely to be the primary sources
of both organochlorines and mercury to most of the small inland lakes. Data collected with
randomized site selection would, thus, provide information concerning ecosystem-wide declines
in these chemicals. Similarly, there are localized non-atmospheric sources of mercury to
systems such as the St. Clair and Detroit Rivers. According to the reasoning above, these would
warrant sampling at fixed stations that would obtain data applicable only to that site.

Nonetheless, the simplistic delineation has utility. Organochlorine concentrations in fish from
most inland systems of Lake Michigan are often too low to be easily measured or to be of much
regulatory concern. There are also ongoing programs, such as the Integrated Atmospheric
Deposition Network, that directly monitor trends of air concentrations of these chemicals in the
Michigan airshed. Consequently, Exponent cannot recommend that MDEQ expend resources to
track whole ecosystem declines of organochlorines by sampling these chemicals in the tissue of
fish from inland lakes and rivers. In contrast, mercury concentrations in the tissue of fish from
small, isolated lakes are generally high enough to be easily monitored, of regulatory concern,
and the target of ongoing regulation. Given this background, Exponent can recommend that
whole ecosystem levels of mercury be tracked by measuring concentrations in tissue of fish
from inland lakes.

Currently, MDEQ has 26 fixed stations. Exponent recommends that inland fixed stations that
are primarily tracking dispersed sources of mercury be reallocated to randomized sites. Those
fixed stations primarily tracking localized sources of organochlorine or mercury, or both, should
be retained as fixed stations.
4.2 In general, should variability be controlled with stratified sampling or post hoc with statistical methods?

FCMPs dedicated to assessing trends generally employ stratified sampling to reduce variability and increase statistical power. For example, the Great Lakes Fish Monitoring Program samples a limited size range of lake trout or limited age class of coho salmon. Similarly, YOY sampling programs sample a limited range of fish sizes/ages, and one element of the Indiana FCMP plans to sample a limited size of small fish. All of the FCMPs described above analyze fish composites, but sampling for some FCMPs devoted to trends analyses does not use composites. Notably, MDEQ’s whole fish sampling, which is devoted primarily to assessment of trends, analyzes whole fish, and, upon the recommendation of statisticians, Minnesota’s has stopped collecting composites in lieu of analyzing individual fish.

In contrast, some FCMPs collect and analyze chemicals in a wide range of fish ages and sizes. This sampling, unstratified with respect to fish size, is usually employed by FCMPs collecting data for fish consumption advisories, because chemical concentration and resulting advice are generally a function of fish size. The DFO has an FCMP devoted to trends analyses; it collects a wide range of fish sizes and ages. When the unstratified data are used for trends analyses, potential confounding effects of different sizes/ages are controlled with post hoc methods. DFO, for example, generally focuses its trends analyses on a subset of the collected data (e.g., 4-year-old fish). Alternately, the entire range of fish ages can be analyzed with statistical techniques such as multiple regression analyses (Borgmann and Whittle 1991b), ANCOVA (e.g., see Ward and Newman [1999]), and ANOVA (e.g., Stow 1995a).

The examples of stratified vs. unstratified sampling focused upon size/age effects because this source of variability is widely controlled, by either stratified sampling or post hoc methods, in many trend analyses. However, myriad other factors affect bioaccumulation of PTS in fish (see Section 2), and sampling could conceivably be stratified or unstratified with respect to each of these factors. Thus, fish sampling is neither totally stratified nor totally unstratified with respect to all important factors. Many FCMPs stratify sampling based on fish size and sampling location. Very few stratify sampling by gender or lipid concentrations, and few stratify by age. If the latter factors are considered at all, they are considered in post hoc statistical analyses.

In addition, the pros and cons of stratified sampling vs. unstratified sampling are similar to those discussed with respect to fixed versus randomized sampling locations. Taking fish size as an example, focusing sampling upon a small size range of fish should reduce variability, and enhance statistical power, in those cases in which size significantly affects chemical concentrations in fish tissue. However, while stratification increases the ability to detect trends for the size range or population that the fitted trend applies to, it reduces the applicability of those trends to other size ranges. This may not be problematic in the case of size. MDEQ tends to sample larger adult fish, and it seems unlikely that significant effects would be seen in one size without being observed in monitored adult fish.

As with the question of randomized versus fixed sampling sites—which really concerns whether sampling location should be stratified or not—the most appropriate method will depend on the goals and logistics of the FCMP. With respect to goals, the FCMP must decide whether precision is more important than applicability. Sampling a small range of sizes or a single
gender may improve the ability to see trends, but it reduces the population to which that trend applies. One might be very sure about trends in that particular size of that species, but inferences to other sizes become tenuous.

Another important issue is the complexity of subsequent analyses and results. Results of sophisticated statistical techniques are considerably less transparent than results of simpler techniques. It is often difficult, for example, to display the results of multivariate analyses graphically, and many viewers, understandably, are suspicious of highly manipulated data. In addition, concentration data frequently do not adhere to the underlying assumptions of ANCOVA (DeVault et al. 1986). Therefore, some trend analyses of unstratified data have used regression analyses to determine concentrations at a standardized size. Trend analyses are then conducted on these estimated concentrations (Sorensen et al. 1990), but this method greatly reduces the number of data points and the resulting power of the test.

Other important determinants are technical feasibility and cost. Stratifying sampling by fish size, sampling location, season is fairly simple and is widely employed. Stratification on the basis of limnological characteristics is also tractable, and some FCMPs stratify sampling on this basis, especially those concerned about trends in mercury concentrations. On the other hand, sampling fish on the basis of gender and age is, with notable exceptions such as spawning salmon, very difficult. It is probably impossible to stratify sampling on the basis of other important factors such as food chain dynamics, growth efficiency, lipid levels, etc. Thus, FCMPs rarely stratify by any of the latter characteristics. Cost is also paramount to this decision. Analytical costs are the primary component of total FCMP costs. Stratified sampling is generally less expensive, because variance is controlled prior to chemical analyses. Thus, in all but a few cases (fish size, limnology, sampling location), the decision to stratify or not is dictated by logistics, cost, and goals of the sampling.

4.3 Should MDEQ stratify sampling by fish size or consider size in post-sampling statistical analyses?

As described above, most FCMPs devoted to trend analyses stratify sampling on the basis of fish size. The pros and cons of stratified sampling by size are discussed above. The primary problem with stratifying by size is the difficulty of finding fish of the appropriate size. As with any stratified sampling, there is also some loss of information because the results pertain only to the size sampled. The advantages of unstratified sampling are ease of sampling and more complete information about trends across fish sizes.

As demonstrated by MDEQ’s sampling of lake trout in Traverse Bay in Lake Michigan, finding the targeted fish size is sometimes difficult at some sites. In this case, samplers took the closest size range available. The resulting size differences that occur from sample to sample sometimes compromise resulting trends analyses. For example, the data from whole lake trout from Traverse Bay between 1990 and 1998 showed highly significant increasing trends, in PCBs (8.5 percent per year), mercury (12 percent per year), and sum of DDT and its breakdown products (5 percent per year). However, the length and age of the fish also increased during this period, so these trends in contaminants are potentially attributable to changes in fish length and age.
This problem can potentially be addressed by adding length into a multiple regression, but the results of these analyses are difficult to interpret because length and year are themselves correlated. In addition, there is generally not a sufficient range of sizes in any one year to determine if the statistically-derived coefficient is reasonable.

Given this background, Exponent recommends that MDEQ switch to selecting a wider range of fish sizes each year and accounting for size with statistical techniques. Current attempts to collect a targeted size range often end up collecting a range of fish sizes anyway. This change in methods should make sampling easier and will help resolve the problems addressed above. This recommendation is also cost effective because it allows MDEQ to continue to rely on other agencies to collect fish.

This recommendation pertains to those sampling sites where MDEQ has trouble collecting fish of the targeted size. There are apparently other sites at which the targeted size is always or almost always available. To retain the benefits of stratified sampling, Exponent recommends that MDEQ continue to stratify sampling by size at these non-problematic sites.

4.4 Should MDEQ consider gender in its sampling or statistical analyses?

Gender has significant effects on PTS concentrations in some species under some circumstances. Thus, stratified sampling by gender of walleye has been recommended (Madenjian et al. 1998b) to improve statistical power of trend analyses, and at least one FCMP currently targets specific genders for different analyses (HELCOM 2002). MDEQ does not control for fish gender in its trends analyses. This is unlikely to be problematic for some of the species monitored by MDEQ, because gender does not appear to have significant effects on PTS concentrations in coho salmon (Williams et al. 1989) or lake trout (Figure 4-1).

However, based on MDEQ and MOE data from multiple locations, male walleye tend to have 50 percent or more PCBs than similarly-sized female walleye (Figure 4-2). Walleye gender may also affect mercury concentrations (Figure 4-2). Similarly, inspection of MDEQ data suggests that male carp tend to have higher concentrations of PCBs and mercury than do female carp.

This hypothesis was tested with whole carp and walleye data from MDEQ and walleye fillet data from MOE. In all three cases, the data from multiple sites were analyzed in a mixed regression model using location and gender as factors, and length as a continuous variable. These variables were regressed against log transformed concentrations of mercury and PCBs. Taking gender into account significantly improves the fit of the models for both PCBs and mercury in both fish, after accounting for fish length and location. This effect is reasonably large in walleye. Male walleye are estimated to have approximately 60 percent more PCBs and mercury than female walleye. Male carp are estimated to have approximately 28 to 45 percent more mercury and PCBs, respectively, than female carp.

MDEQ does not now control for fish gender and does not routinely assess gender. However, given the potential impact of gender on bioaccumulation, Exponent recommends that gender be
assayed in the future and considered in future trends analyses. We believe it is not practical to assess gender in the field, so consideration of gender is recommended for post-sampling statistical analyses.

In making this recommendation, it is recognized that the gender differences described above pertain to fish of the same size, but data were not sufficient to test whether there was a gender effect for fish of the same age. In their analysis, Johnston et al. (2002) found that gender-related differences in PCB and DDE concentrations varied depending on which covariate—age, body mass, or length—was used. In the case of largemouth bass, gender differences noted for fish of the same size virtually disappeared when male and female fish of the same age were compared (Lange et al. 1994). Thus, the issue of gender differences should be revisited once MDEQ gathers more fish age data, as is recommended elsewhere in this report, to see if collection of gender data provides important information once fish age is accounted for.

4.5 Should MDEQ monitor YOY fish?

Compared to FCMPs using older fish, YOY FCMPs are thought to monitor PTS concentrations over smaller temporal and spatial scales. YOY fish are generally less mobile than adult fish, so they are thought to better reflect local PTS concentrations. Similarly, concentrations of PTS in very young fish are also assumed to reflect current conditions, whereas older, larger fish are assumed to integrate the effects of ecosystem concentrations over many years. YOY sampling would, therefore, have advantages for trend analyses concerning hot spots/localized contamination or recent changes resulting from a new source or remediation. YOY fish offer FCMPs other advantages and disadvantages. For example, YOY fish are generally numerous and small, making compositing many small fish easier than compositing large fish. YOY fish also provide information on trends of chemicals at low trophic levels, which may be important in assessing food chain effects. On the other hand, concentrations of PTS in small fish are often too low to be detected with conventional detection limits. At most sites on the Great Lakes, for example, only PCBs and DDT are currently detectable in YOY spottail shiners. Even these chemicals are becoming progressively harder to detect as concentrations continue to decline over time. (The problem with undetected data applies to the use of these data for trends analyses. This is likely not a problem if the intent of monitoring is assessment of risk or status, as detection limits for most chemicals are generally below concentrations that represent a problem.)

Because YOY fish are small and generally available in large numbers, FCMPs using YOY often employ a very high level of compositing. When sufficient fish are available for example, organochlorine concentrations in New York State Department of Environmental Conservation’s YOY fish program are based on a total of 105 fish (7 composite samples of 15 fish). Data for MOE’s spottail shiner collections are based on 60 to 100 fish (6 to 10 composite samples of 10 fish). Because of this high level of compositing, YOY programs are notable in having low levels of variability within a single year (Figure 4-3). In contrast, the variability of mean PTS concentrations over many years is as high or higher than that of other fish, such as lake trout (Figure 4-4). This occurs even though the precision of the yearly samples for the lake trout appears to be lower than that for the spottail shiners (Figure 4-3 vs. Figure 4-4).
Both types of variability—the intra-year and inter-year variability—will affect statistical power, but they may be the results of different phenomena and, thus, controllable by different methods. Although this is simplistic, it could be assumed that most of the intra-year variance results from endogenous factors such as differences in fish size, age, and lipid content. As demonstrated by the spottail shiners, the intra-year variance from these factors can be minimized by stratified sampling (by age and size), compositing, and replication. On the other hand, inter-year variability may be dominated by exogenous factors—food chain dynamics, differential growth rates between years, weather effects, etc. This year-to-year variation, as demonstrated by the spottail shiners, cannot be eliminated through stratified sampling or compositing and replication. (By analogy, a similar dichotomy could be proposed for intra-site and inter-site variability, with respect to spatial trends.)

Given this proposed dichotomy between intra-year and inter-year variability, it could be useful to consider YOY and other species in terms of both intra-year and inter-year variability. Thus, the inter-year and intra-year variability were estimated for various fish species and sampling programs. Intra-year variability of a sampling program was defined as the average of the standard errors for each year. To allow comparison between different species, locations, and sampling events, the average standard error was standardized by the mean value.

Inter-year variability was defined as the standard deviation of yearly means after the long-term trend was removed. As with the intra-year variability, this index of inter-year variability was standardized by the mean. Standardization allows comparison among different samples of fish/locations because the absolute value of the inter-year variation, as defined above, will depend on the magnitude of the mean value. The process of detrending and resulting data are depicted in Figures 4-3 and 4-4.

Some portion of inter-year variance is a result of inevitable imprecision in sampling associated with intra-year variability of the sample. If inter-year variability were caused solely by sampling imprecision, then the expected inter-year variance could be estimated from the central limit theorem. That is, in the absence of factors causing real differences in concentrations across years, the standard deviation of the yearly averages, after the long-term trend is removed, would be approximately equal to the standard deviation of all measurements divided by the square root of N. The latter value, N, is the average number of samples taken during a single year. This inter-year variability resulting from sampling imprecision is approximately equal to the intra-year variability factor as defined above.

Thus, the portion of inter-year variability attributable to intra-year variability can be defined as equal to, on average, the variability resulting from sampling imprecision. This baseline value should, on average, be about equal to the intra-year variability, as defined above. An index of true inter-year variability—excluding that component caused by the sampling imprecision that is a result of intra-year variability—can now be defined as the difference between the observed inter-year variability and the baseline inter-year variability (the average intra-year variability).

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3 First, the long-term trend is estimated with least-squares regression of ln concentration on time. Then, this equation is used to estimate concentrations and variability of data if there had been no trend. Thus, for example, if the long-term trend is 7 percent decline per year, observed data collected in the first, second, third, and fourth year would be divided by 1.0, 0.93, .86, and .80, respectively. These are equal to 0.93 raised to the zero, first, second, and third power respectively.
This is the estimated extra variability in the data caused by real year to year differences in concentrations as opposed to that attributed to sampling error. Subtracting the baseline inter-year variability allows comparison across samples of differing intra-year precision.

The intra-year variability and true inter-year variability were estimated as described above for a number of recent sampling events for which critical data (means, standard deviations, N) were available. Sampling events considered were spottail shiner sampling of the Niagara River and other Great Lakes sites, sampling of Great Lakes lake trout and walleye by both DFO and GLFMP, sampling of Lake Michigan coho salmon by GLFMP and MDEQ, and MDEQ sampling of carp. The resulting intra-year and true inter-year estimates for each of the samples are presented in Figure 4-5. What is clear from this figure is something that is apparent, in a non-quantitative fashion, from visual inspection of the original and detrended data (Figures 4-3 and 4-4). Specifically, some biomonitors/samples, notably the spottail shiners, are less stable from year to year than others. Notably, while the chemical concentrations in YOY and coho salmon can be estimated with a high degree of precision for a single year, this precision does not carry over to estimates for year to year. On the other hand, other species, such as carp, lake trout, and walleye, tend to be less variable from year to year, even though intra-year variability of the sampling can be high.

The analysis of inter-year vs. intra-year variability suggests that concentrations of PTS in YOY fish are less stable, from year to year, than those in larger fish. This observation can be explained based on an understanding of the mechanisms of bioaccumulation. Total bioaccumulation is likely to be a function of size, age, growth efficiency, and prey type. Each of these factors can change appreciably from year to year, and this effect would be especially pronounced in very young fish. In addition, the high year to year variability of PTS concentrations in YOY fish may also reflect variability of chemical concentrations over short-periods.

MDEQ periodically collects data on YOY perch from a number of sites. As with YOY collections for other species, these samples suggest that most chemicals cannot be detected in YOY perch at most sites. Although mercury and PCBs were detected in 100 percent and 71 percent of samples, respectively, most other chemicals (DDT, DDE, octachlorostyrene, dieldrin, and hexachlorobenzene) were rarely or never detected in YOY perch.

Given this background, continued sampling of YOY fish cannot be recommended at this time for use in trends analyses. Previous experience with small, young fish suggests that they have high inter-year variability that reduces the power of subsequent trend analyses. In addition, experience by others and MDEQ suggests that concentrations of many chemicals cannot be detected in very small fish from many sites. It should be noted that this recommendation pertains only to YOY sampling with respect to trend analyses. YOY sampling is appropriate for estimation of risks to piscivorous wildlife. For this use, the high inter-year variability is relevant because it probably reflects real changes in risk. Similarly, the inability to detect chemicals is not generally problematic with respect to risk assessment, because risks at these low concentrations are usually tolerable.
4.6 Should hydrophobic PTS concentrations be lipid-normalized?

MDEQ performs some analyses, especially with the caged fish portion of the program, on lipid-normalized data, using the ratio method. For other analyses, MDEQ examines concentration as wet weight or on a lipid basis. Based on the best available information, lipid normalization appears to be justified when bioconcentration is the primary mechanism of bioaccumulation. This would pertain to those chemicals that do not biomagnify or those situations, such as with the caged fish, where exposure across the gills is the primary exposure. Thus, MDEQ’s current methods for caged fish are appropriate, assuming that the caged fish are not consuming significant amounts of food during the 28 days they are in the cages.

However, lipid normalization, using the ratio method, does not appear warranted under most situations for biomagnifying chemicals for naturally caught fish. While concentrations of lipids may affect excretion and absorption of biomagnifying hydrophobic chemicals, the available theoretical and empirical evidence indicates that lipids play only a minor to moderate role in total bioaccumulation of hydrophobic PTS. Lipids should be considered as a potential covariate in multivariate statistical analyses with the following caveats. First, lipid concentrations tend to co-vary with other factors affecting bioaccumulation (e.g., size, trophic level, growth efficiency). Analysts should recognize that these other factors could be causal. Statistically significant relationships between lipids and PTS concentrations should be checked for legitimacy. Second, analysts should be wary of data sets, and resulting trend analyses, in which lipids vary dramatically across time or space. Lipid levels in fish are a complex function of growth efficiency and food type. Both of these also may profoundly affect bioaccumulation. Very different lipid levels across time and space potentially indicate very different conditions for bioaccumulation. Under these conditions, it is recommended that analysts conduct trends analyses with and without lipid levels as a covariate. But they should be wary of instances where the two methods yield different conclusions.

MDEQ’s current methods are very close to those recommended. In cases in which lipid concentration has changed over time, MDEQ does analyses with and without lipid concentrations. The latter analyses use lipid-normalized data. Based on the information presented in Section 2, however, it can be concluded that lipid normalization probably overcompensates for the likely moderate to small effects of lipids. Thus, it is recommended that MDEQ attempt another statistical technique, such as ANCOVA or multiple regression, to control for lipids.

4.7 Should analyses be controlling for age rather than, or in addition to, length/weight?

While many analysts and FCMPs believe that fish age, rather than fish size, is critical to bioaccumulation, only a few, notably DFO, actually assess fish age on a regular basis. Also, some FCMPs, such as YOY and fall run coho sampling, rely on fish behavior and size as a way to focus on a limited age range. Because aging fish in the field is generally impractical, most FCMPs and trends analyses rely on fish size as the primary covariate. Similarly, while there are
some mathematical models and empirical evidence that suggest that age effects are important above and beyond size effects (e.g., Borgmann and Whittle 1991b; Stow and Carpenter 1994a; Eby et al. 1997), other mathematical models adequately predict concentrations in fish based on size alone (e.g., Campfens and Mackay 1997).

The available empirical evidence is inconclusive regarding whether age is important once fish size is considered. Borgmann and Whittle (1991b) found evidence of age effects after effects of size were controlled. Eby et al.’s (1997) analysis suggests that greatly slowed growth of bloater caused higher than expected concentrations. However, the evidence for this effect is based on predictions of a model that produces age-specific effects, not independent empirical verification. As described in Section 2, age contributes significantly to the variability of PCB concentrations in size-selected lake trout, but the overall effect is small.

Moreover, even if age is a critical determinant of bioaccumulation, two caveats should be remembered. First, age is a secondary indicator of the causal mechanism, which is growth efficiency. Age is not always a good indicator of growth efficiency. In the early years of restocking of lake trout in Lake Ontario, the trout had access to abundant prey. Amidst this bounty, the trout grew quickly but not necessarily efficiently (Borgmann and Whittle 1992a). Second, age-controlled fish may provide a better, though still imperfect, estimate of underlying trends in the ecosystem. The relative accuracy of age-controlled samples vs. size-controlled samples was estimated with the predictions of the model of Eby et al. (1997) for bloater under fast and slow growth conditions. According to this model, significant decreases in the growth rates would produce higher concentrations at any particular size, but lower concentrations for any age (Figure 4-6).

This predicted relationship was applied to the following scenario to illustrate the benefits of assaying fish age. PCBs were assumed to be declining at a steady 7 percent per year over a 10-year period, and this decline was being tracked by monitoring PCB concentrations in bloater every year. Midway in the ten-year period, growth rates for bloater slowed dramatically. This decrease in growth rate caused changes in PCB concentrations, as a function of fish size and fish age, that are predicted by the model of Eby et al. (1997) and depicted in Figure 4-6.

The resulting temporal trends of bloater PCBs were then assessed for a sampling program focused on a constant size (143 g) vs. one focused on three-year-old fish (Figure 4-7). Compared to the underlying ecosystem trend of 7 percent decline per year, best-fit exponential declines are about 3.3 and 9.5 percent per year for the same size and same age sampling, respectively. Although the same age data do track the “real” trend more closely, data from neither sampling protocol accurately tracks the “real” trend. Moreover, it should be noted that this analysis is based on predictions of a model that produces age effects in a situation in which growth rate is drastically decreased.

In summary, it is uncertain whether fish age has significant effects on bioaccumulation beyond those associated with fish size. In view of the difficulty of aging fish in the field, it cannot be recommended that stratified sampling be conducted for certain age-classes. On the other hand, there is evidence, mostly based on modeling, that age may significantly affect bioaccumulation beyond those effects associated with fish size. If this hypothesis is correct, differences in fish age over time and space could significantly affect observed trends (Borgmann and Whittle
1991a; Eby et al. 1997; Whittle 2001, pers. comm.). Given the feasibility of assessing fish age in the lab, it is prudent to also assay age for analyzed fish data. This information could then be considered on a post hoc basis when trend analyses are conducted and evaluated. Therefore, Exponent recommends that fish age be assessed in the lab and be considered as a covariate in subsequent trends analyses.

4.8 Should limnological factors such as pH, temperature, oxygen concentration, and productivity be considered as part of the trend-monitoring program design and/or post hoc statistics?

As discussed in Section 2, limnological factors including lake chemical characteristics such as pH and DOC and lake physical characteristics such as surface area and watershed size are often correlated with fish mercury concentration. Data on these characteristics are generally easy to acquire and have the potential to assist in post-sampling analysis of fish mercury concentration data. For example, trends analysts can stratify the data based on chemical or physical characteristics in order to identify trends in subsets of lakes, or these factors could be covariates in multiple regressions.

The primary lake chemical characteristics that influence fish mercury concentration are pH and DOC. Several studies also evaluated and found significant relationships with alkalinity, conductivity, hardness, total nitrogen, total phosphorus, and various ions, some of which are measures of productivity. Some of these parameters could be easily measured with field equipment while sampling for fish. For example, the Hydrolab® will measure pH, conductivity, temperature, dissolved oxygen, redox potential, and salinity. Of these, pH and conductivity would be the most useful.

If possible, water samples could also be collected separately and submitted to a laboratory for DOC and total phosphorus or chlorophyll a analysis. Total phosphorus and chlorophyll a concentrations provide an indication of phytoplankton standing stock. These chemical data may already be collected for a state water quality monitoring program that coincides with lakes monitored for fish contamination.

Several lake physical characteristics are generally found to correlate with fish mercury concentration and are recommended for consideration in subsequent trend analyses concerning mercury. These data should be readily available from USGS or state databases. These characteristics are surface area of lake, type of lake (i.e., seepage, drainage, or impoundment), presence/absence of annual anoxic hypolimnion, watershed area, and land use in watershed (i.e., percent wetlands, forest, and farmland).

Exponent recommends that MDEQ collect data on basic water chemistry, notably pH and conductivity. These data are easily obtained at nominal cost when sampling fish. In contrast, unless it can be coordinated with a water quality program, Exponent cannot recommend collecting water samples for chlorophyll a, total phosphorus, and DOC. These first two parameters are known to vary considerably over the season and with depth (Wetzel 1983),
limiting the usefulness of a small number of grab samples taken at different times of the year. In contrast, DOC concentrations are apparently quite stable over time and depth (Wetzel 1983), so a small number of grab samples at different times of the year might accurately characterize relative concentrations across lakes. However, DOC generally has a small effect on mercury bioaccumulation, thus, the cost-effectiveness of DOC data are questionable. If these water chemistry data, and data on physical parameters for each lake or system are easily available, then Exponent recommends that they be considered in statistical analyses. Historical data on pH and alkalinity should be used cautiously for lake systems potentially affected by acidification.

This recommendation applies to trends analyses for mercury concentrations in fish from inland lakes, but not from inland rivers or Great Lakes systems. This recommendation also does not apply to trends of hydrophobic PTS because these limnological factors are not known to affect their concentrations.

4.9 Should sampling be stratified by season?

Seasonal effects have been noted for bioaccumulation of some contaminants in some fish, notably mercury (Ward and Newman 1999). Given the potential influence of seasonal factors on bioaccumulation, it may also be important to consider the impact of season on observed concentrations. Summer collection would be ideal to avoid spikes in fish mercury concentration during spring and fall that have been noted in the literature. However, limiting sampling to only summer could be burdensome to the samplers.

Except in the case of sampling fall run coho and chinook salmon, MDEQ does not now focus sampling on any particular season. Exponent recommends that MDEQ continue to sample throughout the year. Limiting sampling to a particular season of the year could constrain sampling. MDEQ should determine whether time of sampling significantly affects concentrations with post-sampling statistical analyses. Rather than designing a special study, Exponent recommends that such analyses be conducted on data collected previously and with the current sampling design.

4.10 Should abiotic concentrations or external loading be monitored along with fish data?

Given all the confounding factors affecting bioaccumulation, several analysts have conducted multimedia comparisons to lend credence to trends observed in biota. Thus, for example, analysts have compared PTS trends in fish with PTS trends in water (DeVault et al. 1996), trends in sediments (Rasmussen et al. 1990; Rowan and Rasmussen 1992), trends in overlying atmosphere (Simcik et al. 2000), and trends in gull eggs, fish, water, sediments, suspended sediments, and overlying air (Smith 2000; Offenberg and Baker 2000). When trends across media are concurrent, multi-media comparisons support the legitimacy of trends found in all media. When trends across media are divergent (DeVault et al. 1996), however, it suggests the importance of confounding factors. Consequently, multimedia comparisons are a robust method to determine if trends observed in biota are real.
Exponent cannot recommend, however, that MDEQ’s FCMP also collect concurrent abiotic data on air or water. The sampling methods, necessary equipment, and analytical methods used to assay PTS concentrations in air and water are very different from those used to sample fish. In addition, given the very low ambient concentrations, the methods and sampling equipment for PTS in air and water are consistent with state-of-the-art academic research. It involves equipment that few people possess and methods that few people have mastered and it is likely to be cost-prohibitive. In addition, some of these data are already being collected by other investigators.

These attributes do not, however, apply to sediments. Concentrations of most PTS chemicals can often be measured in surficial sediments and the methods of sampling are relatively easy. Thus, data on surficial sediments could potentially be obtained at minimal additional cost. Other FCMPs, such as Indiana’s trend program, collect sediment samples along with fish samples. Consequently, Exponent recommends that MDEQ begin to collect a surficial sediment sample at each fish collection station if this can easily be done.

4.11 Should food chain exposure be monitored directly or indirectly?

Changes in a food chain can have very significant effects on PTS concentrations in fish. Consequently, identification of changes in the underlying food chain is a critical factor in understanding trends of PTS in fish across time and space. Several methods have been used in the past to monitor food chain effects, including direct measures such as assessing gut contents and measuring concentrations in prey fish (Madenjian et al. 1999). Indirect methods for identifying food web changes include correlation between changes in PTS concentrations and prey stocks dynamics (Borgmann and Whittle 1991a,b; Smith 1995b) or food chain length (Rasmussen et al. 1990, Rowan and Rasmussen 1992), and changes in delta nitrogen and delta carbon signature (Whittle et al. 2000). Analysts have also considered multichemical, multifish, multimedia, and multimethod approaches. The method compares the behavior of different chemicals in the same organisms over time or space (Smith 1995a,b,c). The multifish method—comparisons of trends across different fish species (e.g., Stow et al. 1995; Lamon et al. 1999)—also represents a way to deduce potential food chain effects that affect different species. The benefits of multifish comparisons are persuasively described in Lamon et al. (1999). Comparisons of trends across media are described above. Lastly, some analysts compare empirically derived trends versus those derived from theory or modeling (e.g., Endicott et al. 1992a; Gobas et al. 1995; Smith 2000).

Each of these methods offers certain advantages. Simultaneously monitoring gut contents and concentrations in prey fish is probably the most direct and convincing method. This method may also provide a quantitative correction to trend analyses. However, it is also quite labor and data intensive. Isotopes of nitrogen and carbon have shown utility in explaining differences in

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4 During the preparation of this document, Exponent was informed by MDEQ that it has recently begun to monitor PTS concentrations in sediment cores from inland lakes. In addition, MDEQ has also begun to monitor contaminant concentrations in blood and feathers of bald eagles, PTS concentrations in herring gull eggs, and contaminant concentrations in water.
bioaccumulation over both time and space (e.g., see Whittle et al. 2000). However, delta nitrogen is not always successful in clearly identifying food web linkages (Kucklick and Baker 1998). In addition, delta nitrogen is only able to detect changes in the trophic level of the prey. However, there can be significant differences in PTS concentrations among forage species at the same trophic level and similarity in PTS concentrations in prey at different trophic levels (Madenjian et al. 1999). Consequently, delta nitrogen is an imperfect indicator of PTS exposure via the food chain.

Multifish comparisons are useful but potentially limited, because many species that are monitored are eating the same prey and, thus, may respond to the same prey dynamics. For example, short-term changes in PCBs in both coho and lake trout correlated with dynamics of alewife, the primary prey of both species (Smith 1995b). Multichemical analyses are based on a potentially problematic assumption that external source dynamics are different for different PTS (Smith 1995a,c). On the other hand, multichemical comparisons are very inexpensive because the data are readily available, and this method has shown success in identifying food chain effects (Smith 1995b,c). Multimethod comparisons are useful but have not been widely adopted.

For biomagnifying substances, differences in the food chain over time and space are potentially the most important confounding effect not controlled by current sampling or considered in post hoc analyses. As discussed elsewhere, there is considerable speculation that changes in the food chain have significantly affected both long- and short-term trends. Consequently, MDEQ is encouraged to make more use of multichemical analyses. Currently, MDEQ will compare trends of mercury and PCBs, for example, but these multichemical comparisons should also include DDT and other hydrophobic compounds. While there are potential problems with the underlying assumption of multichemical comparisons, concurrent short-term trends among chemicals in the same biota often present evidence that the cause is not external loading. This is especially true of large aquatic systems whose internal chemical inventories are inertial. Moreover, the data are readily available so the cost of this information is minimal.

Exponent recommends that MDEQ continue to employ multifish comparisons. Currently, MDEQ will plot trends of the same chemical in different fish species. This provides valuable information at minimal cost.

Exponent also recommends that MDEQ begin to monitor delta nitrogen and delta carbon or archive tissue so that these isotopes can be analyzed to investigate potential effects of food chain disruption. The isotopes have generally been used as qualitative indicators of food chain length and position, but they could potentially be used as quantitative variables in multivariate analyses of trends. Once sufficient isotope data are collected, Exponent recommends that MDEQ investigate how these data can be most effectively used. As with multi-chemical analyses, however, MDEQ should proceed cautiously given the potentially limited use of the subsequent data.

Sampling of prey fish and prey fish concentrations is not recommended. MDEQ relies on other agencies for sampling, and it is uncertain whether these agencies would be willing or able to sample small prey fish. Determination of concentrations in prey fish would also dramatically increase the expenditure on analyses.
4.12 Can edible-portion samples be used to supplement whole fish trend-monitoring data?

MDEQ collects data on PTS concentrations in fillets for use in fish consumption advisories but does not routinely use these data in trend analyses. However, the fillet data represent a significant source of information that could potentially be used in conjunction with the whole fish monitoring program to better deduce trends. With respect to its potential utility, it is notable that other analysts have used MDEQ fillet data for trend analyses (e.g., see Stow et al. 1995).

There are several ways that fillet data could be used to augment the whole fish monitoring program. First, separate analyses on fillets and whole fish could be run in parallel, and trends compared between the two tissue types. Second, data from one tissue type could be converted to the other type by some sort of conversion factor, and analyses run on the combined data set. Third, trend analyses could be run with the two types of data in a mixed linear model, with tissue type used as a covariate. A fourth method, lipid normalization, is not recommended with data that will be used in trend analyses for reasons discussed in the section on lipid normalization.

In addition to the obvious differences in tissue type, PTS concentration data obtained from MDEQ fillet samples differ in two other ways from data generated with the whole fish program. First, fillet data are generally based on a wide range of fish sizes, whereas the whole fish monitoring program targets a limited size range. In addition, some fillets samples are sometimes composites of several fish, while individual whole fish are analyzed. Analyses that tried to combine these data in a single analysis would also have to deal with these issues.

The potential problems and solutions to several of these problems can be illustrated with MDEQ data for lake trout from Grand Traverse Bay. Grand Traverse Bay is one of MDEQ’s fixed whole fish sampling sites, and whole fish from it were sampled in 1990, 1992, 1995, and 1998. Lake trout fillets were also sampled in 1983, 1984, 1997, and 1998. PCB concentrations vs. length for both tissue types for fish collected in 1997 and 1998 were plotted, and log-linear best fit lines were estimated (Figure 4-8). Based on the best fit regressions, these data suggest that fillets should have about 80 percent of the PCB concentrations of whole fish. However, data from fillets and whole fish from the same fish suggest that fillets have about 65 percent of the PCB concentration found in whole fish (Figure 4-9). From this, a whole fish to fillet correction factor of 65 percent can be estimated. This value was used to convert whole fish concentrations to fillet concentrations and the resulting data set was run in a multiple regression of log PCBs vs. time and length. A similar analysis was conducted with the entire data set except that whole fish concentrations were not converted to account for tissue type, and tissue type was added into the regression analysis as a variable.

The results illustrate potential problems with the multiple regression approach. When tissue type was used as a covariate, the multiple regression estimated that tissue type was a highly significant predictor of PCB concentrations. However, the analysis estimated that whole fish

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5 Analyses were conducted on the fillet. The remaining fillet tissue was then combined with the carcass and analyzed. Whole fish concentrations were calculated based on the relative weights of the tissues.
were about 16 percent less contaminated than fillets, contrary to the real data and considerable experience (e.g., Amrhein et al. 1999). The adjusted \( R^2 \) value was 52 percent. In the regression in which whole fish concentrations were converted with the constant conversion factor prior to the regression, the adjusted \( R^2 \) value was 48 percent. In both cases, the long-term decline was insignificantly different from zero. In the third case in which separate analyses were conducted, multiple regression of log PCB concentration in fillets on year and length with the fillet data yielded no significant slope. In contrast, the whole fish data produced a significant slope of about 7 percent increase per year with the same regression.

This analysis illustrates both the promise and problems with combining data. Adding fillet data can enhance the validity of observed trends by increasing the spatial and temporal spread of the data set and the total number of observations. On the other hand, combining data from different tissue types data adds variability and potential biases that may also affect trends. Multiple regression may produce coefficients that are counter-intuitive.

After consideration of these issues, Exponent recommends that MDEQ combine data from both tissue types. To protect the information contained in previously collected whole fish data, Exponent recommends that MDEQ continue to collect whole fish at the fixed sites. These data can then be supplemented with data on edible portions collected from randomly selected sites. In the example above, the most effective strategy was probably the use of a conversion factor prior to statistical analysis. This is also the most transparent method in terms of reporting results. Conversion factors for PCBs and other species can be found in the literature (e.g., Amrhein et al. 1999), but correction factors for other hydrophobic compounds and mercury will likely have to be derived from available data. These analyses should proceed cautiously, however, to ensure that potential bias from combining tissues is not significantly affecting the observed trends.

**4.13 Should fish samples be archived?**

Some FCMPs archive their fish samples. Archived material can be useful when new analytical methods are adopted (e.g., Glassmeyer et al. 1997) and when other information is sought. Examples of the utility of archived samples are found in several papers based on tissue archived by DFO. For example, Huestis et al. (1996) reanalyzed archived extracts with new analytical methods and with the original analytical methods to determine precision across time and analytical method. Archived material was subjected to delta nitrogen analyses in several studies to determine if trends were affected by changes in trophic level (Whittle et al. 2000).

Exponent makes no recommendation concerning archival of tissue or extracts. Archiving tissue has requirements, in terms of money, space, and manpower, that could not be estimated based on our review. In addition, other agencies in the area are archiving tissue, and these tissues will be available to identify any emerging issues that affect the entire area. Thus, the benefits of MDEQ also archiving tissue are difficult to estimate.
4.14 What model for PTS decline should be assumed in temporal trend analyses?

Determination of an appropriate model for ecosystem decline is a critical but often overlooked requirement for defensible analyses of temporal trends of PTS in biota, especially when results of these analyses are used, explicitly or implicitly, to track effectiveness of pollution prevention and remediation actions. Before deciding whether rates of decline are too slow or too fast, or whether those rates of decline are significantly increasing or decreasing over time, it is necessary to have an expectation for how concentrations should decline over time. Statistically significant deviations from this expected decline produce results that can then be appropriately interpreted as significant in a policy sense.

One source for such an expected decline is the modeling literature. Mathematical models of PTS dynamics in whole ecosystems often produce predictions about the long-term decline of PTS in biota expected under different loading scenarios, including the extreme case of system depuration with no further external loading (e.g., see Endicott et al. 1990, 1992a,b; Mackay et al. 1992, 1994; Gobas et al. 1995). The predictions of these models provide information about both the shape and the likely rate of decline of PTS in aquatic biota. According to these models, declines of PTS in aquatic biota are expected to follow a first-order or pseudo-first order process because the underlying loss processes are first-order. Alternately termed exponential decline, this type of decline always traces a concave upward path when plotted on the linear Y-axis. In addition, these models generally suggest that declines of PTS in most aquatic systems will be, at most, between 5 and 20 percent per year even under the extreme case of no further external loading. With respect to PCBs, the rates are generally at the lower end of this range, between 5 and 15 percent per year.

Thus, available theory would suggest that a reasonable expectation for decline of PCBs in biota, for the extreme case of no external loading, would be a first order decline of between 5 and 15 percent per year (Figure 4-10). Models of whole system response to mercury were not reviewed in this analysis, but mercury’s long-term dynamics may be similar those of PTS in both shape and speed, with one important distinction. As a naturally-occurring compound, the exponential decline will eventually decline into an asymptote determined by background mercury concentrations.

Many trend analyses with biomonitoring data are problematic because no model of ecosystem decline is explicitly identified and, worse, the model that is implicitly used is wrong. For example, much of the early literature claiming that PTS concentrations are stabilizing is apparently based on an implicit assumption that PTS decline should follow zero order kinetics. The analyses conclude that concentrations are stabilizing based on visual inspection of the inevitable concave upward path of the data plotted on the linear Y-axis (e.g., see Environment Canada 1991). The apparent stabilization of concentrations seen on the linear Y-axis is only meaningful against an expectation that declines should follow a straight line decline in linear space. The implicit use of first order kinetics, a trivial expectation, is also found in more recent analyses (e.g., Environment Canada et al. 1998; IJC 2002).

Later “stabilization” analyses (e.g., 58 Fed. Reg. 20,806–20,809; Stow 1995b; Stow and Carpenter 1994b; Stow et al. 1995) are more robust. These analyses implicitly assume a
constant first-order decline, and the deviations from the underlying model are tested statistically as opposed to visually deduced. The underlying model of these analyses, which is again not explicitly stated, is that PTS in biota will be a smooth exponential decline over the long-term. Specifically, these analyses test whether most recent declines are as fast as those seen in the past.

However, this underlying model of constant first-order decline is inconsistent with theory and experience. The current theory of bioaccumulation indicates that bioaccumulation will be an intimate function of prey dynamics and growth efficiency. Thus, trends of PTS in biota are known to respond to a number of factors, such as food chain effects (Borgmann and Whittle 1991a,b; Smith 1995b; Madenjian et al. 1999; Whittle et al. 2000), differences in fish growth rates and growth efficiency (Stow and Carpenter 1994a; Eby et al. 1997), and weather effects (Smith 1995c; Hebert et al. 1997). These factors are known to vary over time (Stow and Carpenter 1994b; Stow et al. 1995). There is, therefore, no expectation that declines of biota PTS should be constant over time or track a smooth exponential decline over time, even in the total absence of external loading. Consequently, these later trend analyses also test an inappropriate model for PTS decline.

This background illustrates the importance of both picking a defensible model for ecosystem decline and explicitly stating what that model is so that it can be critically examined. At this point, the most defensible model for declines of PTS in biota in the absence of external loading is a bumpy exponential decline (Figures 4-10 and 4-11). For mercury, the most defensible null hypothesis is a bumpy exponential decline to some asymptote. Exponent recommends that MDEQ adopt these as default null hypotheses in its temporal trend analyses.

Adoption of the model of bumpy exponential decline has several implications for MDEQ’s FCMP. First, Exponent recommends that temporal trends routinely be graphed on the non-linear Y-axis. As discussed above, depicting exponential decline on a linear Y-axis frequently confuses viewers into believing that the rate of decline has slowed and that concentrations are approaching an asymptote. The linear Y-axis also obscured the short-term variability in more recent samples, which misleads viewers into believing that the data are more stable than they really are, and obscures important information about recent short-term trends. The non-linear Y-axis is most appropriate for depicting exponential processes and rates of decline over time. However, it is recognized that plotting temporal trends of PTS on the non-linear Y-axis is also problematic. This axis is not well understood by many non-scientists. The non-linear Y-axis also tends to de-emphasize the dramatic declines noted in most PTS concentrations over the last several decades. Thus, depiction of temporal trends of biota PTS presents a dilemma. Analysts can plot their data on the linear Y-axis with the potential of misleading many viewers. Alternately, these data can be plotted on the non-linear Y-axis with the potential of confusing viewers. The latter seems the lesser of two evils (i.e., better to leave viewers uncertain than certain of something that is untrue). Currently, MDEQ plots data from their own FCMP on a non-linear Y-axis, and data from some other programs on a linear Y-axis. Exponent recommends that MDEQ consider plotting all these data on the non-linear Y-axis.

As with all of our recommendations, Exponent believes that utility should guide MDEQ’s adoption of this recommendation. As shown in Figure 4-10, the optical illusion of stabilization only occurs after concentrations have declined about 50 percent or more. For data sets showing minimal changes over time, use of a non-linear Y-axis will actually obscure information. On
the other hand, when total declines approach 50 percent or more, plots of data on the linear Y-axis give the illusion of stabilization. Thus, a general rule of thumb would be to graph declines of 50 percent or less on a linear Y-axis and those with greater total declines on a non-linear Y-axis.

The second implication for MDEQ’s FCMP is that statistical methods applied to temporal trend analyses must be appropriate for the expected rates and shapes of decline. Thus, trend analyses should be conducted on the entire data set, unless compelling reasons can be advanced for dividing the time trend (Smith 1995b). Although the bumps in fish data do not have a well-defined period, preference should be given to long-term data sets that contain many bumps. In addition, analytical methods and visual methods that key in on short-term perturbations and outlier data should be avoided. For example, recent analyses (IJC 2002) inappropriately focus on very short-term periods of rapid decline as a de facto model of decline to which most recent declines should be compared. In addition, the double exponential and non-zero asymptote methods (Stow and Carpenter 1994b; Stow et al. 1995) are also too responsive to short term trends, and have proven unreliable predictors (Table 4-1).

Third, in those cases in which MDEQ wants to estimate the magnitude of load reductions by tracking concentrations in fish, the agency should explore more realistic models of decline than the default declines expected with zero loading. Due to a combination of new releases and redistribution of previously released compounds, external loading of all of these compounds continues. A more defensible null hypothesis for gauging regulatory effectiveness might be declines expected with no new releases but continuing loading inventories dispersed throughout the environment. In addition, the expected declines of mercury over time are not as well studied as the hydrophobic PTS. Rather than assuming that mercury will behave like hydrophobic PTS, MDEQ should define a defensible model for mercury declines in PTS.

Fourth, MDEQ should explicitly state its model for expected decline of PTS in biota. Explicit recognition would allow this hypothesis to be more critically examined and adjusted, if appropriate. With respect to the latter, it is presumed that most of the bumps in a temporal trend are related to changes in food chain (e.g., Madenjian et al. 1999; Whittle et al. 2000), and growth dynamics (e.g., see Eby et al. 1997). At some point in the future, it may be possible to account for these factors, which could produce a smoother model of decline to which observed data could be compared.

These recommendations suggest some changes to MDEQ’s current methods. Currently, concentrations for temporal trends are log normalized, which effectively assumes exponential decline. MDEQ conducts its analyses on entire data sets, which is appropriately insensitive to short-term changes. However, MDEQ has not explicitly identified how it expects PTS concentrations in fish to decline as a result of decreasing external loading. MDEQ should explicitly present this hypothesis. In addition, MDEQ should begin to plot most of their time trends data on non-linear Y-axes when declines are greater than about 50 percent.
4.15 Should MDEQ assess or control for fish movement/migration?

Fish migration can significantly impact fish concentrations (see Section 2.1.8). MDEQ has historically been concerned about the potential impacts of fish movement on detection of spatial and temporal trends in PTS concentrations. For example, many of its fixed sampling sites were specifically chosen to minimize migration.

However, the literature provided few solutions to this problem. Methods used to control migration— sampling fish near barriers to fish movement or using YOY fish—are too limiting and not recommended for other reasons, respectively. Randomized selection of sampling sites effectively avoids the problem, but Exponent does not recommend this for all of MDEQ’s sampling. Methods found in the literature to estimate recent migration—fish tagging and assaying salinity gradients in otoliths—are very time consuming and/or not applicable to freshwater systems. Differences in the delta nitrogen concentrations of fish collected from many sites were used as evidence of lack of migration between sites (Hansson et al. 1997), but the applicability of this method cannot be assessed with current data. Fish from different areas will likely have different ratios of PTS corresponding to the local conditions, suggesting that ratios of PTS in different fish might be one method to distinguish recent migrants from long-term resident fish. However, no examples of this method were found.

In summary, while recognizing the potential importance of migration, Exponent cannot recommend any changes to MDEQ’s current sampling to either control or assess fish movement/migration. Exponent is recommending that delta nitrogen and delta carbon be collected in future samples. Once sufficient data are collected, MDEQ should analyze them to determine if they could be useful in providing information on recent migration.

4.16 Should MDEQ continue to freely release its data?

MDEQ has a policy of freely releasing its data to outside interested parties. In turn, these data have been used in many published analyses by independent researchers (e.g., see Stow 1995a; Stow et al. 1995, 1997; Lamon et al. 1999; Lamon and Stow 1999). These analyses, in turn, have contributed to our understanding of factors important to bioaccumulation and analyses of trends, in general, and with specific reference to MDEQ’s data. Similarly, analyses presented in Section 2 and this chapter also benefited from data graciously supplied by FCMPs, notably DFO and MDEQ. The Canadian Wildlife Service’s gull egg sampling also has a policy of open access to its data. This open access has also spawned a rich and divergent literature that has enhanced our understanding of those data specifically and the limits of biomonitoring in general (e.g., see Smith 1995c; Stow 1995b; Hebert et al. 1997; Pekarik and Weseloh 1998).

These examples suggest that open access to data enhances the value of the data and FCMPs in general. Thus, Exponent recommends that MDEQ continue its policy of open access to its data.
4.17 Should MDEQ continue to maintain rigorous quality control for its analytical methods?

Analytical precision is essential for successfully determining trends of PTS. Several respondents to the FCMP survey stressed the importance of analytical precision and quality control. MDEQ currently has a quality control system. Exponent recommends that this system be continued.

4.18 Feasibility of Recommendations

The following section examines Exponent’s recommendations in terms of their logistical feasibility and potential effects on statistical power.

Recommendation: MDEQ should use randomized site selection for monitoring of inland lakes and rivers.

This recommendation applies specifically to monitoring geared to dispersed sources and not to hot spots. Randomized site selection represents a change from MDEQ’s current sampling for inland lakes and rivers, which are mostly based on fixed sites. This recommendation is quite feasible. The Fisheries Division of MDNR is now instituting a stratified random site selection for use in its analyses of stocking, and Exponent believes that MDEQ’s inland sampling can be tied to this sampling plan.

The intent of this recommendation is to increase the applicability of observed trends to sites across the state. It is expected that there will be reductions in statistical power because the sampling will include site-to-site variability. However, available data were not sufficient to estimate the reductions in power expected with random site selection.

Recommendation: MDEQ should target a range of sizes as opposed to a small size interval.

This recommendation applies to those sites at which MDEQ has trouble obtaining the targeted size. This represents a change in MDEQ method, which is currently devoted to a specific size range. Based on sampling of the edible portion, it is believed that finding a range of fish sizes will generally be quite feasible. Taking lake trout for example, the average length coefficient of variation for single samples of whole lake trout was about 4.5 percent, while single samples for the edible tissues averaged about 8 percent. Similarly, sampling of Lake Erie walleye whole fish had an average coefficient of variation of 4 percent, whereas the food fish samples from Lake Erie averaged about 10 percent. Thus, it appears that samplers should be able to routinely find a moderate size range of fish at each sampling.

The impact of this recommendation on power is difficult to predict. The current method of sampling a small size range is specifically intended to limit variability and increase statistical power. However, this stratified sampling only works well in species that show a strong relationship between size and PTS concentration and in those situations in which the targeted size category can be obtained consistently. MDEQ has, in the past, had trouble consistently
obtaining the desired size range, resulting in samples in which fish size varied with time, confounding analysis of temporal trends.

If a range of fish sizes is sampled, size would be considered in a multivariate analysis. Ideally, then, post-sampling statistical methods would allow variability to be characterized as effectively as stratified sampling for those species that show a strong relationship between size and PTS concentrations. Thus, the effect on statistical power is estimated to be neutral. However, because MDEQ currently has problems consistently finding the targeted size range, targeting a broader range of fish sizes and employing appropriate statistical methods would be preferable to the current approach.

**Recommendation:** MDEQ should consider gender in post-sample statistical analyses.

Fish gender is reasonably easy to assess in adult fish, and MDEQ occasionally assays gender already. This recommendation represents only a partial change for MDEQ. MDEQ does not now consider gender in its statistical analyses, but analyses conducted by Exponent suggest that this can be done easily with multiple regression. Consequently, this recommendation appears to be quite feasible.

A qualitative estimate of the amount of improvement gained by inclusion of gender in the trend evaluation was conducted for whole carp and walleye data and fillet walleye data, and for the whole carp data from all locations included in the power assessments. The same regression model was fit to each data set, with and without the gender included as a factor. In all cases for mercury and PCBs, the gender factor was significant ($p < 0.05$). Because all models with more independent variables will explain more of the variability of the data and have higher $R^2$ values, models with and without the gender factor were compared based on their adjusted $R^2$ values. Adjusted $R^2$ is an adjustment made to the model’s $R^2$ value to compensate for the number of independent variables in the model.

In all cases, the adjusted $R^2$ value was higher for the model with gender included than the model without. The largest improvement was for whole walleye mercury concentrations. Adding gender to the model increased the adjusted $R^2$ value from 0.31 to 0.48. For all of the data sets evaluated in this comparison mercury was always more improved by inclusion of gender than PCBs. The minimum improvement was for whole carp PCBs, for which adding gender increased the adjusted $R^2$ from 0.74 to only 0.75. Power is related to the amount of variability the model explains, which, in turn, is represented by $R^2$. Thus, the power to detect a trend will increase just as the adjusted $R^2$ value does.

**Recommendation:** Potential effects of lipids should be considered with qualitative comparisons or multivariate methods, not lipid normalization (e.g., dividing concentrations by lipid concentrations.)

Exponent recommended that lipid normalization (i.e., dividing the PTS concentration by the lipid concentration) not be done or be done only in qualitative paired analyses along with wet weight analyses. Instead of normalization, effects of lipids should be addressed with multivariate methods. This recommendation is considered to be feasible.
As described in Section 3, lipid-normalized values often have higher power than wet weight concentrations. However, considering lipids with multivariate methods should compensate for lipid effects as well or better than normalization. Thus, this recommendation is expected to have a neutral or positive effect on power.

**Recommendation:** MDEQ should assay fish age and consider this factor in trend analyses.

Exponent recommended that MDEQ routinely assay fish age and integrate this information into its statistical analyses. MDEQ measures fish age on some occasions, so this recommendation is quite feasible, although aging some fish species can be difficult. Consideration of fish age can also be done in subsequent trend analyses. For example, Borgmann and Whittle (1991b) conducted a multiple regression of PTS trends in lake trout with age, using time and sampling locations as independent variables.

Measuring age is expected to account for differences in growth rate, which likely causes some of the year-to-year variability seen in PTS concentrations in fish. Thus, measurement and consideration of fish age is expected to increase the power of subsequent trend analyses. Available data suggest that age effects, beyond those associated with size, are generally small; thus, consideration of age is generally expected to have little to no effect on power to detect trends. However, age effects could potentially be important in some data sets. In these cases, consideration of age could improve statistical power.

**Recommendation:** Limnological factors such as pH and conductivity should be monitored as of the trend-monitoring program design and considered in post-sampling analyses.

The specific recommendation—to gather water quality data on pH and conductivity—was based, in part, on ease of implementation. The recommendation regarding data to be gathered is based on information which can be collected by widely available meters that are easy and quick to use. Thus, this recommendation has a very high feasibility. Exponent also recommended that available data on other limnological factors (productivity, oxygen climate, depth, watershed area, etc.) be gathered and considered in statistical analyses. Again, the intent here is to have MDEQ use available information.

Water quality factors can have a significant effect on PTS concentrations, most notably mercury. Therefore, consideration of them in subsequent analyses is likely to increase the power of trend analyses.

**Recommendation:** Sediment chemistry should be monitored along with fish data.

Exponent has recommended that MDEQ monitor sediment chemistry. As envisioned by Exponent, this sampling is intended to be easy. Specifically, surficial sediments would be sampled with a simple sampling device (e.g., an Ekman dredge or Petite Ponar). These devices are easy to use and widely available. In systems where these devices do not work well (i.e., very deep lakes), it is recommended that no sample be taken. Moreover, as the costs for analyses of sediment chemistry should be close to those for biota, this recommendation has only a small effect on total costs of analysis.
During the development of our report, Exponent was informed by MDEQ that it has begun to monitor sediment chemistry in inland lakes. Consequently, this recommendation represents no increase in effort.

The intent of this recommendation is to support trends observed in PTS concentrations in fish. It has no likely effect on the power of the fish tissue sampling, but should support the legitimacy of any trends that are observed.

**Recommendation: Food chain effects should be monitored indirectly.**

Exponent recommends that potential food chain effects be indirectly monitored in several ways. First, MDEQ is encouraged to increase its use of multifish and multichemical comparisons. The data for these comparisons are readily available and these sorts of comparisons are already done by MDEQ. Therefore, this recommendation is easy to implement.

Exponent also recommended that delta nitrogen and delta carbon be monitored routinely or sufficient tissue be archived to allow delta nitrogen to be measured after sampling for specific data sets. Analyses for delta nitrogen in fish tissue immediately and on archived tissue are both feasible, as outlined in Kucklick and Baker (1998) and Whittle et al. (2000).

Potentially, the delta nitrogen and delta carbon results can be used in a multivariate analysis of trends, although these data may ultimately be useful only as qualitative indicators of food chain length. In cases where significant variability in PTS concentrations is caused by differences in trophic level, consideration of the delta nitrogen or delta carbon result could increase the power of trend analyses. The other indirect methods—multichemical and multifish comparisons—do not affect the power of trend analyses, but can be used to support the legitimacy of any trends that are observed.

**Recommendation: Data from edible tissue should be used to supplement whole fish trend-monitoring data.**

This recommendation is highly feasible. Several analysts have considered whole body to fillet conversion factors (e.g., Amrhein et al. 1999), and MDEQ has some unpublished data on whole fish and fillet concentrations, from the same fish, that were generated specifically to estimate this factor. MDEQ also has ample data on both fillets and whole fish from the same water body, and these data can be used to estimate a conversion factor for various chemicals.

In terms of power, inclusion of data from the edible tissue could dramatically increase both the trends data set in terms of both number of points and breadth of sampling sites. Inclusion of additional data should increase statistical power as long as the addition of another tissue type does not add significantly to the variability. Addition of more sites would add to the variability of the data, reducing statistical power but increasing the applicability of the trends. Thus, the net effect on power is difficult to assess, but inclusion of additional data will bolster the applicability and legitimacy of any observed trends.
4.18.1 Impacts of Exponent’s Recommendations on Historical Data

In choosing recommendations, Exponent tried to balance cost and feasibility along with potential gains in information compared to previous sampling methods. An important component of the latter was protecting the information contained in previously collected data, an obviously paramount factor to temporal trends analyses. Thus, most recommendations were for additional data or additional analyses that did not impact the value of previously collected data. A notable exception to this is Exponent’s recommendation to discontinue sampling of YOY. However, the attendant loss of information is not likely to be substantial. The YOY data were not extensive, and the high proportion of concentrations below the detection limit further limited the value of the YOY data.

An equally important consideration is how old data can be combined with new data as they are collected. If the recommendations are followed, data collected hereafter will include information on age, gender, and delta carbon and nitrogen. Unfortunately, there does not appear to be any way to back-calculate these factors for previously collected data. Anticipated increases in information will apply only to those concentration data for which these data elements are available. Nonetheless, the value of already collected data is maintained because it can always be combined with subsequently collected data in analyses of temporal trends, albeit without the benefit of information on age, gender, and delta carbon and nitrogen.

As for temporal trends analyses for data from random vs. fixed sampling sites or changes over time in targeted size vs. a wide range of sizes, Exponent believes that old and new data can be combined, with caution. Many of the inland fixed monitoring locations were chosen for reasons other than proximity to a local source of chemicals or high chemical levels in fish. The location of these fixed sites with respect to local sources of chemical concentrations and other factors affecting bioaccumulation (e.g., food chain length, limnological factors, etc.) can be assumed to be random. Analyses combining data from fixed and randomly selected sites should, therefore, be possible. Similarly, it should be possible to combine data from samples on limited sizes of fish with data on samples of a range of fish sizes. In estimating the effect of fish size on PTS concentrations, multiple regressions of fish concentrations on time and fish size will likely key on data sets having a wide range of sizes. In both cases, old and new data can be combined, although MDEQ should be cautious with the results because the underlying assumptions are not truly being met.


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Figures
Figure 1-1. Concentrations of DDT and breakdown products in whole lake trout from Lake Ontario
Figure 1-2. PCBs, DDE, and Mirex in gull eggs from the Snake Island colony on Lake Ontario

Source: Canadian Wildlife Service
LEGEND

- From gull colony at Agawa Rock, Lake Superior
- From gull colony at Granite Island, Lake Superior
- EPA U.S. Environmental Protection Agency

Notes: Bars represent the standard error. Wisconsin, EPA, and Michigan are rate constants based on lake trout sampling programs of Wisconsin, Michigan, and EPA, respectively. Sediment(J) is based on estimated rates of decline within three cores sampled by Jeremiason (1994), while Sediment (2 cm) is based on concentrations in the top 2 cm of different cores collected between 1977 and 1990.

Figure 1-3. Declines of PCBs in different Lake Superior media
Figure 2-1. Fish size versus concentrations of PCB, mercury, and lipid

Source: Data from MDEQ sampling of Lake Huron lake trout
Figure 2-2. Toxaphene in Lake Superior lake trout from various FCMPs and analytical methods

**LEGEND**
- Michigan Department of Environmental Quality data
- Canadian Department of Fisheries and Oceans, Hercules
- Canadian Department of Fisheries and Oceans, Parlar
- U.S. Environmental Protection Agency

Note: MDEQ data are unpublished data for whole fish and fillets, with the latter multiplied by 1.8 to account for differences between whole fish and fillets. DFO data are from Whittle et al. (2000) based on the Parlar method and Hercules standards. See that reference for further explanations. EPA data are from DeVault et al. (1996) and unpublished data from EPA’s sampling of lake trout.
Figure 2-3. Trends of PCB concentrations in Lake Ontario lake trout controlling for fish size

Note: Data are based on all fish collected between 600 cm and 700 cm in length, irrespective of age. Fish average about 5.7 years old.

Source: Canadian Department of Fisheries and Oceans
Figure 2-4. Trends of PCB concentrations in Lake Ontario lake trout controlling for fish age (five-year-old fish)

Note: Data are taken from same set as Figure 3, except that only five-year-old fish were considered.

ln(PCB) = 2.08 - 0.097*(year–1977)

Source: Canadian Department of Fisheries and Oceans
Figure 2-5. Trends of PCB concentrations in Lake Ontario lake trout controlling for fish age (six-year-old fish)

\[ \ln(\text{PCB}) = 2.33 - 0.101 \times (\text{year} - 1977) \]

\[ R^2 = 0.54 \]

Note: Data are taken from same set as Figure 3, except that only six-year-old fish were considered.

Source: Canadian Department of Fisheries and Oceans
Figure 2-6. PCB concentrations in Lake Michigan coho salmon and lake trout over time

Source: EPA’s lake trout and coho sampling program
Figure 2-7. Magnitude and variability of mercury concentrations versus trophic level of fish species from several Florida Everglades lakes

Source: Lange et al. (1994)
Figure 4-1. PCBs versus length for male and female lake trout from Lake Ontario, from 1998 to 2001

Source: Department of Fisheries and Oceans
Figure 4-2. PCBs and mercury concentrations versus length in male and female walleye

Source: Ministry of Environment
Figure 4-3. Original and detrended PCB concentrations in spottail shiners from the Niagara-on-the-Lake site on the Niagara River

Source: Ministry of Environment
Figure 4-4. Original and detrended PCB concentrations in lake trout from Lake Ontario.
Figure 4-5. Intra-year versus true inter-year variability for different species/samples.
Figure 4-6. PCBs versus size for fast- and slow-growing bloater

Source: Eby et al. (1997)
Figure 4-7. PCBs versus size for fast- and slow-growing bloater (constant age and size)

Source: Eby et al. (1997)
Figure 4-8. PCBs versus length in whole and fillet lake trout

Source: Michigan Department of Environmental Quality
Figure 4-9. PCBs in whole fish and fillets from the same fish

Source: Michigan Department of Environmental Quality
Figure 4-10. Potential models of PTS decline in biota (linear Y-axis)

LEGEND
- Zero order
- First order
- Bumpy first order

Note: Zero order—absolute 7.5 percent decrease per year. First order, 7.5 percent decrease per year. Bumpy 1st order, 7.5 percent decrease per year with semi-periodic perturbations.
Figure 4-11. Potential models of PTS decline in biota (non-linear Y-axis)

LEGEND
- Zero order
- First order
- Bumpy first order

Note: Zero order—absolute 7.5 percent decrease per year. First order, 7.5 percent decrease per year. Bumpy 1st order, 7.5 percent decrease per year with semi-periodic perturbations.
Tables
Table 3-1. Power and trends for mercury, PCBs, and lipid standardized PCBs in current data

<table>
<thead>
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<th>Location</th>
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<th>Trend</th>
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<th></th>
<th>PCB</th>
<th></th>
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<td>Trend (percent)</td>
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Note: PCB - polychlorinated biphenyl

Trend is percent change in concentration per year; negative indicates decrease, positive indicates increase.
Table 3-2. Summary of power levels for range of trend levels

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<tr>
<th>Location - Species</th>
<th>Mercury Avg.Trend (percent)</th>
<th>Mercury Power (percent)</th>
<th>PCB Avg.Trend (percent)</th>
<th>PCB Power (percent)</th>
<th>PCB-Lipid Avg.Trend (percent)</th>
<th>PCB-Lipid Power (percent)</th>
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<td>PCB Avg.Trend (percent)</td>
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**Note:** Average trend is the average percent decrease in concentration per year.

Not estimable indicates the data are too variable to detect a trend of this level.
### Table 4-1. Comparisons of 5-year average gull egg concentrations of PCBs (mg/kg) to steady-state concentrations predicted by Stow (1995b)

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<td>11.7 (13.9)</td>
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<td>Lake Huron³</td>
<td>10.6</td>
<td><strong>10.5 (11.9)</strong></td>
<td>9.7 (11.6)</td>
<td>8.1 (10.1)</td>
<td>7.0 (8.6)</td>
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<td>15.7 (17.2)</td>
<td><strong>14.6 (16.0)</strong></td>
<td>13.4 (14.6)</td>
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</table>

**Source:** Canadian Wildlife Service

**Note:** Values are means and 95 percent UCLs.
- Concentrations below predicted asymptote are bolded.
- Bolded 95 percent UCLs are significantly below the asymptote.
- PCB - polychlorinated biphenyl
- UCL - upper confidence limit

³ Colonies without Channel Shelter Island colony.
Appendix A

Literature Review of Endogenous Factors That Affect Bioaccumulation of Persistent Toxic Substances and Statistical Methods for Trend Analyses: Relevance to Fish Contaminant Monitoring Programs
This literature review is presented in three sections. The first section presents a review of papers that address endogenous (lipid, species, size, etc.) and food chain factors affecting bioaccumulation of persistent toxic substances in large fish. The second section contains a review of fish contaminant monitoring programs (FCMPs) that use young-of-the-year fish. The final section is a review of papers that address statistics and sampling design of fish monitoring studies.

**Endogenous and Food Chain Factors Affecting Bioaccumulation in Large Fish**


**Summary:** The authors assert that bioaccumulation factor methods have major limitations “because they fail to represent the primary mechanisms of bioaccumulation process.” Food chain and food web analyses can estimate concentrations as functions of accumulation, uptake, release, metabolism, ration size, and dietary makeup. These factors will vary as functions of “geography, season, and species.” The paper presents a food-web model that can be fairly complex if necessary—it can model up to 16 different aquatic species. The model is a discrete time-step model. The model was tested in experimental stream systems that were 110 m long, with six 8-m square pools that were 1 m deep at the center. Inputs to the stream system were from natural streams. Water from the streams passed through 1-mm mesh to screen out small fish and large food. Benthos in the stream tanks were allowed to equilibrate to conditions prior to adding largemouth bass, golden shiners, and catfish. The fish were stocked and then sampled (three catfish and three bass) at four or five intervals during the test. Stomach contents were examined, and it was determined that bass were much less piscivorous than anticipated; therefore, a lower rate of piscivory was added to the model. Using the default assumption of piscivory caused the model to overpredict bass concentrations. The authors suggest that the relative importance of food vs. water as sources of contaminants will vary with feeding rate. Therefore, it is necessary to consider this variability when sampling and interpreting data.

**Relevance to FCMP:** The authors emphasize that models to predict fish concentrations must consider actual processes of bioaccumulation. They also emphasize the variability of such processes over time and space. Based on model results, they suggest that bioaccumulation of
TCDD/Fs is very sensitive to actual feeding rate and food preferences. At low feeding and growth rates, bioconcentration is more important than at high feeding rates. In effect, low feeding rates approach bioconcentration (i.e., direct uptake from water) experiments when fish were not fed. Predictions of bass bioaccumulation were too high when default feeding preferences (piscivory) were used. Most TCDD/F exposure comes from food.


Summary: Analysts often need whole fish concentrations for a variety of analyses (e.g., estimation of bioaccumulation, assimilation efficiency), but sometimes have only concentration data from fillets. Therefore, conversion factors from whole fish to fillet would be useful. This study examined factors affecting the ratio of polychlorinated biphenyl (PCB) concentrations in fillets vs. whole fish. The researchers took coho and rainbow trout caught in two tributaries to Lake Michigan and analyzed 50 g of tissue taken from fillet and from the rest of the fish. They considered the potential bias, on whole fish concentrations, of taking 50 g of fillet from the whole fish and concluded that it was likely to be small. PCB concentrations were higher in coho fillets and whole fish, but lipid content was higher in rainbow. They regressed PCB concentrations vs. length, without transformation of either variable. There were significant relationships between PCB concentration and length, for both whole fish and fillets, for both species. Significant relationships were also found between lipid content and PCB concentrations in both species, although the correlation coefficient for lipid and PCBs in coho fillets was low (r = 0.12). Ratios of lipid content in whole fish vs. fillets (1.75 in coho and 1.70 in rainbow) were about the same as ratios of PCB concentration in whole fish vs. fillets (1.70 in coho and 1.47 in rainbow). The authors used classification and regression tree (CART) models to determine the most important factors—fillet PCB concentrations, fillet lipid concentrations, and length—in predicting whole fish concentrations. For both species, fillet concentrations were, by far, the most important predictor. Similarly, with multiple regression, fillet concentrations were the most important predictor. Fillet lipids were a weak, but negative predictor of PCB levels in whole fish. The authors suggest that lipids are not an important predictor of total PCB concentrations in whole fish or fillets when size is taken into account.

Relevance to FCMP: PCB concentrations in fish are a function of tissue type, species, and the interactions between the two. They provide a method to convert fillet data to whole fish concentrations. The results suggest that disposition of PCBs within a fish is based on lipid partitioning, but that partitioning from the environment into the fish itself is not a function of lipid content. The authors advise against lipid normalization.

Summary: PCB concentrations increase with age of lake trout. The best-fit equation for the regression is PCB = 1.031 exp(0.259*age). The authors note that variability of PCB concentrations increased with age.

Relevance to FCMP: Organochlorine concentrations in fish are a function of age, length, and weight. Variability of PCBs increases with age, so the precision of estimates decreases with larger fish. Based on data provided (not analyses done by Bache et al.), length was a better predictor, as measured by $r^2$ and stepwise regression, than either weight or age. Also, variability increased with age. The coefficient of variation was about 0.33 for trout younger than 4 years, but was about double that for trout older than 8 years.


Summary: The authors present a mechanistic model of bioaccumulation of PCBs from water and food. In this model, assimilation efficiency is a function of predator and prey concentrations of PCBs, based on the assumption of passive uptake of PCBs. The study presents data from other analyses, which show that assimilation efficiency for PCBs decreases as body burdens increase, implying a passive diffusion mechanism. They conducted modeling runs with salmonids eating prey with the same PCB concentration and salmonids eating prey with increasing concentrations. Their simulations show little difference in PCB concentrations in salmonids with fish size when prey concentrations are kept constant. Even with increasing prey PCB concentrations, the increase in PCB concentrations in salmonids with size is slight and dependent on the PCB isomer. These predictions are not that consistent with observed data. Relative uptake from the gills vs. from the food depends on relative concentrations in water and food. However, even with water concentrations elevated above what is likely, accumulation from water is still subordinate to that obtained from food, but more than other studies speculate.

Relevance to FCMP: This study suggests that water uptake across gills may be larger than estimated by other models, perhaps by as much as 16 percent of total PCB accumulation. This model differs from most other models in that it assumes that PCB assimilation is a function of predator’s body concentration, with lowered assimilation at higher body burdens. Simulations seem to suggest that higher prey concentrations are the primary mechanism of increased concentration of PCBs with age. However, the model’s predicted relationships between size and PCB concentrations are flatter than observed.


Summary: This article critically reviews the assumption used in bioconcentration theory that partitioning of hydrophobic chemicals between animal and water column is as simple as between lipid and water. Bioconcentration is defined as the process by which fish accumulate...
water-borne chemicals other than from dietary sources. Most estimators of bioconcentration, or bioconcentration factors (BCFs) correlate to hydrophobicity or $K_{ow}$. However, while there is good relationship between BCF and $K_{ow}$ over a wide range of $K_{ow}$ values, there is also a wide range, one to two orders of magnitude, of BCF values over a relatively constant $K_{ow}$. Furthermore, most regressions of BCF vs. $K_{ow}$ are based on chemicals that are poorly metabolized. The author critically evaluates lipid normalization for bioconcentration. He suggests that much evidence linking lipid content and bioconcentration is confounded by other factors. Other factors will also cause BCF to vary from simple lipid partitioning. For example, many very large molecules may be too large to pass freely across the gills. Other chemicals are rapidly metabolized, so their BCFs are lower than predicted by simple lipid partitioning. In addition, there are differences in bioavailability of chemicals because of adsorption to particulate and dissolved organic carbon. The author suggests that fish size and temperature affect bioconcentration.

Relevance to FCMP: Bioconcentration is more complicated than simple lipid partitioning. The author recommends mechanistic models to estimate bioconcentration. The arguments made here are similar, although not as extensive, as arguments made concerning bioaccumulation.


Summary: The authors point out that Annex 11 and 12 of the Great Lakes Water Quality Agreement call for joint surveillance and monitoring by both parties, including standardized sampling and analytical methodology. The authors considered data from Canadian Department of Fisheries and Oceans sampling, which focuses upon age-controlled whole fish, and the U.S. Environmental Protection Agency’s (EPA’s) lake trout sampling, which focuses upon composites with average fish size from 620 to 640 mm. They also analyzed data from EPA’s coho salmon sampling, which uses age 3 salmon on spawning runs, and the spottail shiners collected by the Canadian Ministry of the Environment. Analyses of temporal trends were based on visual inspection of data on linear Y-axis, which represents implicit use of zero order kinetics. Analysts compare long-term trends of both PCBs and DDT in the same media and indicate that they show the same long-term trends—fast declines followed by slow to no declines. The authors also considered DDT and PCBs in spottail shiners and smelt.

Relevance to FCMP: These analyses demonstrate the value of looking at several media and several chemicals at once, although the value of this information is not recognized by the authors. The authors employ trends analyses without statistics or null hypotheses.


Summary: This paper presents a comprehensive review of changes of PCB concentrations in aquatic biota collected from Lake Ontario between 1977 and 1993. The researchers collected
data on lake trout, other salmonids, prey fish, and zooplankton and benthos. Based on inspection of the data on the linear Y-axis, the authors suggest that PCBs declined from the early 1980s but changes in recent years are masked by interannual variability. PCB concentrations were consistent among the surveys after consideration of fish lipid content, age or size, and analytical protocol. The authors suggest that variability of 20 to 30 percent in annual average estimates for a given size of lake trout is attributable to both analytical and in situ sources of variability. They also recommend that future monitoring consist of annual sampling of key species supplemented with periodic intensive sampling of the entire food web (e.g., every 5 years). There is implicit use of zero order kinetics—interpretation of declines based on inspection of data on linear Y-axis—along with explicit use of first order kinetics.

Relevance to FCMP: PCBs in biota are a function of lipid, size and/or age, tissue type (fillet with skin vs. whole fish), species, food chain position, and food chain structure. The authors state that detecting trends in underlying loading will require more extensive sampling, of the entire food chain, as well as modeling, to understand changes in the biotic and abiotic system. The authors recommend multimedia (different biota) and multi-method (modeling and sampling) approach to trends analyses.


Summary: This paper addresses the importance of age and lipid content on bioaccumulation. A bioenergetics model was developed based on observed growth rates of lake trout. Two models were considered—one in which contaminant elimination was assumed to be a function of body burden and one in which elimination was independent of body burden. The model also integrated predation on large prey sizes as the lake trout grew, and changes in prey species, as the lake trout increased in size. These changes had antagonistic effects on lake trout body burdens. Although larger prey tended to have higher contaminant burdens, young lake trout prefer sculpins, which are more contaminated at any size, than alewives, the preferred prey of large lake trout. The two basic models were fitted to observed data (from Borgmann and Whittle 1991b) for lake trout size and chemical concentrations, which allowed estimation of consumption rate. That is, prey consumption rate was based on observed contaminant concentrations. To address the importance of lipid content, both models were run with excretion as a function of lipid content or excretion independent of lipid content. Adding lipid to the models had a minor effect on PCB and DDE concentrations. Increasing lipid content by about 50 percent, from 14.2 to 21 percent, only increased predicted DDE concentrations by about 6.6 percent, from 0.75 to 0.80 mg/kg. According to the authors, the response of PCB and DDE body burdens to increasing lipid levels is small because, even though excretion is inversely proportional to lipid concentrations, “excretion rates are slow and have only a limited impact on final concentrations.” The authors also use a model to predict effects of prey selection. As expected, selection of less contaminated prey led to less contaminated predators. The models were also used to predict the rate of response of individual fish to changing concentrations of PCBs. Prey concentrations of PCBs were doubled for 3 years and then reset back to the original concentrations. Younger fish (2 to 4 years) responded almost immediately
to the changes, but 7- to 8-year-old fish had half response times of about 1 year. Most of the response was growth dilution, with lesser amounts due to excretion of PCBs.

**Relevance to FCMP:** By combining modeling and empirical data, the analysis addresses several questions of importance to FCMPs. The analysis suggests that predator lipid concentrations have limited impact on body burdens of DDE and PCBs. It also demonstrates that changes in prey concentrations affect concentrations in predators.


**Summary:** The authors collected lake trout from various points in Lake Ontario. They conducted a multiple regression of log chemical concentrations (DDE, PCB, chlordane, dieldrin, Mirex, and mercury) on log weight, year, age, and site of capture. Concentrations of all chemicals tended to be a function of age, weight, and year. There were significant declines in all contaminant concentrations, including mercury, DDT, dieldrin, and PCBs. The authors assessed temporal trend data on log Y-axis, which represents implicit use of first order decline. When they repeated regressions with lipid-normalized data, they found that regressions were not greatly improved, but lipid was a significant predictor. However, the authors caution that lipid and weight covary, so it is inappropriate to use both of them in linear regression. The authors contend that there are both size and age effects, and the slope of concentration vs. size, within an age, will be flatter than the slope of concentrations vs. size for variable age fish. Specifically, they state: “Lipid levels are not the major factor directly controlling contaminant concentrations although they have some influence on excretion rates. There is, therefore, no advantage to expressing contaminant concentrations on a lipid basis for those contaminants which are accumulated primarily through the food web.” They compare PCBs declines across lakes, and conclude that trends observed in Lake Ontario are unique.

**Relevance to FCMP:** Data from various age classes, sizes, locations, and sampling times can be analyzed with multivariate regression models. These models can, potentially, control for other sources of variability and focus upon specific factors, such as spatial and temporal trends. The authors demonstrate that bioaccumulation is a function of weight, age, location, and probably food chain. They suggest that there are effects attributable to age in addition to size, and also that lipid is not an important factor. They specifically argue that lipid normalization is not appropriate.


**Summary:** Heavier nitrogen isotopes tend to become more concentrated at higher trophic levels, with delta nitrogen increasing 3 to 5 percent per trophic transfer. This study evaluated trophic transfer of dioxins in two food webs of the Baltic Sea. The first food web was a pelagic food web, with fish as top predator, and the other was a littoral food chain with the duck as top predator.
According to delta nitrogen analysis, the phytoplankton have the lowest delta nitrogen, with higher amounts in the seston. Particle grazers, mussels, and zooplankton had about 3 percent more than phytoplankton, but not much more than seston, suggesting that phytoplankton was the primary source of food for both grazers. The predators on grazers—the eiders in the littoral region and herring in the pelagic—have about 2 and 5 percent more delta nitrogen than their prey. Cod, a top predator that eats herring, had about 1.6 percent more delta nitrogen than their prey. The authors examined the relationship between different dioxins and delta nitrogen. They found that some dioxins, mostly more chlorinated dioxins and furans, decreased with trophic levels, whereas others, including the higher chlorinated 2,3,7,8-substituted congener, increased with trophic levels. Therefore, the total toxicity equivalent increased with trophic levels. Although total PCDD/PCDF concentrations increased with trophic level, the concentrations of dioxins/furans declined as delta nitrogen increased, indicating that the dioxins and furans did not biomagnify. The authors did not find any significant relationship between the lipid concentrations in fish and the concentration of any of the compounds.

**Relevance to FCMP:** The work illustrates the complexity of bioaccumulation. The authors argue that biomagnification is a function of chlorine substitution pattern, such that some dioxins/furans biomagnify but others do not. They also argue that food chain exposure is the dominant source for most of the dioxins and furans, but some dioxins/furans do not biomagnify because they are readily metabolized. For these congeners, the food chain is still the primary source, but there is no biomagnification. The study also illustrates the value of delta nitrogen in determining position of consumer in the food chain.


**Summary:** The study addresses the problem of considering long-term trends of PCBs when the analytical method has changed. According to the authors, “valid interpretation of historical trends of PCB concentrations cannot be made without consideration of the effects of the change in analytical methods.” Originally, they analyzed PCBs by comparison to only a few peaks of Aroclor® standards with packed column analyses, and the peaks selected changed over time. Now, they measure individual congeners. Mathematically, they estimate the amount of Aroclor® 1016 and 1254 that would be measured with congener analyses. Their estimates suggest that Aroclor® analyses prior to 1977 overestimated total PCB congener concentrations by about 25 percent, but total Aroclor® methods used in 1979 and 1983 tended to underestimate total congener concentrations by about 13 and 14 percent. The effect was more pronounced for Aroclor® 1016 and relatively slight for Aroclor® 1254. Based on historical data, which included the effects of the change in technique, Aroclor® 1016 concentrations fell 70 percent between 1978 and 1980. However, their analysis suggests that most of this decline (40 of the 70 percent) was due to the change in methods alone.

**Relevance to FCMP:** Changes in analytical method can have significant effects on long-term and short-term trends.

Summary: A fugacity-based model was developed to simulate bioconcentration and biomagnification of hydrophobic organic contaminants in complex food webs in aquatic systems with contaminated water and sediment. The expressions reduce to a single equation involving an N×N matrix of food preference parameters that is readily solved to give concentrations and fluxes throughout the food web. The model uses available data for different PCB congeners in the Lake Ontario ecosystem, and is able to approximate the observed data. The data suggest that bioaccumulation is a function of PCB congener, trophic level, organism size and metabolism, and whether its food chain is water column- or sediment-based. The sediment food chain has a higher fugacity, and sediment-based organisms are predicted to have higher PCB concentrations. Modeling indicates that food chain exposure becomes progressively more important as the organism increases in size and trophic level.

Relevance to FCMP: A substantial amount of PCBs comes from the food chain, even for forage fish and mysids. Therefore, food chain structure is paramount to measured bioaccumulation, even with lower food chain species. Results suggest that transition from water column to benthic food chain entails significant increases in bioaccumulation because fugacity of sediments is higher than water. In the model, it is assumed that the assimilation efficiency for a compound is independent of the consumer’s lipid levels, yet the model still produces good predictions for high lipid species.


Summary: This study is a manipulative experiment to test effects of predator and prey (i.e., food) lipid levels on bioaccumulation of PCBs. Small perch and rainbow trout were fed high and low fat diets to get the fish themselves to be high and low fat. High fat perch had about twice the fat as low fat fish (5.5 vs. 3 percent), whereas high fat rainbow trout had only 7.7 percent fat vs. 5.6 percent for low fat fish. An unfortunate confounding factor between the treatments was size. High fat fish tended to be about 30 percent larger, by weight, than low fat fish (i.e., about 11.3 g compared with about 8.5 g) in both species. After this initial period, the fish were fed high and low fat diets contaminated with PCBs. The researchers looked at net assimilation efficiency as function of fat of the fish and fat of the diet. After feeding with contaminated food was discontinued, they then looked at excretion rate. Results were sometimes consistent with predictions and sometimes inconsistent. The authors conclude that lipid levels in predators and their prey can affect net bioaccumulation, but the effect is inconsistently observed, potentially due to differences between species. The effect of predator lipid levels was mostly on excretion; absorption efficiency for this PCB appeared to be relatively constant across predator lipid levels.

Relevance to FCMP: This study illustrates the complexity of the biomagnification process. Predator and prey lipid levels may affect net bioaccumulation rates, but this effect may not be noticeable under certain situations or with certain species.

Summary: This is the first published report of EPA (and the U.S. Fish and Wildlife Service [FWS]) sampling of lake trout from the Great Lakes. Lake trout were collected from offshore sites away from point sources. There was one collection site per lake, and 60 fish were taken per collection site. Twenty fish were placed into three size categories: 300 to 450 mm, 451 to 650 mm, and > 650 mm, divided into four composite samples of five fish each. As originally planned, the sampling was intended to compare concentrations across time and space in the three size categories using analysis of covariance (ANCOVA). However, the ANCOVA required that there be a relationship between size and concentrations and, to test for differences, the slopes between size and concentration could not be significantly different. Analysis of data from the early years showed that only about half of the regressions of size vs. concentration were significant at the 95 percent confidence level, and some of the slopes exhibited differences. Therefore, ANCOVA was dismissed and the analysis proceeded with mean statistics based on one size category (600 to 700 mm). For long-term trends, data from 1970 to 1976 in Lake Michigan were from FWS analyses of single fish. From 1977 to 1981, the data are from composites of medium and large fish. Average sizes for these composites ranged from 620 to 640 mm. After 1981, the fish collected were between 600 and 700 mm and composited. Temporal trends analyses were based on the assumption of first order decline.

Relevance to FCMP: Results illustrate potential problems with ANCOVA and real data sets. The paper provides an explicit description of sampling being modified to increase precision and power. Data demonstrate the increase in precision associated with using composites vs. single fish. For temporal trends, the authors assumed first order decline over time.


Summary: Fillets of fall run coho salmon from each of the Great Lakes were analyzed for pesticides and industrial compounds. The sampling focused on a single life stage (i.e., cohos swimming upstream to spawn). Data were based on multiple replicates of composites of fillets with skins. Most fish were 3 years old, but length, weight, and lipid content varied from site to site. Comparison of coho salmon collected in 1980 through 1984 shows that PCB and DDT in coho from Lakes Erie and Michigan have declined, following first order loss kinetics. However, DDT and PCBs did not decline between 1983 and 1984, which the authors suggest may signal a new equilibrium due to constant external sources. The authors looked at declines of several chemicals, but did not compare trends of different chemicals.

Relevance to FCMP: The U.S. Environmental Protection Agency’s sampling protocol concentrates upon a single life stage/age, using both composites and replication at multiple sites for some lakes. The method controls for age, season, and site. Temporal trends were analyzed using a single exponential model for decline over time. As with lake trout sampling, a surrogate measure (breeding behavior) is used to age fish.

Summary: Lake trout or walleye were collected from one to three locations in each of the Great Lakes in 1984. Generally, five composites of five fish each were taken from each sampling site, and analyzed for dioxins and furans. An attempt was apparently made to make sure the fish were the same size across all lakes, but Lake Superior lake trout tended to be somewhat smaller than those caught in other Great Lakes. The authors concentrated upon the distribution of different dioxin/furan congeners, which presumably are less affected by age and size. They compared the relative proportion of different dioxin/furan congeners across lakes, and in relation to PCB concentrations in the same fish, and found a unique pattern in Lake Ontario.

Relevance to FCMP: Comparisons of the relative concentrations of different chemicals can potentially illuminate the importance of different source dynamics. High variability can be addressed with stratified sampling (small size range), compositing of several fish, and replication of composites.


Summary: This paper is a periodic report of EPA’s sampling of lake trout and walleye from the Great Lakes. According to the authors, “while it is understood that both size and age affect contaminant concentrations,” limited resources precluded aging of fish or analyses on individuals. Therefore, sampling tried to focus upon a narrow size range of fish—600 to 700 mm for lake trout and 400 to 500 mm for walleye. However, fish of an appropriate size were not always available in Lake Ontario. These fish were analyzed as five-fish composites. The composites were then replicated from 3 to 30 times per lake, with replication varying over time and space. The authors analyzed temporal trends with single and change point regression models, the latter pertaining to a change in the decay rate “corresponding to a change in the food web.” There was a weak and inconsistent relationship between contaminants and lipid levels; therefore, data were not lipid normalized. According to visual inspection of data, both DDT and PCBs stopped declining after the early 1980s. When a change point regression was applied to the data, regression was unable to pick a specific change point, although several change points in the mid-1980s gave significant results. The authors compared declines in the water column to those in fish and concluded that they were similar over the long term. However, this finding was largely due to calculation error for Lake Superior water column. First order decline was actually −0.19/year, not −0.09/year, as estimated by the authors. The authors did note that water column PCBs declined while fish PCBs remained stable in the second half of the time period, and suggested the latter was due to a food chain event. They suggest that changes in the rate of decline over time was not due to different contaminant pools, but rather to food web changes.

Relevance to FCMP: The EPA sampling of lake trout addresses variability by sampling same site and limited size of fish, compositing fish (five per sample), and incorporating a moderate level of replication of composites. Variability was still quite high over time due to some
unknown factor. Trends analyses of PCBs in fish was aided by multimedia comparison with PCBs in water and fish. The authors assumed that changes in decline were due to food chain instead of source dynamics. The authors noticed that declines were not similar over the short term, but missed that they are also not similar over long term, in Lake Superior, because of the calculation error. The analysis suggests change-point regression as another model of potential decline over time.


Summary: Concentrations of PCBs in Lake Michigan bloaters (Coregonus hoyi) declined during the 1970s but have stabilized since 1980. During the same period, bloater population also increased 40-fold during 1970 through 1984, the diet shifted, growth rate declined, and lipid levels declined. With a bioenergetics-based PCB bioaccumulation model, this study examined the effects of diet shift and decreased growth on PCB bioaccumulation. For this model, it was assumed that assimilation efficiency was independent of lipid, but that excretion was dependent on lipid. The effect of changing lipid was incorporated into the model. The concentrations of prey were estimated with back-calculated concentrations in fish, and therefore PCBs in prey showed the same stabilization shown in the fish stocks. PCB concentrations increased little when the amount of more contaminated prey was increased, and concentrations at any age declined with the low growth scenario. However, the PCB concentration at any specific size of bloater increased. Thus, the modeling showed that lower growth rates during the 1980s placed older, more contaminated bloaters in the size range most vulnerable to predators and in the size range sampled by PCB monitoring programs. The authors recommend that sampling of a constant age class is better to assay trends and that several fish species should be sampled because any single species might be impacted by such things as diet shifts and changes in growth rates.

Relevance to FCMP: Observed trends were apparently affected by both diet shifts and differences in growth rates of bloater (which might have also impacted trends of PCBs in the predators of the bloaters). When designing sampling program for trends analyses, analysts must consider the ecological processes that affect bioaccumulation.


Summary: A mass balance bioaccumulation model for 2,3,7,8-TCDD (TCDD) is presented. The steady-state model is a combination of the WASP fate and transport model with a bioaccumulation model. The model was run under dynamic conditions to predict the response of Lake Ontario to changes in external loading. According to the model, TCDD concentrations in the water column will respond quickly to reductions in external loading. In contrast, TCDD
concentrations in the sediments are predicted to respond slowly. Half-response times of TCDD concentrations were estimated to be 0.5 year for the water column and about 10 years for the sediments.

Relevance to FCMP: The analysis provides a null hypothesis for shape and speed of declines of TCDD in Lake Ontario. In the absence of external loading, declines of TCDD for the sediment-based food chain are predicted to be a first-order decline of about 7 percent per year. The model also predicts non-steady-state periods when decreases of TCDD are independent of external loading—if external loading decreases faster than about 7 percent per year.


Summary: This study describes a steady-state and dynamic model of persistent toxic substances for Lake Michigan. MICHTOX is a steady-state bioaccumulation model hooked into a WASP chemical fate model. Although data on loading and ambient concentrations in several environmental media are limited, the model was verified against available data. However, the authors caution that available data are too limited and uncertain to adequately verify or calibrate the model, so the results are useful only for qualitative predictions. The most important result of the modeling was the description of the lag between system response and changes in loading. Because the sediments are slow to respond and serve as major reservoirs for chemicals, rates of decline of in-lake concentrations may be independent of external loading if loading has recently declined faster than internal sediment inventories can respond. Available data for declines of PCB, DDT, and TCDD in lake trout were compared against the predicted rates of decline when loading had been reduced to zero. These data showed that rates of decline were as fast as those predicted with no external loading, suggesting that the lake was at non-steady-state with external loading for these compounds.

Relevance to FCMP: The model provides null hypotheses for temporal trends of chemicals in aquatic ecosystems. With respect to the shape, the model predicts that declines of chemicals over time should approximate first-order declines. With respect to potential rates of decline of these chemicals, the model predicts that maximum rates of decline of these chemicals are likely to be on the order of 5 to 15 percent per year. The rate of decline will depend on both the chemical and the lake. In conjunction with Endicott et al. (1990), this analysis indicates that maximum declines of specific chemicals will differ from lake to lake.


Summary: This study is an analysis of toxaphene in archived fish tissue of lake trout and walleye, collected from the Great Lakes by EPA in 1982 and 1992. Tissues from smelt collected from three of the lakes (Superior, Huron, and Ontario) in 1982 and 1994 were also analyzed. Toxaphene concentrations in 1982 lake trout were similar across lakes, between 4.5 and 5.2 mg/kg, wet weight. Concentrations of toxaphene in whole walleye from Lake Erie
were much lower, about 0.25 mg/kg. Except for lake trout from Lake Superior, toxaphene concentrations in fish collected in 1992 were 50 percent or less of those reported in 1992. However, wet weight concentrations of toxaphene in Lake Superior lake trout either increased or remained constant depending on how an outlier value was included, and lipid-normalized concentrations of toxaphene in smelt also did not decrease in Lake Superior smelt. (However, wet weight concentrations of toxaphene decreased significantly over time in smelt.) The researchers considered whether the lake trout in Lake Superior were too old to have responded to the ban on toxaphene, but dismiss this hypothesis because concentrations of DDT and PCBs decreased over time in these same trout. (However, while this is true for all data between 1982 and 1992, it is not true for only these two dates.) Thus, the authors conclude that Lake Superior must have local sources of toxaphene.

Relevance to FCMP: This analysis illustrates the value of tissue archival. Analysts employ multiple lake and multiple chemical comparisons, although the latter is done incorrectly. Analysis illustrates the dangers of basin trends on small amounts of data, even when the data are spread out over time. Analysis also illustrates potential dangers of lipid normalization.


Summary: Bioconcentration data based on laboratory experiments were reported for a series of super-hydrophobic chemicals including polybrominated biphenyls, chlorinated benzenes, Mirex, and PCBs in the guppy. Experiments were done in small aquaria with periods of bioaccumulation and then depuration. Authors observed that bioconcentration factors follow a linear relationship with the log $K_{ow}$ for chemicals with a log $K_{ow}$ up to 6. For chemicals with higher $K_{ow}$ values, the bioconcentration factors were lower than expected. This loss of linear correlation is shown to be caused by a) a low fraction of bioavailable chemical in the water, b) elimination of chemical into the feces, c) an insufficient exposure time to achieve equilibrium, and d) fish growth. The study controlled for these effects mathematically. The paper presents procedures by which the magnitudes and relative contributions of these factors to reducing the apparent bioconcentration factor from linearity can be determined. Authors specifically address whether some molecules are too large to get into the fish, and reject this hypothesis for a number of reasons—large, less hydrophobic molecules were bioaccumulated as quickly as small, more hydrophobic molecules. Moreover, the tendency for very hydrophobic molecules to show reduced bioaccumulation was eliminated when the bioaccumulation was corrected mathematically for lower dissolved concentrations and higher fecal excretion.

Relevance to FCMP: Bioaccumulation of very hydrophobic molecules may be limited by their very low solubility, especially in the presence of suspended sediments and dissolved organic carbon. These results may suggest that lake trophic status could affect bioaccumulation of some very hydrophobic compounds. However, these experiments pertain to bioconcentration.

Summary: The authors conducted bioaccumulation experiments with chlorobenzenes, PCBs, and Mirex in guppies and goldfish. Experiments consisted of short bioaccumulation period followed by a depuration period, during which the fugacity in the food, feces, and fish were measured. For chemicals with log $K_{ow} > 6$, the fugacity in the feces was elevated above the fugacity in the consumed food as a result of digestion and absorption. The fugacities observed in fecal matter were up to 4.6 times the fugacity in the administered food for the more hydrophobic compounds, but the fugacity of less hydrophobic chemicals (log $K_{ow} < 5.5$) in fecal matter was less than food. These data suggest that, due to digestion and absorption, the fugacity in the food bolus increases as it travels through the gut. This observation supports the hypothesis that increases in fugacity as the food bolus is digested is the driving force of the biomagnification and food chain accumulation of hydrophobic organic chemicals. Authors also showed that the fugacity of the feces was higher than the food during the depuration experiments, showing that losses as well as gains of organochlorines can occur across the gut.

Relevance to FCMP: This study provides data to support passive diffusion, as opposed to active absorption, as a mechanism for food chain biomagnification. Passive diffusion as a mechanism of biomagnification suggests that lipids in the body and in the food should affect bioaccumulation.


Summary: This analysis presents an integrated time-dependent, model of environmental fate and food-chain bioaccumulation of PCB concentrations in various media and organisms of Lake Ontario. The model specifically considers the time responses of internal concentrations of PCBs to changes in external loading to the lake. Along with the model, observed PCB concentration time trends in herring gull eggs, lake trout, sculpin, smelt, water, and sediment data are used to reconstruct the time response and PCB loading history for Lake Ontario. The model is also used to assess the past and future time response of PCB concentrations in Lake Ontario. The model predicts non-steady state conditions between external loading and in-lake concentrations, primarily because the sediments are slow to respond to changes in external loading. The model predicts disequilibrium between water and sediments, which will have an effect on food chain bioaccumulation, because the total bioaccumulation will be different for steady-state and non-steady-state systems.

Relevance to FCMP: When external loading of PCBs increases or decreases faster than sediment inventories can respond, rates of decline of PCBs in sediments may become independent of external loading. As sediment stores are major source of PCBs to lake biota, trends in biota may be virtually independent of trends in external loading. Also, bioaccumulation processes may be different for steady-state and non-steady-state conditions, because the relative importance of the sediment vs. the water column food chain will vary.

Summary: The authors argue that normalization to lipids is only reasonable when there is a statistically significant relationship between lipid and the organochlorine concentration. Usually, this correction is accomplished by the “ratio approach,” dividing tissue contaminant concentration by lipid concentrations to determine lipid-normalized data. The ratio-based approach is satisfactory when contaminant concentration varies in direct proportion to lipid content. However, when such an isometric relationship does not exist, erroneous conclusions may be reached. Three examples are presented, and an alternative approach based upon the use of an analysis of covariance is suggested.

Relevance to FCMP: The authors provide a statistical basis for when and when not to lipid normalize and when to normalize with an analysis of covariance or simple division. This analysis implies that lipid normalization is legitimate when statistical relationship exists, irrespective of causal relationship between lipid and contaminant concentrations.


Summary: This paper is a report of the Canadian Department of Fisheries and Oceans sampling of lake trout from Lake Ontario. The researchers collected a number of fish but based temporal analyses on a single age class (4+) from a single species from a single location. They analyzed individual fish, and after aging, had 5 to 12 individual fish in the age group per year. They also looked at effects of age on persistent toxic substance concentrations from fish taken from single site. For age vs. persistent toxic substance concentration, they had 3 to 5 fish per age group. These analyses were based on historical analyses as well as more recent analyses based on archived material. Coefficients of variance for total PCBs were generally less than 0.5. Lipid levels changed over the period (1977 to 1993), but “lipid normalization did not alter the trends or interpretation of data.” A number of other chemicals were also considered in the analyses. The authors noted that most chemicals exhibited a sharp drop in concentrations from 1971 to 1981 followed by a gradual decline, but failed to consider the importance of concurrence between chemicals. This observation was based on data on the linear Y-axis. The percent of hexa- and heptachlorinated PCBs increased slightly while the percent of penta- and tetrachlorinated PCBs decreased slightly over time. PCBs and other organochlorines increased with fish age, both on a wet-weight and lipid-normalized basis. The relative proportion of different PCB homologues was constant across fish age. Archived samples had less variance than historical samples, but about the same mean. According to the authors, this was not attributable to different analytical methods, but was likely due to year to year differences in “laboratory methods and performance.”

Relevance to FCMP: Concentrations of PCBs and other organochlorines increase with fish age. Lipids are not important when age effects are considered. This sampling program controls for potential age effects by focusing upon a single age group from a single site. Frequent
discussion of trends viewed on a linear Y-axis represents implicit use of zero order kinetics, but overt statistics conducted on log transformed concentration data, explicitly suggesting first order kinetics. There may be differences in declines for different PCB congeners. There was some variability due to factors other than instrumentation and analytical method (e.g., variability between analysts and the performance of the same analyst over time).


Summary: This is a companion paper to Huestis et al. (1996). The results are based on the same methods except the results were all generated from archived tissue. Results showed that concentrations of PCBs and dioxins and furans declined over time. All discussions of trends pertain to graphs plotted on a linear Y-axis, indicating the implicit use of zero order kinetics. There were significant trends in dioxin/furan concentrations with age of fish for lower chlorinated dioxin/furans, but trends were less clear for more chlorinated dioxins/furans. There were differences in rates of decline over time for different dioxin/furan congeners.

Relevance to FCMP: Concentrations of most dioxins/furans are a function of fish age, but slope of concentration vs. age depends on the specific congener. The Canadian Department of Fisheries and Oceans controls for age effects by sampling single age group from same site. Trends in agglomerated chemicals, such as PCBs and dioxin/furans, may mask differences in trends of individual congeners.


Summary: The authors present estimates of net PCB transfer efficiencies (i.e., the ratio of PCB in predator to PCB in prey multiplied by the gross growth efficiency) for Lake Michigan coho salmon, chinook salmon, and lake trout based on 15 years of PCB concentration data in these fish and their prey. Despite large changes in the food web, diet, and growth rate for some of these species, the transfer efficiency remained relatively constant over the 1980s. The authors found apparent changes in transfer efficiency in the 1970s, but these changes are dismissed as likely due to data variability and not being “ecologically significant.” After 1983, PCB transfer efficiencies remained relatively constant at ~0.55 for lake trout and at ~0.60 for chinook salmon and increased slightly to ~0.50 for coho salmon. PCB transfer efficiencies appear to be little affected by changes in prey PCB concentration, shifts in prey type, and shifts in predator gross growth efficiency.

Relevance to FCMP: Transfer efficiency appears to be relatively constant across different prey types, prey PCB concentrations, predator growth rates, and potentially, predator/prey lipid levels.

Summary: The authors applied a bioenergetics model to describe uptake of PCBs by lake trout, brown trout, lake whitefish, coho salmon, and chinook salmon in Lake Michigan; lake trout and lake whitefish in Green Bay; and lake trout in Cayuga Lake, New York. The model described PCB uptake in terms of metabolism, food consumption, size, and growth, all of which are themselves related. Uptake and loss terms were not dependent on lipid content, and the only loss process for PCBs was across the gills. Sensitivity analyses were conducted to show that shape of PCB vs. age depended on the relationship between metabolism and the relationship between size and metabolism. The authors were able to produce various shapes of PCBs vs. weight that differed from species to species. Thus, for example, PCBs in brown trout were shown to increase rapidly and then reach an asymptote, whereas concentrations in lake trout tended to increase continuously with size. Simulation studies indicate that differences in PCB concentrations among species and in the same species among different environments result from differences in metabolic parameters, exposure, size, and rate of growth.

Relevance to FCMP: The shape of the relationship between size and PCB concentrations may vary from species to species. Thus, one type of regression may not be applicable to all species. An analysis of covariance might work for one species but not another. The paper also illustrates how models can be parameterized to fit most any data set and that similar patterns can be obtained with different combinations of parameters. Therefore, concurrence between models and observed data is not necessarily proof of validity of models of parameters used.


Summary: The authors suggest that organochlorine concentrations are a function of lipid levels, position in food chain, and relative effect of water column vs. sediment food chain. Organisms, except phytoplankton, were collected from the benthos and the water column in 1994. The study determined feeding relationships among species with delta nitrogen. According to theory, delta nitrogen should increase an absolute amount of about 3 to 3.5 percent per trophic level. However, the herring and bloaters had about the same percent delta nitrogen (7.71 and 7.51 percent) as lake trout predators (8.9 percent), all of which had lower numbers than the sculpin (10.2 percent). Delta nitrogen concentrations were consistent with theory for herring and bloater and their prey, amphipods and small mysids, which had 4.6 and 4.9 percent. Sculpins also had about 3 percent more delta nitrogen than their prey, deepwater mysids (7.7 percent). In addition, lake trout, with 8.9 percent, had more delta nitrogen than smelt, with 6.3 and 7.0 percent for moderate sized and large smelt, respectively. PCB concentrations increased with size for all species of fish, as did lipid content. Both log lipid and log PCBs correlated with delta nitrogen. That is, species higher in the food chain had higher lipid and PCB levels. Moreover, there was a significant relationship between lipid-normalized PCBs and delta nitrogen. Based on comparisons of multiple vs. single regressions, the authors estimated that trophic position was responsible for 37 to 42 percent of the variance in total PCBs, whereas lipid levels are responsible for 42 and 81 percent of the variance. Contrary to the authors’
hypothesis, there was no relationship between hydrophobicity of PCB congener and bioaccumulation, which suggests that lipid content is not important. To determine relative importance of sediment and water food chains, the authors constructed biota–sediment accumulation factors (BSAFs) for bottom sediments and settling sediments. The BSAF for bottom sediments averaged about 10, with no relationship to $K_{ow}$, whereas the BSAF for settling sediments averaged 0.5 and increased with log $K_{ow}$. The authors interpret this to suggest that settling particles are the primary source of PCB to amphipods.

**Relevance to FCMP:** Based on regression models, the authors suggest that lipid levels and trophic position affect bioaccumulation of PCBs across the food chain. Their analyses suggest that lipid levels are more important than trophic position, although the two factors are themselves correlated. There did not appear to be a relationship between trophic status and progressive bioaccumulation of more hydrophobic PCBs, as found in other lakes. Therefore, bioaccumulation of individual congeners in this lake was not an apparent function of the $K_{ow}$ of the chemical.


**Summary:** The authors used dynamic linear modeling (DLM), a regression technique that allows parameters, such as the slope of decline, to vary over time. The method is able to accommodate variance and changing slopes over time, which is necessary because rates of decline might change over time from variability in the ecosystem and food chains. The DLM method weights more recent data more heavily to make predictions into the future. Using log-normalized concentrations from the Lake Michigan salmonid database, the authors fit the DLM models to data up to 1994, and then forecast concentrations between 1994 and 2004. According to these models, PCB concentrations between 1994 and 2004 were predicted to fall about 15 percent per year in rainbow trout, about 10 percent per year in coho and brown trout, about 8 percent per year in chinook, and 4 percent per year in lake trout. The analysis does not consider effects of size or other confounding factors on trends.

**Relevance to FCMP:** The method recognizes the potential instability of ecosystem and food chain effects on bioaccumulation, and provides a totally empirical method to fit the model to data and forecast to future. However, as an empirical method, the model makes no attempt to determine which factors are important.


**Summary:** The authors applied an atypical statistical method, classification and regression tree (CART) models, to evaluate factors that affect concentrations of PCBs in Lake Michigan salmonids. CART models can identify nonlinear patterns of variability in data. The authors applied the CART model to log PCB concentration, collected from 1972 to 1994 by both the Michigan and Wisconsin Departments of Natural Resources, for lake trout, coho, chinook,
brown trout, and rainbow. Factors entered into the analysis included longitude, latitude, state, lipid, length, and species. The date of collection was not included in this analysis because the intent was to determine factors that were critical for temporal trends evaluation. For coho, chinook, and lake trout, length was the major source of variability, followed by location. For rainbow trout and brown trout, location was the primary factor affecting variability; latitude was most important for rainbow trout and longitude was most important for brown trout. Generally, higher PCB concentrations were found in southern Lake Michigan, and a hot spot for several species was found near Milwaukee. Lipid was not generally an important factor affecting PCB concentrations. The authors suggest that the analyses have two implications for analyses of trends. First, factors important to variability can be controlled for statistically after data are collected. Second, future monitoring can be directed toward those species, locations, and sizes that most closely approximate average conditions.

Relevance to FCMP: PCB concentrations are affected by species, size, location of capture, and collector/analyzer. However, after size is considered, lipid levels are often not important. Therefore, trends analysts should consider these factors post-hoc when evaluating data for trends. Alternately, sampling could focus on those areas, sizes, and species that are representative of the population.


Summary: Organochlorine (PCB, DDT, lindane) concentrations in the muscle tissue of female northern pike decreased with age, while gonad tissue increased linearly with body weight and made up about 10.6 percent of total body weight. Therefore, there was a negative correlation between percent of body weight in gonad vs. most organochlorine concentrations, but not lindane. The fat content of gonad was about seven times higher than fat content of muscle. Male gonad tissue made up only 1–2 percent of body weight. There was no relationship between age and organochlorine concentration in male muscle tissue. Females were significantly less contaminated than males. The authors suggest that there was likely a positive trend between organochlorine concentrations and fish age in males, but in this case it was not due to limited range of fish size.

Relevance to FCMP: Final bioaccumulation of chemicals is a function of inputs from food and gills, and losses, via reproduction, degradation, stomach, and gills. Consequently, total bioaccumulation will vary from species to species and from gender to gender and, within a gender, from age to age. A positive relationship between fish size and organochlorine concentrations is not ubiquitous across all species.

**Summary:** Lake trout released into Tadenac Lake as juveniles have a pronounced acceleration in growth rate, beginning at about age 6 (or 30 cm in length), coinciding with a change in diet from benthic invertebrates to rainbow smelt. There is simultaneously an abrupt increase in the rate of mercury accumulation in muscle of these fish. Across lakes, there was a linear relationship between ln mercury and length of lake trout in each lake, but the relationship varied from lake to lake. There was also a relationship between mercury levels in lake trout and co-resident smelt across lakes, but this relationship also varied across lakes. To control for differences in fish size, the authors considered the relationship between smelt mercury concentrations and lake trout mercury measured at a standard length of 60 cm. Because the growth rates are similar in the different lakes, differences in mercury accumulation can be attributed to differences in mercury availability among lakes. In view of a strong correlation (r = 0.96) between mercury levels in smelt and trout calculated at the standardized length, it is proposed that the smelt is an appropriate indicator species for the ranking of cold-water lakes relative to the availability of mercury for uptake by lake trout and other living aquatic organisms.

**Relevance to FCMP:** This study demonstrates the importance of the food chain to mercury accumulation, but also demonstrates that limnological factors are very important to mercury bioaccumulation. There is a linear relationship between ln mercury concentration and length. However, there did not seem to be a strong relationship between length and mercury concentrations while trout were eating invertebrates. These findings are consistent with other data, which show that the relationship between mercury bioaccumulation and length is relatively constant when prey are low on the food chain.


**Summary:** This paper presents another use of the Individual Based Model (IBM) to estimate growth and bioaccumulation as a function of stocking time and size. Based on these models, concentrations of organochlorines in fish vary as a function of size at stocking. Fish stocked at a large size start eating larger, more contaminated prey earlier. Therefore, after a set amount of time in the lake (e.g., 4 years), fish from larger fingerlings have higher concentrations. However, fish stocked as larger fingerlings are larger than those stocked as small fingerlings, and the models suggest that fish stocked as large fingerlings have lower concentrations at any size.

**Relevance to FCMP:** Models suggest that the relationship between PCB concentrations and fish age and fish size are functions of size at stocking, through the causal relationship between size and types of prey consumed.

Summary: As opposed to previous modeling, which estimated mean PCB concentrations, this modeling attempted to estimate observed variation of PCB concentrations in fish. The authors modeled the PCB accumulation of many individual fish based on available bioenergetics models, prey encounter models, and PCB bioaccumulation. According to their model, excretion efficiency was an inverse function of lipid levels, although assimilation efficiency was not a function of lipid. Growth and PCB bioaccumulation of individual fish was based on methods similar to Monte Carlo. For example, the number of prey–predator encounters for each fish each day was based on random sampling from a distribution of likely prey–predator interactions. The model accurately represented the variation in growth exhibited by the different age classes of lake trout, but not the variability in PCB concentrations. The model was rerun with subpopulations of lake trout exposed to different PCB concentrations, after which the variability of PCB concentrations was approximated when an extreme subpopulation ate prey with +40 percent of the average. Subsequent analyses were also conducted to determine the sensitivity of the PCB concentrations to doubling of the predator lipid levels, organochlorine losses during spawning, and a diet composed entirely of adult alewife. These had minor effects on average PCB concentrations. The authors suggest that to estimate average PCBs in lake trout, a wide range of lake trout sizes and locations should be sampled.

Relevance to FCMP: In addition to age and/or size, spatial variation of PCBs within the food chain may be an important factor underlying PCB concentrations in lake trout. According to the model results, lipid levels in predators did not have a significant effect on PCB accumulation. However, their model assumed that only excretion but not absorption was a function of predator lipid level.


Summary: This study is a follow-up paper to Madenjian et al. (1993) using about the same methods except that assimilation efficiency was adjusted to fit the data and lipid content was assumed to be 8.5 percent. Based on observations of Lake Michigan rainbows, the trout were divided into different “life history forms,” which varied in the time they spent in tributaries, time they spent in Lake Michigan before spawning, and number of times they had spawned. In the modeling, several parameters were estimated by fitting to observed data. The model was able to simulate the range in sizes of different life history forms observed in the lake, and when the prey variability factor was adjusted to 60 percent, it simulated the observed range of PCB concentrations. Sixty percent means that extreme groups in a cohort ate prey that deviated 60 percent from average. Other life history effects—years spent in streams before going to the lake and numbers of times spawned—had only minor effects on the estimated PCB concentrations. Absolute PCB concentrations were well modeled with a gross assimilation efficiency of 0.5, compared to 0.8 used for lake trout. Although rainbows and lake trout gained PCBs at similar rates per year and had about the same concentrations at the same age, rainbow
trout caught by anglers had lower concentrations because trout were much larger, and recruited to the recreational anglers, at an early age. PCBs in rainbow trout were more variable than in lake trout because the rainbow trout probably had more variable diets.

**Relevance to FCMP:** Bioaccumulation of PCBs is largely a function of PCB content of food, which varies from individual to individual. Effects such as spawning history are not significant. Concentrations are also a function of age, size, and species, and the interaction between these three factors. Other important factors are gross growth efficiency, assimilation efficiency of PCBs from food, and variability in types of prey, which affect both PCB concentrations and growth rates.


**Summary:** Many factors affect PCB accumulation. As growth efficiency increases, PCB concentrations decrease due to growth dilution, and vice versa. For a limited number of fish, such as northern pike, losses of PCBs during spawning can reduce PCB concentrations in female but not male fish. Concentrations in prey also affect bioaccumulation. Female walleye older than 6 years from Saginaw River have about 40 percent of the PCB concentrations of male walleye. This difference could be due to 1) higher growth efficiency in females, 2) spawning losses, and 3) less contaminated prey. This study sampled female and male walleye. PCB concentrations in older males increased with age, but remained constant with age in females. Lipids were nominally, but not statistically, lower in males (7.1 vs. 7.7 percent). Based on observational evidence comparing the relative mass of tissue and PCBs in the ovaries and testes compared to whole body, the authors conclude that spawning had little effect on PCB concentrations in the female or male. Similarly, modeling suggested that growth efficiency of the males and females did not differ enough to produce the large differences in PCB concentrations. The remaining hypothesis, different food concentrations, was suggested by mark-recapture studies. Mature females were more likely to leave the Saginaw River and Bay and inhabit areas where prey concentrations were presumably lower.

**Relevance to FCMP:** Because PCB concentrations in female fish are much lower, stratified sampling provides much higher precision at lower numbers of fish. The standard error of the estimated mean PCB concentration was the same with a sample of two female and five male fish as with a random sample of 20 fish. This finding suggests that stratified random sampling is more efficient in cases where the fish fit into definable strata.


**Summary:** The authors state that “[l]ake trout diet surveys and PCB concentrations of prey fish population are valuable tools in interpreting long-term trends in lake trout PCB concentrations.” The lake trout diet is different at different sites in Lake Michigan. Near Saugatuck, Michigan,
small lake trout (<400 mm) eat mostly slimy sculpins, while large lake trout (>600 mm) eat about half large alewife and half large bloater. Near Sturgeon Bay, Wisconsin, small lake trout eat predominantly small alewife and large lake trout eat mostly large alewife. There were also data on diet for lake trout off Sheboygan reef. The goal of this study was to document diets at different spots in the lake, document PCB concentrations in prey, and use results to determine if they caused observable spatial differences in lake trout PCB. An analysis of variance showed that lake trout PCB concentrations were higher at Saugatuck than at the other two sites, but concentrations in alewife and smelt were not statistically different across sites. PCB concentrations in bloater, slimy sculpin, and deepwater sculpin were higher at the site near Sheboygan reef than at the other sites, but there was no difference in these species’ concentrations between the other two sites. For prey fish, the relationship between PCBs and length was best described without transformation, but the best fit, as determined by $r^2$, tended to vary from site to site for the prey fish except alewives. Prey fish also varied in PCB concentrations by species as follows: large bloater > large alewife > deepwater sculpin = slimy sculpin > rainbow smelt. To correct for differences in size of lake trout across the three sites, the authors compared regressions of length vs. log PCBs, which provided the best fit to data. The analyses suggested that slopes of ln PCB vs. length were similar for all sites, but the intercept for Saugatuck was higher than for the other two sites. Observation of the same slope but different intercept for lake trout at Saugatuck suggests that the prey of both small and large Saugatuck lake trout had greater PCBs than prey of trout at the other two spots. However, PCB concentrations in prey fish at Saugatuck were not higher than those at Sturgeon Bay. The observed differences in diet explained the difference between PCB concentrations at the two sites. The diet of half sculpin–half small alewife was more contaminated than a diet entirely composed of all small alewife, and the larger lake trout’s diet from Saugatuck’s, which consisted of half large alewife and half large bloater, was more contaminated than a diet of only large alewife. The authors state that the diet of lake trout at Saugatuck shifted sometime in mid-1980s to a more contaminated mix of prey. This temporal shift in diet explains why the EPA sampling data, which includes samples of lake trout from Saugatuck, tended to show a cessation of decline of PCBs, whereas concentrations of PCBs in lake trout from Wisconsin tended to show ongoing declines over the 1980s.

**Relevance to FCMP:** Bioaccumulation of PCBs depends on diet, which varies over time and space. Therefore, interpreting trends analyses may require monitoring of fish diet and PCB concentrations in prey over time. Analyses of spatial trends of PCBs can use ANCOVA-like statistics to control for differences in length or age. The relationship between PCBs and length was best defined by ln PCB concentrations vs. length for lake trout, but by untransformed PCB concentrations vs. length for prey fish. Relationship between size and length varies from site to site for both prey fish and lake trout. Therefore, extrapolation of trends from one site in a lake to the entire lake may not always be valid.

**Summary:** Evidence suggests that dioxin-like PCBs have different uptake and depuration kinetics and greater affinity for organic matter than other congeners. The authors state that the efficiency of uptake from water and diet, and the efficiency of excretion are dependent on $K_{ow}$. The distribution of PCB congeners in the food web of Lake Ontario was studied to determine the parameters that control the trophodynamics of individual PCB congeners. To determine the effect of hydrophobicity on biomagnification, the authors compared lipid-normalized concentrations of congeners in lower vs. upper levels of the food chain. Generally, there was a positive relationship to $K_{ow}$; however, there was no evidence of biomagnification between forage fish and piscivorous fish. Non-ortho congeners 77 and 126 and congener 151 were not biomagnified to the extent of other congeners with a similar $K_{ow}$, which was attributed to high rates of metabolic clearance of these compounds. Nonetheless, the toxicity equivalents associated with non-ortho and mono-ortho PCBs increased throughout the Lake Ontario food web. The high toxicity equivalent observed in herring gull eggs was primarily the result of very high concentrations of congener 126 in this component of the food web. Bioaccumulation of individual PCBs depends on food web, lipid content of biota, route of exposure (aqueous vs. dietary), rates of uptake and clearance as a function of hydrophobicity, and metabolic clearance rate.

**Relevance to FCMP:** Bioaccumulation is a complex function of many factors (e.g., food web; lipid levels; metabolic rates, which vary across taxa and chemical; and net uptake, which also varies across chemical and taxa). These factors could affect trends analyses.


**Summary:** Siscowet lake trout are fattier and more contaminated at the same length than lean lake trout. Lipid, DDT, and PCB levels in five siscowet and their eggs were assessed. Although lipid levels in the muscle was significantly greater than that of lean lake trout from Lake Superior and Lake Michigan, eggs from siscowet had about the same lipid concentrations as the leans. Also, while lipid levels in muscles of both leans and siscowet trout tended to increase with age, lipid levels in egg concentrations tended to change little across species or across sizes. There was generally a tight relationship between PCBs in muscle and eggs for both leans and siscowet. A similar relationship occurred with DDT.

**Relevance to FCMP:** Potential loss rates of contaminants with spawning is different for lake trout and siscowet. Disposition of organochlorines within an organism follows the same trend as lipids.

Summary: Lean lake trout and the endemic siscowet are ecologically distinct phenotypes of char in Lake Superior. The authors examined growth and contaminant concentrations using data on whole fish and fillets with skins collected by the Wisconsin Department of Natural Resources. Fish were not aged, so the ages were estimated from age-length data from 1989 fish (lean lake trout) and 1993 (siscowet). Lean lake trout grew faster in length and weight than siscowet, but siscowets had higher lipid concentrations. Lipid levels increased with age/length in siscowet. This was also initially true with lean lake trout, but lipid levels reached a maximum for lean lake trout at about 11 years or 70 cm length. After this, lipid levels began to decline for very large/old lake trout. However, PCBs and other organochlorine compounds tended to increase with size/age for both types. The authors regressed ln concentration vs. both length and age, but no explanation was given for this choice. The two types of fish have very similar PCB concentrations at the same age, but siscowet had about twice the concentrations as lean lake trout at a specific length. PCBs and other organochlorine compounds positively correlated with lipids in siscowet, but negatively correlated with lipids in lean lake trout.

Relevance to FCMP: Lipids were inversely correlated with organochlorines in larger, older lean lake trout. The results imply that lipids are not a controlling factor on total bioaccumulation of organochlorine compounds. The analysis also suggests that interpretation of bioaccumulation dynamics depends on underlying assumptions of about how bioaccumulation works. Plotting organochlorine concentrations vs. age gives a very different interpretation than plotting organochlorine concentrations vs. length. The authors assume that age is critical to bioaccumulation, but ages were estimated based on fish length, not on actual age measurements. The authors regressed log concentration of organochlorines and lipids vs. both age and length for both varieties, except for lipids vs. length and age in lean lake trout, where the relationship was a complex function.


Summary: To investigate spatial and temporal patterns of organochlorine concentrations in lake trout from Wisconsin waters of the Great Lakes, the study examined laboratory contaminant analysis data of fillets with skins from Lake Michigan (n = 317) and Lake Superior (n = 53) fish collected between 1975 and 1990. These data were compared to previous Wisconsin samples to determine temporal trends. Because samples varied in size from year to year, the authors subsampled from their distribution of samples as follows. They excluded large or small fish until the residual samples had an average length between 620 and 640 mm. After this, PCBs were determined to have decreased over time by about 10 percent per year from 1975 to 1990. Dieldrin and chlordane also declined about 12 percent per year from 1983 to 1990. The bioaccumulation rate of PCBs was significantly lower for lake trout inhabiting Lake Michigan’s midlake reef complex, compared to lake trout from the nearshore waters of western Lake Michigan, and organochlorine compound concentrations were greater in Lake Michigan lake trout than Lake Superior fish. Lake Superior lean lake trout and siscowet exhibited similar
rates of PCB bioaccumulation by age, but not by length, despite major differences in lipid content. The authors suggest that the similarity of concentrations by age, but not by lipid, demonstrates a lack of effect of lipids. Relative concentrations of the various organochlorine contaminants found in lake trout were highly correlated, suggesting similar “mass balance processes” for these compounds.

**Relevance to FCMP:** The authors conducted post-hoc virtual sampling to control for size-effects, which provides another method to control for size in addition to multivariate statistics and stratified sampling. They tested temporal trends on log transformed data, assuming first order kinetics. They also compared trends across varieties of lake trout, and suggest that lipid is not important in bioaccumulation of PCBs. However, this assessment is dependent on the assumption that age, rather than size, is paramount to bioaccumulation.


**Summary:** A model of benthic/pelagic food web bioaccumulation was parameterized to predict concentrations of nine nonmetabolized PCB congeners in invertebrates and fish from Lake Ontario. Predicted concentrations were compared with observed concentrations, for those species with observed data, to verify the model. The model was able to reliably predict concentrations for those species: 86 percent of predictions were within a factor of two of observed concentrations. They also used the model to estimate breakdown of 15 PCB, dioxin, and furan congeners that are potentially metabolized. Even for these chemicals, estimated metabolism was slight and was generally much lower that total excretion. The model was also used to predict concentrations of PCB, PCDD, and PCDF congeners in invertebrates and fish species for which no field measured data exist. Predicted bioaccumulation factors for benthic-eating fish such as channel catfish and common carp were generally between those for the young-of-the-year fish and predatory salmonids.

**Relevance to FCMP:** Bioaccumulation is clearly a function of diet, which varies from species to species and time to time. The data also suggest that some species may be tied to the water column food chain, and others may be tied to the sediment food chain. Therefore, there may be different rates of decline if the water and sediments decline at different rates.


**Summary:** The authors argue that bioindicators should be highly contaminated (e.g., chemical concentrations are easily measured), reflective of concentrations in other media in the environment, widely distributed across systems, and easy to sample. They compared dorsal muscle and liver of coho salmon with gull eggs (composite samples of 9 or 10 eggs). They also took prey fish out of the stomachs of the larger fish, homogenized this tissue, and estimated concentrations in them. They then looked at coefficients of variation for each medium. The
coefficients of variation were generally 12 to 20 percent for six herring gull composite samples, whereas coefficients of variation averaged about 50 percent for 28 coho samples. Total weight of fish had about the same coefficient of variation as organochlorine concentrations, but the coefficient of variation for total length was only about 13 percent. For coho muscle and liver, there was no difference between organochlorine concentrations in male and female fish. Organochlorine concentrations were fitted to weight, log weight, lipid, and length. All contaminants except oxychlordane and beta-HCH were significantly correlated to all parameters. Lipid worked as well as weight for most organochlorines, but lipid worked better for HCB, heptachlor epoxide, and dieldrin. Most organochlorine concentrations correlated with all other organochlorine concentrations. The authors compared residues of organochlorines in the different species to DDE (“which is known to be excreted very slowly”), and found that ratios for very hydrophobic organochlorines were fairly constant across species, whereas ratios for less hydrophobic organochlorines tended to be more variable across species. Across species, correlations between lipid and organochlorine concentrations were weaker for more hydrophobic organochlorines (PCBs, DDE, Mirex), which are more difficult to excrete. Muscle had higher organochlorine concentrations than livers, but muscle also had higher lipid levels.

Relevance to FCMP: An ideal biomonitor of trends of organochlorines has certain characteristics: ease of sampling and analysis, high precision, widespread distribution across systems, and ability to reflect concentrations in other media. The authors present multichemical analysis and multimedia analysis, using data across chemicals and media to support inferences about trends in the other chemicals or medium. There was a weaker relationships between lipids and strongly hydrophobic, highly biomagnifying chemicals than between lipids and less hydrophobic/non-biomagnifying chemicals. This finding suggests that lipids are not that important to biomagnification.


Summary: The authors suggest that monitoring of trends is useful for evaluating effectiveness of regulations and control measures. In 1967, they set up a monitoring program for PCBs and DDT in uncontaminated (e.g., no known discharges) freshwater and marine areas of Sweden. They used pike as a monitor of freshwater systems because they are stationary in a lake. For marine systems (the Baltic Sea), herring younger than 4 years were chosen because older herring have higher variability, probably because they migrate more than young herring. Previous work suggests that PCB and DDT levels do not vary as function of age, size, or gender, but these concentrations do vary as a function of lipid. Therefore, all data were expressed on a lipid-weighted basis. The researchers collected 10 to 20 pike from each of two lakes each spring. Herring, 25 fish, were collected in the spring from two marine areas, and 20 fish were also collected from several areas in the fall. Lipid-weighted concentrations in herring tended to be higher in spring, when lipid levels were low, than in fall, when lipid levels were high. The researchers assayed declines by viewing yearly concentrations with 95 percent confidence intervals. They found that the rate of decline of DDT was about the same in all of the systems even though the residence time of the water was very different. They concluded that particle settling, which should be similar across sites, is a dominant factor controlling
decline. They compare and contrast declines of DDT and PCBs across chemicals and across marine and freshwater systems. PCBs started to decline earlier in the lakes than in the Baltic, which they suggest is a local vs. global phenomenon.

**Relevance to FCMP:** Indicator fish species are chosen because they do not migrate and, therefore, have lower variance and greater ability for detecting trends. Comparing trends across systems and across chemicals can illustrate potentially important phenomena about sources and fate processes.


**Summary:** Concentrations of mercury, methylmercury, and organochlorine compounds were measured in the sediment, plankton, roach, and pike of clean and polluted lakes in central Finland. There was a 30-fold difference in the concentration of mercury in sediment of the most contaminated lake compared to the purest lake. DDT and PCB concentrations were also higher in the contaminated lakes than in the pristine lake, where both organochlorines were below detection. Mercury was used as a biocide in pulp mills, and its sediment concentrations correlated with other biocides such as HCB, but did not correlate with DDT. However, total mercury and HCB in sediments also correlated with PCBs, which was never used as a biocide. The researchers collected large plankton, mostly zooplankton, with a 300-µm plankton net and small plankton, mostly phytoplankton, with a 25-µm net. There was no difference in concentrations of mercury, PCB, or DDT in plankton between lakes, but concentrations were very variable over time so the precision of these comparisons was low. Mercury concentrations in roach, a bottom feeder, also did not differ between lakes. Roach from different lakes were different sizes, but “[c]ovariance analysis showed that the weight of the fish [roach] did not have any effect on mercury concentrations.” DDT in roach was highest at the pristine site, but PCBs were highest at the more polluted site. Lipid-normalized values were similar to those not lipid-normalized. Of the three lakes, mercury concentrations in pike were highest at the lake with intermediate mercury concentrations in sediments, even though the pike from the most contaminated lake were about 10 percent larger, by weight, and sediment concentrations were 2 to 3 times higher. With pike, the authors found mercury concentration correlated with weight and age, after log normalization of mercury concentrations. Using lipid normalization, they found evidence of food chain enrichment for DDE, PCBs, and HCB. Roach to pike biomagnification factors (lipid-normalized) were about 2, 1.5, and 8 for DDE, PCBs, and HCB, respectively. The relative enrichment in the food chain was similar for total mercury, which had a biomagnification factor (wet weight) of about 3.

**Relevance to FCMP:** Mercury bioaccumulation as a function of abiotic concentrations is different than with organochlorines. Across different lakes, PCB concentrations in roach had the same pattern as PCBs in sediments, but mercury in fish did not follow the same pattern as mercury in sediments. Bioaccumulation of mercury may be more affected, or affected differently by lake limnology, than bioaccumulation of organochlorines. Based on these lakes, mercury in fish was a poorer bioindicator of ambient ecosystem levels than organochlorine in fish. Analyses suggest that mercury did not vary with size for roach, but did increase with size...
for pike. This might suggest that concentrations in prey additional factor in mercury bioaccumulation along with size effects on net growth efficiency.


Summary: This study measured bioconcentration of a number of metabolizable (e.g., PAHs) and non-metabolizable hydrophobic organic compounds by fish eggs and larvae of a number of species. Given their higher gill to volume ratio, larvae reach equilibrium faster than larger fish, and probably obtain more of their total dose of chemicals from the water. The authors found that biotransformation was higher in warm water than cold water fish. The authors suggest that this is a temperature effect on metabolism and, ultimately, bioconcentration.

Relevance to FCMP: For somewhat metabolizable chemicals, there may be a temperature effect on bioconcentration. However, the effect on bioconcentration was minor, suggesting that this effect may not be noticeable for chemicals that are significantly biomagnified.


Summary: There are chemically different classes of lipids, neutral and polar, the latter primarily associated with membranes. There are also analytically distinct lipid classes based on the degree to which they are extracted with different solvents. Thus, the effect of normalizing to total lipids will depend on how total lipids are defined and extracted. The authors suggest that some lipid extraction should be done at room temperature because lipids are heat sensitive, but some analysts report hot solvent extraction. On the other hand, alcohol extracts components other than lipids, so an alcohol extraction will overestimate lipid levels. To examine the effect of this characteristic, the researchers compared four common extraction methods: acetonitrile, acetonitrile and pentane, acetonitrile and pentane, and methanol and chloroform. They also did intra- and interlaboratory analysis with five replicates of tissue samples sent to three different laboratories who performed the extractions with a single method. The variability within a laboratory on replicate tissues was very low, with a mean coefficient of variation of about 5 percent. Precision among laboratories was also good, with a coefficient of variation of about 10 percent for the three laboratories. The comparison between extraction methods yielded high variability. Generally, two solvent extractions yielded higher lipids levels than the single solvent (acetonitrile alone), and chloroform/methanol yielded about twice the lipid as the other two-solvent extractions and about three times the lipid of the acetonitrile extraction. There might also be effects of tissue type and tissue amount on lipid extraction.

Relevance to FCMP: The total lipid extracted by different extraction methods may vary by factor of 3 to 4. Variability is primarily due to differences among extraction methods, but there is also low level variability due to tissue amount, laboratory, and different performance within the same laboratory. Lipid-normalized values will incorporate this variability. When comparing lipid normalized data, it is especially important to make sure that lipid methods are
the same. In terms of lipid amounts extracted, the solvents can be ranked as follows: acetonitrile < acetonitrile and pentane ≈ acentone and pentane < methanol and chloroform. Therefore, lipid-normalized values using these methods would follow the opposite trend, assuming that persistent toxic substances extraction was equal.


Summary: The authors extracted lipids, PCBs, and DDT from white croaker muscle using hexane and mixture of chloroform and methanol. The hexane is supposed to extract the neutral lipid, while chloroform/methanol will also extract membrane-bound lipids. Chloroform/methanol extracted about 1.4 times more PCB than hexane but an identical amount of DDE. Chloroform/methanol extracted about 4 times more lipid than hexane. Muscle was about 1.25 percent lipid vs. 0.3 percent lipid with chloroform extraction, so lipid-normalized concentrations could be very different depending upon which lipid extraction method was used. If different lipid methods are used for chemical and lipid extraction, the differences in lipid-normalized concentrations across studies could be as high as 4- to 5-fold.

Relevance to FCMP: Concentrations of both lipid and organochlorine are a function of the solvent extraction method. This adds to potential pitfalls of lipid normalization and comparisons of lipid and organochlorine concentrations across methods. However, this study was done on a lipid-poor tissue (muscle) of a low-lipid fish. The membrane bound vs. neutral lipid components might not vary so much in lipid-rich tissues and lipid-rich fish.


Summary: This study attempted to test the importance of dietary sources, as opposed to bioconcentration, on PCB uptake, by comparing PCB concentrations in the same species in different lakes with the same loading but different trophic chain lengths. The authors noticed a wide difference in PCB levels in lake trout in the many lakes in Canada, and also noted wide differences in trophic chain lengths. They classed the lakes with lake trout but no mysids and forage fish (e.g., smelt) as Class 1, those with lake trout and smelt but no mysids as Class 2, and those with all three as Class 3. Based on analyses in some of the Class 3 lakes, they conclude that lake trout eat forage fish, which eat mysids, which eat mostly smaller zooplankton. In Class 2 lakes, the forage fish eat mostly smaller zooplankton. In Class 1 lakes, the lake trout must eat zooplankton and benthic invertebrates for much of the year because they are isolated from warm water forage fish by the thermocline. A total of 83 lakes with PCB concentrations were classified, with about one-third in each class. They also divided the lakes into southern lakes (those close to sources) and northern lakes, which were far from sources. PCB concentrations in southern lakes were about two to three times higher than in northern lakes. PCB concentrations in Class 3 were about twice those in Class 2, which were about twice those
in Class 1. However, different classes of lakes also varied in terms of fish size and lipid content, so they conducted multiple linear regression, which found that both fish size and lipid content were also significant predictors, along with trophic length and location. Regressions were conducted with log PCBs vs. log length or log lipid concentrations.

**Relevance to FCMP:** Food chain length had significant effects on PCB levels. When controlled for differences in lipid levels or fish length, an extra link in the food chain added 40–60 percent more to the PCB concentrations. Trends across time and space must control for food chain length.


**Summary:** A technique was developed using mercury-labeled methylmercury as a tracer. According to the authors, methylmercury permits direct determination of the assimilation efficiency from food and the biological half-life of chlorinated hydrocarbons of individual fish. By comparing amounts of chlordane in the fish, which could respond due to lipid levels, to amounts of mercury in the fish, which should not respond to amount of lipid in the fish, the method should show differential absorption of the hydrophobic substance. The method also might be able to account for confounding factors such as differential growth dilution over the depuration periods, because this should affect mercury and chlordane similarly. Data show that absorption of chlordane was generally related to lipid content. However, the relationship was not quantitatively very strong; tripling lipid levels from 1.5 to 4.5 percent increased net absorption by only 30 percent. A similar effect was seen with white sucker. Fish with triple the lipids had body burdens that were only about 20 percent higher. The authors suggest that depuration was also dependent on lipid levels, but they did not account for higher body burdens in the more lipid-rich fish at the outset. There was, however, potentially an effect seen on the rate of depuration.

**Relevance to FCMP:** Lipid levels may impact both net absorption and depuration, although the effect is unlikely to be strong and is not isometric. Lipid normalization is therefore, probably not appropriate, but statistical methods that include lipid as an independent variable may be appropriate.


**Summary:** The authors reviewed the literature on PCBs and DDT in the Great Lakes ecosystem in an attempt to explain between-basin and between-species variation in fish contamination. Empirical models were developed, using log-linear multiple regressions, to link tissue contaminant concentrations in a wide variety of fish species to environmental levels.
(water and sediments) as well as basin-specific ecological attributes such as fish lipid content, trophic level of the fish, and the trophic structure of the food chain. Lipid levels were the best predictor of contamination levels when only a single predictor was used. Abiotic levels were not useful predictors until lipid levels were entered, after which other factors such as concentrations in water, trophic level, and predator yield/primary productivity, and log primary productivity increased. The authors note that lipid relationship between species is not linear. They suggest that shorter food chains (i.e., higher predator yield/primary productivity) produce lower fish concentrations. They compare the biota–sediment accumulation factor for PCBs to that for DDT and find very high correspondence, which “shows that the between basin variability in fish contaminant concentrations reflects major differences in the way persistent organic contaminants are partitioned.” It is not simply experimental or random error.

Relevance to FCMP: When different species of fish are compared, bioaccumulation is a weak function of abiotic environmental levels. The effects of the latter can be overwhelmed by other factors including lipid levels, trophic levels, and food chain length. The authors apply a multi-chemical analysis in which similarity of biota–sediment accumulation factors for PCBs and DDT, at different locations, is used as evidence that something other than loading is affecting concentrations.


Summary: Although gastrointestinal absorption of hydrophobic chemicals is critical to addressing the risk of these chemicals, little is known about factors controlling absorption. The authors did a mass balance study with seven human volunteers, measuring PCBs and dioxins in food and feces over three trial periods, each lasting 3 days. In one study, all volunteers were given the same food, which had the same concentrations of chemicals. In the second and third trials, the volunteers ate what they wanted, so concentrations of chemicals in food and feces varied from person to person. They also measured chemicals in blood of all the subjects 3 weeks after the end of the third trial. Mass consumption of PCBs and dioxins during the first trial, when everybody at the same food, were fairly similar for all seven subjects. For example, the dioxin toxicity equivalent of food ranged from 82 to 122 pg/kg with a coefficient of variation of 13 percent. Fecal concentrations were much higher and much more variable among subjects, with a mean toxicity equivalent of 1,507 pg/kg and a coefficient of variation of 56 percent. Net absorption tended to be negative for very highly chlorinated dioxins and furans and for older volunteers, but was positive for less chlorinated dioxins and furans and PCBs, especially for younger subjects. There was often a strong negative relationship between absorption of a compound and the blood levels of that compound. There were some exceptions—some rapidly metabolized PCBs did not show a relationship, but were efficiently absorbed by all subjects, probably because the blood levels were well below equilibrium with past food items. The generally strong negative relationship between blood levels and absorption and the increases in concentration between food and feces supported the hypothesis that absorption is due to passive diffusion. However, the authors found net absorption for compounds with a negative diffusion gradient, even when increases of fugacity due to digestion
were considered. The authors present a fat flush hypothesis, which suggests that fugacity of hydrophobic chemicals in blood near the intestine may be locally suppressed because the blood is absorbing fat from the intestine. This may provide a local diffusive gradient that allows passive transport of hydrophobic chemicals. This also suggests that the body may be simultaneously absorbing hydrophobic chemicals at one spot and excreting them at another.

**Relevance to FCMP:** This analysis provides evidence that absorption of chemicals is due to passive diffusion and that, when diet changes, excretion across the gut can exceed absorption. Analysis suggests that blood lipids are a critical component of dietary absorption and that the amount of lipid in food and lipid digestibility will also affect absorption of hydrophobic chemicals. In short, lipids are important to absorption of chemicals, but other factors—current body burdens, lipids in diet, lipid digestibility, and distribution of lipids in blood vs. body—are also important.


**Summary:** This paper presents a description of the FCMP for Wisconsin. In the early 1970s, the program primarily responded to instances of known contamination and problem areas. In 1976, statewide surveillance was begun. This FCMP has two elements: statewide screening and consumption advisories. For statewide screening, as part of the national water quality survey, fish from 29 sites across the state were monitored, and 17 additional screening stations were selected on tributaries to Lake Michigan. Carp (two five-carp composites per site) was selected as the monitoring species because it is fatty, long-lived, abundant, widely distributed, easily caught, and seldom sought by sports fisherman. For the consumption advisories, this FCMP tries to collect a wide range of sizes and species of sport caught fish.

**Relevance to FCMP:** The study describes how FCMPs often have different elements—statewide monitoring and issuance of consumption advisories. The study also proposes carp as an ideal biomonitor.


**Summary:** The dioxin congener 2,8-dichlorodibenzo-p-dioxin (DCDD) does not usually bioaccumulate or cause toxicity. To determine if this was due to lack of toxicity or lack of exposure (e.g., minimal bioaccumulation), the authors studied bioaccumulation and toxicity of DCDD in combination with an inhibitor of metabolism. DCDD alone was eliminated very rapidly from fish not treated with the inhibitor, piperonyl-butoxide, which did not cause toxicity by itself in control experiments. However, DCDD was not metabolized and was accumulated when the metabolic inhibitor was added. Therefore, metabolism decreased the amount of DCDD in goldfish and prevented DCDD from becoming lethal at the concentration used. DCDD, when not metabolized and bioaccumulated, appeared to be 60 times less toxic than TCDD. By comparison, in mouse and guinea pigs, DCDD is about 1,000,000 times less toxic.
suggesting that metabolism and excretion account for most of the decrease in toxicity. The bioaccumulation factor measured for DCDD was 17.2 with metabolism, compared to about 1,700 for TCDD, which is not metabolized.

Relevance to FCMP: Bioaccumulation of different dioxin congeners varies dramatically, probably due to differential metabolism. Metabolic function should vary across individuals and species.


Summary: The author presents a multimedia, multichemical, and multimethod analysis to address potential problems with confounding factors affecting PCB concentrations in any one medium. Multichemical analyses are based on two basic assumptions, which are supported by data and theory. First, inventories of abiotic PCB and other organochlorines in large lakes are inherently inertial (i.e., resistant to rapid changes over time). Second, external sources of different organochlorines are unlikely to be coordinated with each other or be sufficiently large to significantly affect total inventories in the lake. Therefore, synchronized year-to-year or short-term changes of two or more organochlorines are indicative of changes in loading or abiotic inventories. Rather, these synchronized changes are likely due to some internal factor, such as food chain events, that affect final bioaccumulation of several organochlorines in a synchronous manner. The highly concurrent year-to-year and medium-term dynamics of both DDT and PCB concentrations in lake trout and gull eggs, for example, is evidence for the importance of internal factors, likely changes in the food chain. There is also value in comparing trends across media, because the factors that confound temporal or spatial trends in one medium are unlikely to be coordinated with the confounding factors affecting other media. Lastly, the modeling and theoretical literature should be consulted to determine appropriate null hypotheses for spatial and temporal trends analyses. Contrary to many trends analyses, declines of organochlorines in Great Lakes systems are likely to be a first-order process with maximum long-term declines of 5–15 percent per year, depending on the chemical and lake. Trends analyses that employ zero order kinetics address a trivial null hypothesis.

Relevance to FCMP: Temporal and spatial trends of organochlorines in biota are confounded by a number of factors that affect concentrations more profoundly than potential changes in abiotic inventories or external loading. Comparisons of trends across chemicals and across media are a simple, inexpensive way to identify confounding factors. Trends analyses must employ an appropriate null hypothesis.


Summary: The author suggests that many trends analyses of organochlorines in biota are based on an assumption—concentrations of organochlorines in biota data faithfully reflect levels of abiotic inventories and external loading—that is not supported by theory or data. Because PCB
concentrations in lake trout are largely due to diet, which varies over time and space, there is no expectation that declines of chemicals should be smooth or consistent over time. Because resulting declines of PCB are “wavy,” analysts can profoundly affect results by arbitrary sub-selection of data. Year-to-year trends and long-term trends in lake trout and coho salmon show concurrent waves between DDTs and PCBs, which cannot easily be explained by underlying changes in abiotic concentrations or external loading. In contrast, this concurrence can be explained by underlying changes in the food chain, and short-term changes in PCB concentrations correspond to short-term changes in the density of their primary prey.

Relevance to FCMP: Confounding factors, such as changes in the diet, are often a much better explanation for short-term trends of persistent toxic substances in biota. Comparing trends across chemicals is a method to qualitatively identify effects of confounding factors on short- and long-term trends. Because there are periodic waves in concentration data over time, long-term trends analyses are extremely sensitive to choice of starting and stopping points.


Summary: Mathematical modeling of persistent toxic substances in chemicals predicts that internal inventories (i.e., the sediments) of PCBs are slow to respond to changes in external loading. When loading decreases faster than internal inventories can respond, subsequent declines in PCBs for the slow responding in-lake media are determined by the lake’s ability to depurate PCBs, which is largely independent of external loading. Available data on external loading suggests that this may have occurred in Lake Superior over the last several decades. Available data on external loading, and concentrations of PCBs in the water column, settling sediments, lake trout, smelt, and gull eggs were collected, and first-order rates of decline for each medium were determined. Loading and fast responding in-lake media (water column, settling sediments) were declining significantly faster (about 17 percent per year) than slow-responding media (sediments, lake trout, smelt, and gull eggs) were declining (about 5 to 7 percent per year). Both the qualitative differences and quantitative magnitudes of decline were consistent with predictions of models.

Relevance to FCMP: The method used in this analysis is based on the assumption of a first-order decline for all media. Rates of decline of persistent toxic substances in fish, over times scales of decades, may be completely independent of declines in external loading and of PCBs in the water column. As PCBs in Lake Superior biota are apparently falling at a terminal velocity determined by the interaction of the physical-chemical characteristics of the PCBs and the lake’s limnology, speculation about slowing of declines and new equilibria for PCBs was incorrect.

Summary: Mercury concentrations in precipitation, lake water, sediment, zooplankton, and fish were measured along with extensive watershed and lake chemistry data for 80 lakes in northeastern Minnesota. Atmospheric deposition of mercury, water column lifetimes, and sedimentation in lakes were estimated. The authors controlled for size effects on mercury concentrations by estimating concentrations in a uniform size. Northern pike tended to be less contaminated, about 20 percent, than walleye at the same length. Lakes were then typified by the mercury concentrations estimated in the standard length of walleye and northern pike. These standard lengths were chosen at the lengths of the two species at which their estimated concentrations showed maximum correlation. The authors first did correlation analyses between fish concentrations and other factors, followed by step-wise regression of significant correlates. A total of about 40 factors were considered, including the following: concentrations of several water quality parameters (e.g., pH, conductivity, chloride, magnesium, calcium, sodium); limnological factors such as flow through, mean and maximum depth, water volume, percent forest in watershed, and percent water in watershed; mercury concentrations in water, sediments, and zooplankton; and other factors. The most notable predictors for mercury concentrations in northern pike were mercury concentrations in zooplankton and water, total organic carbon concentration, and pH. The primary source of mercury was found to be of atmospheric origin.

Relevance to FCMP: Instead of sampling the same size, the authors focus upon a uniform size statistically with regression analysis. They determined that log-log transformation produced the best fit between mercury concentrations and fish length, for both walleye and northern pike. Mercury bioaccumulation varies from species to species, size to size within a species, and site to site depending upon physical-chemical-biological factors.


Summary: This report is a description of Indiana’s fish tissue and sediment monitoring plan. Historically, fish tissue sampling was used to issue fish consumption advisories and to “locate reaches of contamination.” According to the authors, coupling contaminant monitoring with a probabilistic random site selection enables estimates of chemical levels over entire watersheds and over time. “Approximately 80 sites will be generated randomly by the USEPA Corvallis lab selection approach.” At each site, whole creek chubs will be collected. Creek chub are proposed as an indicator species because they are widely distributed and are generally abundant at all sites except lakes. They also have small home ranges and indicate local conditions. They are also an important prey fish for larger fish and fish-eating wildlife. On the other hand, they are opportunistic carnivores, eating whatever is available. The author recommends that the mean length of fish in a sample be between 10.2 and 17.8 cm, but the smallest fish should be at least 90 percent of the length of the largest fish in a composite. An ideal composite would be
enough fish to total 400 g of tissue. On average, this corresponds to about seven fish. However, as little as 100 g of tissue would be acceptable, which might be as few as one or two individual fish. There is also targeted sampling of food fish from specific rivers and lakes to support the consumption advisory process. “Sampling sites were selected based on historical data, strategic positioning, and/or consideration of the amount of data” collected on this water body. Fish were analyzed as skin-on fillet composites of three to five fish. Catfish are processed as skinless fillets. A wide variety of fish species are collected and analyzed for a wide variety of contaminants.

Relevance to FCMP: This FCMP has determined that probabilistic site selection is necessary to characterize a site and determine trends. Creek chub were selected as the indicator species.


Summary: The author conducted an analysis of variance on 20 years of PCB concentration data from coho salmon, chinook salmon, brown trout, rainbow trout, and lake trout from Lake Michigan. Log PCB concentrations were the dependent variable. Year, species, length, and location were first order terms, and length times species was considered as a second order term. Length was apparently not log transformed. The location variable was based on state or sampling site. Not all samples had data for lipid, so residuals were regressed vs. lipid concentrations. The author looked at residuals for analysis of variance results without year, location, and species to look at the effect of each of these factors. The analysis showed that year, species, length, location, and length–species were all significant predictors. Residual plots without time showed, on a linear Y-axis, that concentrations “decreased rapidly from 1978 to 1981 and since have remained fairly stable.” There were differences among locations—a hot spot was noted near Sheboygan River. After PCB concentrations were corrected for the effects of time, length, location, and interspecies variability, there was no relationship with percent lipid content. The author suggests that the fast decline of PCBs may have been due to changes in forage stock.

Relevance to FCMP: In addition to year, PCB concentrations in the same lake are significantly affected by fish length, location of capture, species, and, potentially, collector/analyst. After these factors were considered, lipid was not a significant predictor.


Summary: The study used individual-based models to describe PCB accumulation and variability in Lake Michigan coho and chinook salmon. The models are based on a combination of simplified allometric relationships, such as the relationship between predator size and growth, respiration, and prey size. The models also use random elements concerning interactions between predator and prey and the number and size of prey consumed. To achieve the observed level of variability in PCB concentrations, different assimilation efficiencies were assigned to
individual fish. This approach conflicts with the Madenjian papers, which achieved the same goal by assigning different prey PCB concentrations to different fish. Fillet concentrations were converted to whole body concentrations by multiplying by 155 percent. According to simulations, growth rate had little effect on final PCB concentrations for either species for a specific age. However, growth rate profoundly affected fish size. Therefore, fish of a certain size tended to be less contaminated under high growth compared to low growth conditions, although this effect was not large. According to model results, age is a better predictor of contaminant level than is size. Model results suggest that a 10–15 percent reduction in PCB levels for fish of a given size can be obtained by managing for high growth rates. The models also show that prey PCB concentration strongly affects PCB levels in the salmon; however, management options for controlling prey concentrations of PCBs are fairly limited and expensive.

**Relevance to FCMP:** PCB concentrations vary as function of age and, to a lesser extent, fish growth rate. Age may be a better predictor of concentration than size. Variability in prey concentrations over time and space critical to concentrations in predators, but variability of predator PCBs could also be due to individual differences in assimilation efficiency.


**Summary:** The authors considered the following models for PCB decline over time: first-order decline, double exponential, and non-zero asymptote. They suggest that the models imply different sources of PCBs with different long-term fate potential. Specifically, the single exponential suggests one dominant source, the double exponential suggests two different declining sources, and the non-zero asymptote suggests one declining and one essentially constant source. The authors log transformed concentration data prior to analysis, and conducted linear regression for the single exponential model. However, they used a non-linear regression for the other two models because log transformation does not produce a linear model. The degree to which the models fit the data was tested with the extra sum of squares principle, and corrected for transformation bias with a correction factor equal to exp(mse/2), where mse is the mean square error. For both fish species, the double exponential model fit the data best, and the second exponential was positive suggesting that PCB concentrations are going to increase. The rates of decline for the different fish were very similar for the single exponential model but different for the double exponential and non-zero asymptote models. The authors suggest that initial decline due to cessation of inputs after which other factors, such as food chain, become more important.

**Relevance to FCMP:** Models other than single exponential may explain observed data better. Rates of decline may not be constant over time or across species. Factors other than external and internal sources may affect long term trends. Food chain effects may overwhelm trends in abiotic concentrations.

Summary: The authors examined PCB concentration data for seven species of Lake Michigan fishes to determine temporal trends. They looked at three models for decline: single exponential, single exponential with asymptote, and double exponential. The paper provides a detailed description of the methods used to address the mix of data from individual fish and composite samples; the authors conclude that composites should be weighted by number of fish. They conducted regressions of log PCB concentrations vs. time, but did not consider effects of size, agency, analytical method, or location. PCB concentrations in all seven species—lake trout, rainbow trout, brown trout, chinook salmon, coho salmon, alewife, and bloater chub—declined and appeared to stabilize in the mid- to late 1980s. Concentrations in two species, chinook and coho salmon, appear to have increased slightly since the late 1980s. The authors conclude that changes in rates of declines are due to different sources over time, but for the chinook and coho, the changes are also potentially due to food chain and growth effects. The authors present a power analysis of the sampling. They calculated sample size necessary to detect a change in concentration based on variance of concentrations, size of PCB decrease that could be predicted, and the Type 1 and Type II error. According to their estimate, detecting small changes (e.g., 10 to 15 percent) in PCB levels may require samples of 1,000–2,000 fish because of the high variability in PCB concentrations among individuals.

Relevance to FCMP: Declines of chemicals over time may fit models other than simple exponential decay, potentially due to changes in source dynamics, food chain, and/or growth rates. Sampling sizes necessary to detect small changes in PCB concentrations (e.g., 10 percent) may be quite large, as many as several thousand fish. The authors present a detailed discussion of methods for combining data from composites and individual fish.


Summary: The authors examined the relationship between PCBs and lipids in individual salmonids from Lake Michigan from 1984 to 1994. There are strong relationships between mean total PCB concentration and percent lipid for different species. However, within individuals of the same species, there were only modest positive relationships in brown trout, chinook salmon, coho salmon, and rainbow trout during spawning, but even these weak relationships were not apparent among non-spawning individuals. Lake trout never exhibited a discernible PCB:lipid relationship. Lack of lipid:PCB effect might be due to combining fish from different areas and monitoring programs. However, their analyses controlled for this by plotting residuals from log total PCBs on year, log length, and location vs. residuals of log lipid on log length, year, and location. The authors discuss several caveats. Analyses might be more convincing if based on whole fish, and imprecision of lipid measurements might overwhelm any relationship between PCBs and lipids. However, the authors also point out that the positive relationship might be due to analytical variability because lipids and PCBs are often extracted simultaneously from the same sample. Therefore, fish with high extraction efficiency for lipids,
and consequent high lipid concentrations, will tend to occur with fish with high extraction efficiencies for PCBs, and higher PCB concentrations. In their discussion of results, they state: “Possibly, within an individual, contaminants may accumulate in lipids, but lipid concentration may be unimportant in the mechanisms governing contaminant assimilation.” These results suggest that lipids are not a major factor influencing contaminant uptake. With respect to Hebert and Keenleyside’s (1995) recommendations that lipid normalization is warranted only when a significant relationship exists, they state: “But what are the consequences if a relationship exists and the observed correlation is spurious? More precisely, how is mechanistic inference affected if the cause-effect relationship is attributed to an extraneous variable?”

Relevance to FCMP: This paper provides more evidence that lipids are not critical to bioaccumulation, suggesting that lipid normalization is not warranted. There is limited discussion that lipid normalization may obscure important factors. The authors conducted many combinations of regressions and settled on log concentration vs. log length as a predictor, although the rationale for this selection is not clear.


Summary: An age-dependent, food chain model that considers species bioenergetics and toxicant exposure through water and food was developed. The model assumes that absorption efficiency is constant (i.e., stomach absorption and excretion is not a function of concentrations of lipid or chemical in food or lake trout concentrations). The model also uses growth as a \textit{de facto} loss rate. The model suggests that for the top predator, lake trout, PCB exposure through the food chain can account for more than 99 of the observed concentration. The lipid partitioning model was inadequate to describe observed data, even using a high value for $K_{ow}$.

Relevance to FCMP: The model predicts increasing concentrations with increasing sizes for both trout and alewives, even after their prey concentrations had nearly stabilized. The paper suggests that growth dynamics and inverse relationship between body size and gill excretion are important to the size–concentration relationship and that lipid and increasing prey concentrations are less important. Almost all exposure to PCBs was found to be via food, emphasizing the importance of the food chain.


Summary: Twenty sport-caught brown trout (\textit{Salmo trutta}) from Lake Ontario were selected for this study. Total weight, total length, sex, age, and condition factor (K) for each fish were determined. A fillet from one side of each fish, including rib cage and scaled skin, was removed using a standard filleting technique. This is the “standard fillet” used by New York. The opposing fillet was trimmed using the fat-trimming procedures recommended by New York State for reduction of fat-soluble contaminants. Trimming resulted in substantial loss of fish, with a mean loss of 41.6 percent of the mass, compared to the untrimmed “standard fillet.”
However, this loss was mostly composed of what is “generally considered inedible or unpalatable body parts.” The standard and trimmed fillets were analyzed for total fat content and for concentrations of PCBs and Mirex. Trimming significantly reduced total fat (61.8 percent), PCB (45.6 percent), and Mirex (44.2 percent) levels compared to the standard fillet. Reductions in fat tended to be about the same as the reductions in organochlorines. There were negative correlations between weight, length, age, and condition and the observed reductions, with trimming, in both fat content and contaminant concentrations. That is, trimming tended to be less effective for larger, older fish. Sex of brown trout was unrelated to fat content and contaminant levels.

**Relevance to FCMP:** Concentrations of both fat and organochlorines will depend on the method of filleting. Therefore, fillet data from different agencies or even from the same agency at different times may not be comparable. Disposition of organochlorines within a fish tends to follow lipid levels.

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**Summary:** To understand data for fish consumption advisories and trends analyses, it is necessary to understand seasonal variability in mercury concentrations. About 25 largemouth bass were collected from Lake Lillinonah and Pickerel Lake, Connecticut, during three sampling periods in 1996: spring (May 14–15), summer (July 22–30), and fall (October 10–17). Total mercury concentrations, in skinless fillets, ranged from 0.106 to 2.293 µg/g in Lake Lillinonah and from 0.456 to 1.762 µg/g in Pickerel Lake. Within a lake, there was no difference between mercury concentrations in male and female fish. In both lakes, there was a significant positive relationship between log total mercury concentration and length and age during each season. The authors used an analysis of covariance to test for differences in the log concentration-length relationship between seasons. Mean mercury concentrations adjusted for length were 26–43 percent higher ($P < 0.01$) during spring than in summer and fall in both lakes. A similar, but weaker relationship was found with age. In Lake Lillinonah, mean mercury concentrations adjusted for age were significantly higher during spring than during fall ($P < 0.017$), but log-concentration and age relationships were not significantly, but just barely ($P = 0.0615$), different for Pickerel Lake. For any one season, age was about as good a predictor of log mercury as length, as measured by $r^2$. Results similar to other work with other species (pike, mummichog, roach) show higher mercury concentrations during spring, but some other results show no difference with season.

**Relevance to FCMP:** For bass, bioaccumulation may vary by season. This suggests that researchers may want to assess mercury concentrations in largemouth bass during standardized sampling periods so that data can be more accurately compared among water bodies and tracked over time. The authors used an analysis of covariance to compensate for age and length effects. They did regressions of log concentrations vs. length.
Summary: Sampling of chemicals in fish is a compromise between available funding, logistical factors, and the limits of statistical inference. Highly replicated sampling of multiple size classes of multiple species is an ideal that conflicts with the reality of limited resources. The goal of the thesis was to develop a data analysis methodology for monitoring mercury in fish that minimizes replication and sampling costs without sacrificing accuracy. The author developed a statistical model in which log mercury in fish was a function of several components. There is a “community module,” which includes limnological factors, such as lotic or lentic habitat, and endogenous factors, such as tissue type, species, and fish length. There is also a temporal trends module, which considers underlying changes in abiotic concentrations and analytical bias, and an effects of reservoirs module. The community module consists of regressions of log mercury vs. log length segregated by tissue type and fish from lotic vs. lentic habitats. The temporal model used as independent variables year, year$^2$, and year$^3$ as well as a factor for the laboratory that did the analysis. The reservoir module considered both the limnology of the reservoir as well as its age, both of which are thought to affect mercury methylation. The author also added a spatial component to the model. The latter allocated the residual error from the previous model to error that varied with location of sample and that residual error which did not. A data set of 915 samples from 268 sites (353 sampling events) from streams in Indiana was used to test this model, with a maximum likelihood estimate method. The model results were checked for validity before acceptance. For example, if a statistically significant relationship between mercury and size was produced by the statistical methods, the relationship was compared to the literature and theory before being accepted. The model results suggest that fish from lotic environments have considerably higher concentrations than fish from ponds and lakes, but this difference was species specific. Carp tended to show the largest differences between habitats. For mercury, whole fish concentrations exceeded those of fillet with skins, which exceeded those of fillets without skins, but the ratio depended upon fish species. The model also found a positive relationship between mercury concentrations and fish length for all species with adequate data. Again, this relationship tended to vary from species to species. Log-log transformation tended to provide better fits to data than analyses with untransformed data. The relationship between size and mercury concentration differed by trophic level. Detritivores like carp had lower concentrations and lower slopes of mercury concentrations vs. length, than piscivores like largemouth bass. The analysis of Indiana’s data set estimated a decline in statewide biotic-mercury concentrations of 23 percent between 1983 and 1991. The majority of this decline is thought to be due to reductions in regional-scale atmospheric mercury deposition rather than changes at the global or local (point source) scale. The model determined that there were sometimes significant differences in predicted concentrations in several upstream vs. downstream samplings, but all sites predicted to be different when location was considered were also predicted to be different when location was ignored. This result implies that differences were due to other factors, not location downstream of putative source. In addition, this analysis indicates flood control reservoirs are a significant source of mercury contamination in the waterways that receive the discharge of these reservoirs. Lastly, three applications are demonstrated using this model to predict fish tissue concentrations, measure deviations from background conditions, and perform valid hypothesis
testing. The author addresses concern about stability of food chain and effects on trends. He suggests sampling of the food chain to confirm stability if there is evidence of change.

Relevance to FCMP: This study uses multivariate statistical analyses to estimate the importance and quantitative impact of various factors. According to the author, the method uses all data from past sampling and knowledge base to reduce the necessary level of future sampling. Results conform to previous analyses: mercury concentrations vary with fish size, species, trophic position, and limnological factors. Analyses suggest that log-log transformation of mercury concentration vs. length provides the best fit to the data. The author suggests that the model more accurately portrays background, leading to selection of more appropriate background sites and determination of effect over background concentrations. As with all empirical models, the predictions are limited by the assumption of constant ecosystem processes, a problem the author acknowledges with respect to food chain effects.


Summary: The authors considered the effect of size and season on concentrations of PCBs in trimmed, skinned fillets of chinook salmon collected from one site in Lake Michigan in May, June, and August 1988. Fish ranged in length from 15.75 to 37.75 in. and 1.5 to 17.5 lb. Total PCB concentrations ranged from 0.14 to 2.1 µg/g in the trimmed fillets. The mean concentration was 0.94 µg/g with a standard deviation of 0.43. PCB concentrations in spring-caught fish were significantly higher than in summer, but fish caught in fall were intermediate in concentration. However, fish caught in summer were also shorter and smaller, and the difference in PCB concentrations with season disappeared if PCB concentrations were normalized to length or weight. PCB concentrations varied significantly with size class, with smaller fish (<24 in.) having lower PCB concentrations than medium fish (24 to 32 in.), which had lower PCB concentrations than the largest fish (>32 in.). Regression of total PCB concentration against length and weight of the whole fish explained about 45 percent of the variability in PCB concentration. PCB concentrations were not significantly related to lipid levels, nor were lipid levels and fish length significantly related. There were also significant effects of fish gender on PCB concentrations, but these effects also disappeared when size was considered.

Relevance to FCMP: For chinooks, PCB concentrations do not vary with season when fish size is considered. For these samples, there was no relationship between lipid levels and fish length, nor between PCBs and lipid levels. This might be due to the extra variability added due to variability in trimming and skinning. The authors regressed PCBs vs. weight and length, the latter without transformation. However, inspection of residuals suggested that log PCBs vs. length would have yielded a better fit.

Summary: This study considered PCB concentrations in various trophic levels in a small stream contaminated with PCBs to 1) determine spatial and temporal trends, and 2) determine if trophic structure was critical to PCB bioaccumulation. The authors used an analysis of covariance (ANCOVA) with lipid as the covariate, as suggested by Herbert and Keenleyside (1995). They collected data on PCB concentrations in four trophic groups: fish, leeches, crayfish, and oligochaetes and chironomids. Fish were too small to eat either leeches or crayfish. Thus, all three were at about the same trophic level—predators of benthic invertebrates, although crayfish were also herbivores and detritivores like the benthos. The authors compared ln PCB concentration vs. time, which is an explicit use of first order kinetics. ANCOVA results suggested that PCB concentrations were a linear function of lipid, and there were also significant effects of station, time, and trophic level on PCB concentrations. Relationship between ln PCBs and lipid levels (e.g., slopes) were similar across stations (ANCOVA), time, and trophic level. (However, the replication number was small, and slopes were nominally very different with the leech.) Using ANCOVA, the authors corrected for lipid level and found that trophic level affected PCB concentrations. Lipid-adjusted PCB concentrations in the leech and fish were about 6 to 7 times higher than in the oligochaetes and crayfish. The authors conclude that lipid levels are important in PCB bioaccumulation in fish and other aquatic organisms. They suggest that biomonitoring should include several different trophic levels, and collection of the same species at the same time. Using ANCOVA, the authors found differences in PCB vs. lipid at different times of the year, potentially due to differential migration at different times of the year.

Relevance to FCMP: ANCOVA is one method to compare PCB concentrations, over time or space, in individual samples that vary by some important factor such as length, age, or lipid level. The method is sensitive to changes in PCB concentrations and changes in the relationship between PCBs and the covariate. Lipid levels are important to bioaccumulation, but trophic level is also important to bioaccumulation after compensation for lipid levels. However, within trophic level, lipid levels are likely a function of size and age, so a causal effect of lipids was not demonstrated.

Young-of-the-Year Fish Monitoring


Summary: For each year, the authors estimated the concentration of chemicals in fish of a specified size by regression with power curve, which is equivalent to log normalizing both length and concentration. Then, the temporal trends in the estimated concentrations over time were estimated with regression, the type of which (linear, logistic, inverse, power, exponential) was chosen based on the best fit (e.g., highest $r^2$ value). Generally, concentrations of PCBs and
DDT declined in both sportfish and spottail shiners; mercury declined over time in some areas but not at others.

Relevance to FCMP: This is a summary report of data from the Canadian Ministry of the Environment FCMP. In sport fish, the researchers control for some confounders by controlling for size of fish, with statistical analysis and production of estimated average concentrations at a standard length. They log transform both concentration and length, but no rationale is given. They use the $r^2$ value to select the type of regression for temporal trends. Exponential decline is usually but not always chosen as the best fit.


Summary: This paper reports information from New York’s young-of-the-year (YOY) fish program. YOY fish were collected with seine or electrofishing. Samples normally consisted of composites of 15 fish with 10 replicates, but only 7 of the 10 were used for PCBs and organochlorines. The other three were used for mercury and other chemicals. Spottail shiners were not available at 9 of 25 stations, but other minnows were collected when available. The authors compared samples with a $t$-test. They suggest that comparisons between species (e.g., spottail shiners at one site or time vs. emerald shiners at another site or time) may be done with lipid normalization. Coefficients of variance are generally around 20 to 40 percent with seven replicate composites. However, year-to-year variability can be high, in terms of both lipid and organochlorine concentrations. Lipid levels from site to site in any one year may vary as much as three-fold, and data show high unexplained interyear variability that is often concurrent with both PCBs and DDT.

Relevance to FCMP: YOY sampling programs illustrate the trade-offs between sampling of biota and abiotic media. Biota are often suggested as ideal biomonitors because chemicals are detectable with conventional methods and biota integrate concentrations over relatively large time and space. However, YOY fish are recommended because they are representative of a small area, and as very young fish, cannot integrate chemical concentrations for any longer than a short period. In addition, concentrations of several chemicals in YOY fish are too low to be detected with conventional analyses. Samples show relatively low within-sample variability, but variability between samples at the same spot can be high and erratic just as with larger fish.


Summary: This report states that most monitoring of food fish lacks “the ability to identify recent inputs of” chemicals because it integrates chemical concentrations over long term and over a large spatial scale. It also states that sampling of young-of-the-year spottail shiners is “an ideal surveillance tool for monitoring recent additions to the aquatic ecosystem.” The goal of
the monitoring is to measure changes in contaminant levels both spatially and temporally. The sample size is 150 fish from each location, which will be divided into 10 composites of 15 fish each. Both length and composite weight of individual fish are measured. Of the 10 composites, at least 7 are used for lipid, PCBs, and organochlorine analysis. This number was considered sufficient to allow statistical analysis of temporal and spatial trends.

Relevance to FCMP: Short-lived and less migratory fish might be a better bioindicator of recent and localized inputs.


Summary: Young-of-the-year fish were collected at several locations in Cayuga Creek drainage basin near Love Canal in 1987 prior to dredging and in 1990 and 1992 after dredging. The field team tried to collect the same fish species each year, but sometimes the same species was not available. They composited enough fish to obtain a sample of 10 g, if possible, and tried to replicate the composites. Generally replication was about three or less composites per species per site. Data showed reductions in TCDD concentrations before and after dredging.

Relevance to FCMP: Analysis demonstrates some of the problems with before and after sampling of small fish in small stream. Samplers were unable to obtain large numbers of replicates for any species at any site, and species changed in the area that was dredged. Results indicate that samplers need a clear understanding of the ecology and carrying capacity of the system prior to choosing species. At one site, there was about double the concentrations in age 1+ fish vs. young-of-the-year fish, indicating that age analyses must be precise.


Summary: This document is a work plan for ongoing sampling of fish from the Hudson River. The sampling relies on yearling pumpkinseed, which would indicate relatively short-term changes, yet would require a relatively small sample size and be available throughout the system. The young pumpkinseeds have a limited home range, making them good indicators of local conditions. The sampling goal is to collect 25 yearling pumpkinseed, with a minimum of 15 fish. Originally, the goal was to capture 75 fish, and divide them into 25 composites of 3 fish. However, the pumpkinseed had become progressively harder to find in recent years. This report questions whether the sampling itself might have reduced pumpkinseed numbers. Based on previous data, the author concludes that 15 individual analyses were sufficient to detect a 25 percent change in concentration. In years where variability is high, more than 15 individuals will be analyzed.

Relevance to FCMP: The author presents a discussion of the advantages of certain types of fish as indicators. The difficulty in finding their primary bioindicators illustrates problems with focusing upon one species of fish. The agency’s response—a power analysis and phased
analysis geared to satisfy a desired power—represents a methodical, statistics-based approach to planning of sampling.


Summary: Concerned with the effect on lipid normalization of changes in lipids over time and space, the researchers decided to focus on fall-caught yearling pumpkinseed (Lepomis gibbosus) as a standardized tool for monitoring trends in the PCB concentrations. Thus, pumpkinseed were collected and analyzed as composites, usually of three or four fish. The data were collected after cessation of the major PCB discharges at Fort Edward and Hudson Falls. There was a high degree of correlation between PCB concentrations in water and yearling pumpkinseed in the upper Hudson, but not in the lower Hudson River. They looked at the relationship between PCBs and lipid over time and space. Correlation between total PCB and lipid tended to be higher for lipid than for PCBs and length, especially at upstream sites where Aroclor® 1016 was the primary Aroclor®. The authors conclude that the system was reaching “equilibrium” from trends observed on the linear Y-axis.

Relevance to FCMP: The importance of lipid may be a function of water borne vs. sediment exposure, which may be a function of the type of PCBs. To control for changeable lipid concentrations over time, the authors settled upon a single age class of a single species at a particular time. They also compared trends in two media (water and fish) and looked for concurrence between them over time and space.


Summary: To assess the feasibility of alternate species as biomonitors, total PCB, DDT, hexachlorobenzene, and octachlorostyrene concentrations in young-of-the-year spottail shiners (Notropis hudsonilis) were compared with concentrations in emerald shiners (Notropis atherinoides) and yellow perch (Perca flavescens). Same-sized fish were taken from six different collection sites in Ontario, New York, and Michigan waters, and ages were determined. Total DDT concentrations in spottail shiners were significantly different (p < 0.05) from those of emerald shiners at two of the six sites compared. PCBs were significantly different (p < 0.05) at three of six sites, octachlorostyrene at two of six sites, and hexachlorobenzene at four of six sites. Fish size and lipid contents were not significantly (p > 0.05) correlated with contaminant concentrations, but this may be due to little variation in both size and lipid concentration. Contaminant concentrations were also sometimes different at various collection sites when spottail shiner and yellow perch were compared. The authors suggest that lipid content, fish weight, growth, and metabolic factors affect bioaccumulation, but additional factors affect bioaccumulation in the field, including habitat selection and food preferences. The paper states: “In order to reconcile contaminant concentrations differences among fish species, it is necessary to quantify the physico-chemical and biological variables.”
Relevance to FCMP: Lipid normalization between species is not sufficient to address differences in bioaccumulation, and perch and emerald shiners are not satisfactory substitutes for spottail shiners. The results suggest that combining data across species cannot be done by simple lipid normalization. The results also emphasize the risk involved in choosing a single species as a biomonitor because extrapolation across species is not necessarily valid.


Summary: As with other young-of-the-year analyses, intra-sample variability is relatively low, but year-to-year variability and site-to-site variability is high. The authors assume that spottail shiners reflect water concentrations, and attribute all differences in time and space to differences in current loading.

Relevance to FCMP: Given the authors’ assumptions that spottail shiners reflect water concentrations, all changes over time and space are attributed to current inputs.


Summary: This study provides contaminant data from spottail shiner sampling of Lake Ontario. The authors suggest that spottail shiners reflect site-specific levels of “water-borne chemicals.” Spottail shiners of a certain size (41 to 65 mm) were collected in September. Individual fish lengths were measured and 10 were composited into a single sample. Coefficient of variation values for 4 to 10 replicate composites averaged about 20 to 30 percent for PCBs and DDT. Lipid-adjusted results did not change relationships nor add explanatory power. Changes are interpreted as evidence of changes in water column concentrations and current loading. The authors applied linear regression to untransformed concentrations over time to assess changes over time, with the implicit use of zero order kinetics.

Relevance to FCMP: This study controls for confounding effects by sampling same age (actually same size), same place, same time, and same species. The method illustrates the advantages of young-of-the-year sampling. The method has coefficients of variation of 20 to 30 percent with 10 fish composites. However, year-to-year variability is still high, and the confounding effects on spottail shiner concentrations are not well understood.

Summary: This paper is a report of the Canadian Ministry of the Environment young-of-the-year sampling program. For analyses of temporal trends, the “best fit,” probably higher $r^2$, was used to choose whether exponential decline or linear decline should be assumed. Exponential decline worked better for 24 of 29 data sets with significant slopes of concentration over time. Both wet-weight and lipid-normalized values were analyzed, and no differences were found. The authors suggest that the value of lipid normalization is uncertain. They claim that high PCBs near Love Canal demonstrate that it was the source of PCBs. The authors interpret concentrations as evidence of current inputs, not residual sediment concentrations. The paper states: “In terms of absolute values, most pronounced decreases in PCBs were noted during the late 1970’s.” “While PCB and DOT residues continued to decline at most sites, downward trends at some sites have stabilized,” an inference based on perusal of data on the linear Y-axis.

Relevance to FCMP: The null hypothesis for mechanisms of decline (zero order, first order, or other order) was chosen empirically. The authors presented data for several chemicals, but the implications of multichemical analyses were not noted. While within site variability was low, year-to-year and site-to-site variability was very high. Recognizing the potential effects of year-to-year variability, the authors deduced trends on average concentrations over multiple years. This paper presents another method to reduce effects of interyear variability.

Statistics and Sampling Design


Summary: The authors suggest that trends analyses for chemicals (metals, PCB, DDT, PAHs) in cod and flounder have low statistical power because of high variability. The high variance may be due to episodic releases but some is also likely due to a “biological component.” The goal of this study was to identify variance components of contaminant concentrations in fish to devise sampling and/or analysis programs to address the variance. For example, if there are significant differences between sites and/or seasons, variance can be suppressed, and power increased, by sampling at the same time and same season. The authors tried to identify sources of variance with three methods: 1) covariance and multiple regression analyses over data from time series, 2) analysis of spatial and temporal components, and 3) analysis of biomarkers. This study focused on cadmium, zinc, p,p’-DDE, and CB 153 in fish livers and mercury in fish fillets. The data are based on samples of 25 individual fish from same location. An analysis of covariance was performed on log concentrations from fish taken during a single sampling event (same time, same site) relative to various biological variables (age, log length, log normalized weight, gender, lipid levels). Normalized weight for both liver and whole fish is the ratio of the observed weight compared to the average weight expected at the fish’s length. Most factors were significant for livers, but for mercury in fillets, only age and length were significant along
with two characteristics of the liver (liver normalized weight and liver wet weight). Why liver characteristics should be significant predictors of mercury concentrations in fillet is not discussed. After correcting the concentration data for significant covariates, the authors did an analysis of variance to determine whether season of sample and site location affected concentrations. For both PCB and DDT, and potentially mercury, season of sampling appeared to affect concentrations. The authors present a table that suggests that sampling more seasons and/or locations will improve power if between-sample variance is a significant portion of the variance.

Relevance to FCMP: The paper describes a systematic method to determine which factors are important to variability and power of sampling. When this information is available, sampling methods can be modified to improve power.


Summary: According to the author, a trend is detected when a regression analysis has slope different from zero. Incorrectly concluding that trend is occurring is a Type 1 error and is measured by alpha. Incorrectly concluding that no trend is occurring is a Type 2 error and is measured by beta. Power, which is the probability of detecting a trend, is defined as 1-beta. He considers two types of trends, linear and exponential. He presents a power analysis based on n, the number of samples, r, the rate of change, the coefficient of variation of the data, and alpha and beta. The results are applicable to an experimental situation in which samples are taken at regular intervals in time or space. The effects of linear and exponential change and of having sample variability be a function of abundance are investigated. Results are summarized graphically and, as an example, applied to the monitoring of the California sea otter population with aerial surveys. Generally, power increases as the actual trend, the number of samples, and the precision goes up (coefficient of variation goes down). However, the actual relationships are very complex and depend on other factors such as whether the trend is linear or exponential. The author states that the coefficient of variation can be reduced, at least that part of the coefficient of variation due to measurement error, by increased sampling. The author also suggests that it might be better to conduct surveys over longer time periods if the expected rate of decline is slow.

Relevance to FCMP: This paper provides a method to estimate the power of detecting trends using linear regression analysis. The power analysis depends on whether the trend is linear or exponential, whether the coefficient of variation varies with concentration, and other factors.


Summary: This report specifically states that is does not attempt to make statements about trends of environmental contaminants in an area. Such analyses would require, in addition to the observed statistical differences noted in the report, comprehensive knowledge about the
environmental factors and improved knowledge about the factors that affect bioaccumulation and excretion of these compounds by the organisms. The statistical analyses were simple. The yearly average of each contaminant was estimated with the geometric mean value and these geometric mean values were subjected to regression analysis. An analysis of variance was used to test for trends. All contaminants except PCBs were expressed on a wet weight basis; PCBs were expressed on a lipid-weighted basis. Three models were considered: 1) \( \ln \text{conc.} = u + \text{error} \), 2) \( \ln \text{conc.} = u + \beta t + \text{error} \), and 3) \( \ln \text{conc} = ut + \text{error} \), where \( \ln \text{conc} \) is the log transformed concentration, \( u \) is the overall mean for all years, \( t \) is time, \( \beta \) is a slope, and \( ut \) is a mean for year \( t \). The report states that if Model 3 is better than Model 1, there is evidence of significant changes from year to year. If Model 2 is better than Model 1, then there is evidence for a linear trend. (Note: Because concentration is log transformed, linear trend is consistent with exponential decline.) If Model 3 is also better than Model 2, there is additional variance that cannot be explained by a linear trend. These analyses ignored effects of compositing, but the authors recognize that compositing can affect trends analyses and such analyses are identified. An appendix to this report discusses the effects of compositing. Their studies suggest that compositing might move the geometric mean to the mean, but would generally not affect estimation of trends if the numbers and size of individuals in each composite were similar from year to year. When compositing was not consistent from year, some other statistical methods were potentially better, but these authors concluded that the simpler methods were sufficiently robust. However, the results suggest that spurious year-to-year difference could occur if compositing differs from year to year. In a second appendix, the potential effects of bio-physiological factors on trends analyses are considered. The authors considered the effects of a number of covariables—shell length, tissue weight, shell weight, and these variables log transformed—on concentrations of chemicals in mussels. There was, sometimes, a significant relationship between concentrations in mussel tissues (for cadmium, lead, copper, mercury, and zinc) and log shell weight, but the slope was very close to 1.0. That is, there is convincing evidence of a very slight effect. The other covariables were highly correlated with shell length, so the results were similar.

**Relevance to FCMP:** This study used an analysis of variance to test for linear trends. The temporal models are based on ln concentrations vs. time, which implicitly assumes exponential decline (however, no explanation is given for this choice). Analyses also consider whether there are year-to-year differences that are not associated with long-term trends. Non-constant compositing over time (i.e., different number of individuals, changes in length of size of individuals) can affect trends.


**Summary:** The probability (power) of detecting changes in contaminant levels with time depends on both the pattern and magnitude of these changes. Contaminant monitoring programs are more sensitive to some patterns of change than to others. Power is inversely related to the variability but positively related to the size of the sample, the significance level, and the size of the change. This is demonstrated for an annual monitoring program by computing the power for several simple environmental scenarios exhibiting different patterns of
change in contaminant levels. Sharp changes in concentration have a greater chance of being noticed than gradual changes. Alternatively, a gradual change needs to be 60 percent larger to be detected with the same power as a very sharp change. The authors applied this analysis to real data collected from the Baltic, where they collected length-stratified samples of 25 fish from the same area at the same time of year.

**Relevance to FCMP:** At a constant level of replication and variability, the probability of detecting a change will depend on the rapidity of change as well as the size of the change. These North Sea researchers control for effects by sampling a limited size at the same place and time.


**Summary:** This paper is a companion article to U.S. EPA (1989b). For a constant power of 80 percent and type I error of 5 percent, this evaluation considered the effect of sampling replication and data variability (average within group variance) on the minimal detectable difference between groups. The increase in precision with increasing replication declines dramatically as the number of replicates increases, but the point of diminishing return is lower replication for lower variance. For highly variable populations with coefficient of variance equal to 90 percent, the sampling would only be able to distinguish differences of 125 percent with 15 replicates.

**Relevance to FCMP:** Given the low levels of likely environmental response, very high levels of replication may be needed to discern differences between years.


**Summary:** This evaluation considered the effects of composite samples on power based on simulation methods. The analyses were based on random sampling from normally distributed contaminant concentrations, with statistical power estimated for single and composites of various n. There were two analyses. In the first, the simulation methods were used to show the effect of compositing on the sample mean. Based on two hypothetical fish populations with the same mean but coefficients of variation of 45.5 and 101.6 percent, the confidence interval for the mean decreased with more compositing. However, the effect on precision tended to diminish as composite number increased, but the point of diminishing return depended upon variance of the population. The point of diminishing return, or optimal composite number, was lower for low variance populations.
Relevance to FCMP: Compositing is one method to reduce variability and analytical costs, but the optimal number of composites depends on the variability of the population. The analyses may be inapplicable to fish concentrations because 1) they are based on normal distribution and fish concentrations are probably not normally distributed, and 2) they may assume equal mass placed in a composite, whereas most composites analyses may be weighted to the heavier individuals.


Summary: According to this paper, “the objectives of monitoring study have to be defined qualitatively and quantitatively.” However, estimates of precision of estimates cannot be made until some data have been collected. Experience with monitoring suggests that changes can only be observed when they are larger than “natural variation.” Therefore, it is necessary to determine the sources of variance so that the controllable sources can be controlled. The authors suggest that long-term quality control may be necessary because it may take many years to see a change. They state that it is critical to sample the same stock (e.g., same size at same time of year). In terms of covariables, the authors state that it is prudent to measure all of those that are easy to measure, such as lipid and size, and to preserve the data in case it is needed later. They also suggest that one important use of covariable data is for checking data for analytical and transcription errors or abnormalities in the specimen. They list seven guidelines for studying temporal trends. Guideline 1 is to estimate the magnitude of the change to be observed, and then to estimate the likely variance. This information can be used to determine the power of various sampling schemes. Guideline 2 is to develop a statistically valid sampling plan. Guideline 3 is to develop appropriate quality control methods, potentially including specimen banking to estimate “drift” in analytical precision over time. Guideline 4 is to rigorously adhere to the protocol over time. Specifically, the authors suggest that fish length is a critical factor for finfish. Guideline 5 is to identify changes in inputs or dynamics of the system. Guideline 6 is to avoid taking “unrepresentative samples,” such as abnormal fish. Guideline 7 is to recognize that observation of significant trend is not sufficient and that it is necessary to demonstrate that the same trend occurs in other media. The authors also recommend that the ideal biomonitor should be a bioaccumulator, sedentary, abundant, available in multi-year class populations, and sufficiently large to make analyses easy. The paper states that stocks selected should follow “a relatively unchanging pattern of living over time and feed on a relatively consistent food supply.” When using a covariate, such as length or weight, it is necessary to have quality control over time and analysts.

Relevance to FCMP: This paper is a basic primer on using fish as a biomonitor. Several sensible suggestions are provided, including estimating the magnitude of changes that are likely to be observed and designing monitoring programs that are sufficient to test the changes. The authors also specifically recommend multi-media comparisons—trends are not to be believed unless they are corroborated by similar trends in other media.
Survey of Fish Contaminant Monitoring Programs

Introduction

Prior to reviewing the trend monitoring components of the Michigan Department of Environmental Quality’s (MDEQ’s) fish contaminant monitoring programs (FCMP), a survey of FCMPs was conducted to determine the state of the art for contaminant surveys. It was also felt that other FCMPs might have considered many of the same issues that concern MDEQ’s FCMP, and their insights and experiences would be valuable in evaluating Michigan’s FCMP. To that end, managers of several FCMPs, from the Great Lakes regions and elsewhere, were contacted and asked to fill out a questionnaire. The questionnaire (see Attachment B-1) considered the goals, methods, and uses of their FCMPs. Managers were also encouraged to provide additional information pertaining to their FCMPs. Information concerning these FCMPs was also obtained from published reports and the scientific literature.

The information was summarized for all of the FCMPs as a whole and for each FCMP, and these summaries are described below.

Summary of All Surveyed FCMPs

The surveyed FCMPs span a range of program size, longevity, and goals. For example, Pennsylvania’s FCMP is devoted almost entirely to generating fish consumption advisories. Thus, site selection and sampling intensity are determined primarily on the basis of known sources of contamination as opposed to statistical power. The Pennsylvania program analyzes tissues appropriate to consumption advisories (i.e., as fillets or skinless fillets), and the data are not routinely used for trends analyses. In contrast, in other FCMPs, such as the Canadian Department of Fisheries and Oceans (DFO) and the U.S. Great Lakes Fish Monitoring Program (GLFMP), sampling is devoted solely to detection of trends over time and space. Sampling designs for these programs are determined primarily by statistical considerations. Fish are analyzed as whole fish and the data are routinely used in trends analyses. In the middle are the hybrid and multifaceted programs such as those administered by MDEQ and Indiana. Both states have FCMP elements geared toward detection of trends as well as elements geared toward collection of data for fish consumption advisories. The sampling design and analysis of the different elements tend to reflect its goals. Other hybrid programs are primarily geared toward promulgation of fish consumption advisories, but also use data for analysis of trends.

Survey responses were tabulated in a summary table, with more detailed responses contained in Attachments B-2 through B-6. As can be seen from Table B-1, a majority of surveyed FCMPs use their data to monitor trends of chemicals, environmental quality, and effectiveness of remediation or regulation. Of the FCMPs surveyed, about 60 percent use their data to issue fish consumption advisories, and most of these also use the data to track trends. The tissue monitored varies—most FCMPs monitor concentrations in whole fish, but those using data for consumption advisories generally monitor concentrations in fillets, although these data are often
also used in trend analyses. A limited number of FCMPs also monitor concentrations in young-of-year (YOY) fish.

Most FCMPs have been in existence for a relatively long time, an average of about 20 years. Consequently, changes in sampling methods and/or analytical methods have occurred over the lifetimes of most programs. Most FCMPs sample fixed stations at relatively fixed intervals, but two FCMPs, Indiana’s and the National Fish Tissue program, have recently implemented random site selection so that their results can be extrapolated to area-wide trends.

All FCMPs measure the size of their fish, generally both weight and length, and almost all routinely measure lipids. Fewer FCMPs assess fish age, gender, or reproductive condition, and most of those that do monitor these parameters do so on an irregular basis. A variety of fish species are monitored, ranging from top predators such as walleye and lake trout to bottom feeders like carp and smaller panfish, such as perch. Some FCMPs measure concentrations in individual fish, some analyze composites, but most assay concentrations in both composites and individual fish. When fish are composited, all of the FCMPs try to composite with a single species and limited size or age range of fish. All FCMPs monitor polychlorinated biphenyls (PCBs) and mercury, all but one monitor DDT and its breakdown products, and many also monitor a number of other pesticides such as chlordane, dieldrin, and BHC.

To control for confounding factors potentially affecting concentrations and observed trends, all but one of the FCMPs controls for fish species. The only exception, the National Fish Tissue Study monitoring program, will consider a limited number of different species because a single species would likely not be available at all sites across the country. Most FCMPs also resample at the same location. The notable exceptions to this rule are Indiana’s creek chub sampling program and the National Fish Tissue Study FCMP, which select sites randomly. Most FCMPs consider fish size when sampling, while only about one third consider the age of the fish. One respondent, DFO, suggested that controlling for fish age is essential in order to differentiate growth rates between sampling sites and sampling times. Although most FCMPs monitor lipids, only about half normalize chemical concentrations to lipid levels. One FCMP (Wisconsin) will only lipid-normalize when comparing concentrations across species, and other respondents stated that lipid normalization was inappropriate (DFO) or ineffective (Massachusetts Water Resources Authority [MWRA]). Only two FCMPs consider gender when analyzing tissue concentrations, in both cases apparently only on an irregular basis.

Very few FCMPs collect additional limnological data, such as organic carbon or pH, and about half analyze other media, such as sediments or water column, for the chemicals measured in fish. Only one, DFO, specifically analyzes chemical concentrations in prey and the food chain, although some FCMPs monitor concentrations in other biota. MDEQ considers concentrations in eagles, and MWRA monitors chemical concentrations in mussels and lobsters as well as fish.

About half of the FCMPs archive tissue. Most FCMPs issue annual or semi-annual reports, which summarize the data, while others, such as DFO and GLFMP, rely on the scientific literature for exposition of their results. The raw data for most FCMPs are available, generally upon request.
Canadian Department of Fisheries and Oceans

In 1976, the DFO Great Lakes FCMP was developed in response to Annex 11, “Surveillance & Monitoring,” of the Canada/U.S. Great Lakes Water Quality Agreement (GLWQA). DFO’s program was developed jointly with the U.S. Environmental Protection Agency (EPA) and the U.S. Fish and Wildlife Service (FWS) to satisfy the GLWQA requirement that the U.S. and Canada must “provide information for measuring local and whole lake response(s) to control measures using trend analysis.” The DFO monitoring program also addresses the need to identify emerging problems and to provide support for the development of remedial action plans at areas of concern and lakewide management plans for critical pollutants. DFO’s program also maintains a biological tissue bank to permit retrospective analysis of recently identified compounds. The DFO open lake monitoring program has been in place since 1977.

Several monitoring stations located on the lower Great Lakes (Ontario and Erie) are surveyed annually. Two of several designated monitoring stations on the upper Great Lakes (Huron and Superior) are surveyed each year in a rotating pattern. If possible, these rotating stations are sampled for two consecutive years. Although lake trout may migrate throughout the lake after stocking, DFO samples multiple stations in each lake to ensure that the final sample contains a representative mixture of the strains of lake trout in each Lake. An attempt is made to collect fish for two consecutive years at any one station. The contaminants analyzed are PCBs, other pesticides (DDTs, chlordane, dieldrin, endrin, mirex, heptachlor epoxide, hexachlorobenzene), and mercury. Toxaphene, dioxins and furans, PCB isomers, and emerging chemicals (e.g., polybrominated diphenylethers) are measured on a limited subset of samples. DFO also measures a limited number of metals (arsenic, copper, cadmium, lead, nickel, and zinc) in selected forage fish.

The program monitors contaminant trends in whole lake trout, or walleye when appropriate. This FCMP also monitors chemical concentrations in forage base including smelt, alewife, and sculpins, as they are available at any single site. Collections of the invertebrate forage base (Mysis, Diporeia, plankton) are also made periodically at many of the primary monitoring sites.

DFO also maintains a specimen bank that now contains more than 15,000 samples of fish and invertebrate tissues, a small number of sediment samples, and the solvent extracts from prior analyses of samples. These specimens are based on samples taken between 1977 and the present. The specimen bank has several functions. First, archived tissue and extracts can be used for retrospective analyses of chemicals that have become problematic since the original analyses. In addition, analytical methodologies and instrumentation improve over time, which will allow more in-depth analysis of chemical concentrations in archival material. Retrospective analyses can also be used to corroborate historical analyses (e.g., see Huestis et al. 1996). Various hard tissues (fish scales, opercular bones, otoliths) from samples are also retained. These tissues can be analyzed for stable isotopes of carbon and nitrogen to determine if food webs have changed significantly during the sampling period, as was done in Kiriluk et al. (1995, 1999) and Whittle et al. (2000). The specimen bank is described in detail in Hyatt et al. (1993) and Kiriluk et al. (1996a,b,c, 1997). DFO believes strongly that the archive is cost-effective in terms of the information gained.
As illustrated above, this FCMP collects a wide range of information on the fish themselves and on other components of the ecosystem. DFO believes that the costs associated with collecting this extra information are warranted because this FCMP intends not only to describe ongoing trends but to understand why they occur. To that end, DFO data are widely used in the evaluation of trends. Interpreted data have been provided to the IJC Water Quality Boards, for their biennial reports, and have more recently been provided to the biennial SOLEC meetings. DFO data have also been the basis of a number of papers in the peer reviewed literature, as illustrated in Borgmann and Whittle (1991b, 1992b) and Huestis et al. (1996, 1997). These reports, which focus on Lake Ontario, have found significant reductions in mercury, PCBs, dioxin/furans, and pesticides in lake trout smelt. These analyses have typically used linear regression or, in the case of Borgmann and Whittle (1991b, 1992b), sophisticated multiple regression techniques. DFO data are available, with some limitations, to scientists within and outside the Department, and this information has been the basis of a number of peer reviewed publications. In addition to the references listed above, see, for example, Borgmann and Whittle (1992a, 1994), MacEachen et al. (2000), Madenjian et al. (1995), Morrison et al. (1998, 1999, 2000, 2002), and Whittle et al. (1992).

According to DFO scientists, important issues to take into consideration when sampling for trends analysis include the following:

- DFO compares temporal and spatial data for same-aged fish, generally 4-year-old fish. DFO scientists believe that controlling for age is essential because the use of similar size-based data does not account for changes in growth rates over time or between locations.

- Large top predators integrate environmental conditions over large portions of any single water body. Therefore, collecting these fish types at multiple sites within any one lake system to determine whole lake conditions is not necessary. On the other hand, mobile fish species such as salmonids cannot be used to identify fine scale differences in concentrations or localized inputs.

- DFO scientists believe that lipid correction/normalization for contaminant data from individual fish is not effective for statistical analysis of spatial or temporal trends. However, lipid normalization may be appropriate for normalization of concentrations for different tissues within a fish.

**Great Lakes Indian Fish and Wildlife Commission**

The main focus of the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) has been to protect tribal health and environmental resources for the geographic area covered in the 1836, 1837, 1842, and 1854 treaties. Specifically, the GLIFWC provides current data for the creation of fish advisories and the study of temporal trends in mercury concentrations. Since 1989, the GLIFWC has monitored mercury in skin-off fillets of walleye. Inland lake monitoring (ILM) of muskellunge skin-off fillets for mercury began in 1999. The ILM study samples 12 lakes every two years. Other lakes that make up the top 90 percent of tribal harvest are to be sampled once...
every five years. Such lakes include Bay Mills, Fond du Lac, Keweenaw Bay, Lac
du Flambeau, Red Cliff, and others.

In addition to the ILM, there was a detailed study of the Lake Superior commercial harvest in
1999–2000. For this study, sampling sites were selected by their relative importance for the
tribal harvest. While the regular ILM monitoring analyzes only for mercury, the following
chemicals were analyzed for the Lake Superior project: PCBs, DDT, mercury, and pesticides
(chlordane, toxaphene, aldrin, dieldrin, endrin, heptachlor, endosulfans, methoxychlor,
hexachlorobenzene, pentachloroanisole, and mirex). The Lake Superior study was, at this point,
a one time event.

For the ILM project, 12 walleye (3 walleye in 4 size groups) and 6 skin-off muskellunge muscle
samples (2 muskellunge in 3 size groups) are collected from 6 of the long-term study lakes and a
number of other lakes. For the Lake Superior study, composite samples were collected
according to EPA contaminant sampling methods for fish advisories for walleye, herring, lean
lake trout, siscowet lake trout, whitefish, and lake sturgeon. For each species, 4 composites of
12 fish per composite were analyzed.

Temporal trends were determined using multivariate analyses (PROC GLM in SAS) and a
significance level of 0.05). In these analyses, size and age were accounted for within the same
species and tissue type. For the ILM, regression analyses (concentration vs. length) using
12 data points typically provided r^2 values around 0.7 or better. Preliminary work on the
mercury concentrations in walleye fillets have not yielded clear conclusions with respect to
trends. In the Lake Superior study, with the use of 4 composites with 12 fish per composite, the
assumption was that the standard deviation divided by the mean would be no worse than 1.0.
Thus, the estimated power to detect a 50 percent change in concentration would be 80 percent or
better. However, no conclusions have been made.

Great Lakes Fish Monitoring Program

The GLFMP is an interagency monitoring program composed of state and federal agencies
around the Great Lakes. Starting in the late 1960s, FWS began to monitor chemicals in Lake
Michigan lake trout. In 1977, this became a cooperative program between FWS, EPA, and the
National Biological Survey. At that time, the program was expanded to include lake trout from
Lakes Superior, Huron, and Ontario, and walleye from Lake Erie. In 1980, the monitoring
program was expanded further to include state agencies and monitoring of fall run coho salmon,
and later to include monitoring of fall run Chinook salmon on alternate years. The goal of the
GLFMP sampling program is to monitor the status and trends of chemical levels in Great Lakes
fish. The history of this program, sampling methods, and results are described in detail in

Monitoring of chemical concentrations in lake trout/walleye and coho salmon sampling is
intended to address different aspects of status and trends. As trout and walleye are large and
relatively slow-growing predators, they were assumed to represent worst-case concentrations
over an extended period of time. In contrast, chemical concentrations in fast-growing coho
salmon were thought to reflect recent conditions, because these fish spend less than three years
in the lakes. In addition, coho fillets were thought to be more useful in assessing average risks to human consumers.

Lake trout and walleye are collected from an offshore site in each lake. There is a single collection site per Great Lake, which was chosen to be far from known point sources of contamination. Originally 60 fish were to be taken per collection site. Fish were to be collected in each of three size classes, analyzed as individual fish, and the resulting data were to be analyzed by ANCOVA. However, data collected during the first several years often did not conform to the assumptions of ANCOVA. Since 1981, lake trout between 600 and 700 mm and walleye between 400 and 500 mm have been collected and analyzed as composites of 5 whole fish. Depending on the number of fish available, there may be as many as 10 composite samples per lake. Lake trout and walleye are analyzed for PCBs and a range of pesticides (DDT and its breakdown products, dieldrin, oxychlordane, trans-nonchlordane, cis-nonchlordane, alpha and beta chlordane, and toxaphene). Results of this sampling are periodically published in the peer reviewed literature (DeVault et al. 1986, 1996). These results demonstrate that most all compounds have declined over time.

Fall run coho salmon are collected from a number of streams on each of the Great Lakes. Most salmon migrating into streams are 3 years old. Thus, collecting migrating salmon controls for fish age and, to a lesser extent, fish size. Salmon are collected at only one stream apiece in Lakes Superior, Huron, and Ontario, but four streams are sampled in Lake Erie, and ten Lake Michigan tributaries are sampled. When sufficient fish are caught at each site, fifteen fish are divided into three five-fish composites of fillets with skins. Analytes are similar to those measured in the large predators. Trends analyses of these data have been conducted (DeVault et al. 1986), generally using simple regression analysis. These analyses demonstrate declines in most compounds over the early years of this program.

Illinois

The objectives of the Illinois FCMP are to 1) investigate and detect the presence and build-up of toxic and potentially hazardous substances in fish, encompassing both fish toxicity and public health implications; 2) determine the impact of fish contaminants upon the suitability of aquatic environments for supporting abundant, useful, and diverse communities of fish life in streams and impoundments of Illinois; and 3) aid in the location of sources of toxic material discharges and evaluate long-term effects of source controls and land use changes. This program has been in existence since 1976.

Fish are collected from a number of sites, including the Great Lakes, inland rivers lakes, reservoirs, and impoundments. Most waters are sampled at some type of fixed interval. Major rivers and their basins are sampled on a 10-year cycle, water bodies with existing advisories are sampled every 1 or 2 years, and major lakes and reservoirs without existing advisories are sampled every 5 to 8 years.

Contaminants analyzed include PCBs, DDTs and other pesticides (chlordanes, toxaphene, aldrin, dieldrin, endrin, BHC [lindane], heptachlors, methoxychlor, hexachlorobenzene, mirex), and mercury.
Over 20 species of fish are collected across the state. Data from whole fish are primarily used for detecting trends. Whole fish, along with composite samples of fish fillets, are also used to identify lakes and streams with significant fish contaminant problems requiring more intensive investigation. For the composites, three to five or more fish of a single species of about the same size and weight comprise each composite fillet sample (untrimmed fillet with skins). The total weight of the composite must range from 1 to 5 lb. A minimum of four composite fillet samples are obtained at each station (one predator, one omnivorous species, and two bottom feeders). One whole fish sample is provided for each of the composite fillet samples, with the whole fish sample being a representative of the individual fish that comprise the composite fillet sample. Tissues from each sample are archived in a tissue bank.

No formal trend analyses have been conducted on this data. However, contaminant data from 1978−1998 have been analyzed by a graduate student. Preliminary findings suggest that PCB and mercury levels are stable, while chlordane levels are declining.

Indiana

The monitoring of chemicals in fish is one element of a monitoring strategy that assesses the quality of Indiana surface water and identifies factors responsible for impairment. These data are used to assess the ability of surface waters to support designated uses and to effectively communicate this information to internal and external customers. This information is also used to identify trends and make recommendations for the protection of surface water resources of Indiana.

There are two elements to the Indiana FCMP. The first element, in existence since 1977, is based on the collection of adult fish. These data are primarily for use in fish consumption advisories but also to identify areas of contamination. Water bodies to be sampled are chosen on the basis of accessibility, whether there has been previous monitoring, and whether there is evidence of past problems. There are core stations from which contaminants in fish tissue are assessed for trends. These are sites where there have been a number of years of monitoring data. They are sites located on major rivers and in relation to major urban regions. The monitoring strategy calls for a five-year rotation for the inland waters of the state. Each inland basin is sampled every five years. Non-salmonid fish are sampled from Lake Michigan on an annual basis and Indiana also participates in the ongoing EPA Great Lakes National Program Office Coho/Chinook program. Tissue data from the Ohio River are also collected annually. Ideally, the fish collected at each site include a bottom feeder, a top predator, and another fish species representative of the site. Across the state, indicator organisms are the common carp and channel catfish. However, more than 50 species of fish have been collected. Chemicals analyzed are PCBs, mercury, DDTs and a variety of organic pesticides, as well as 22 metals.

A second element of the Indiana FCMP, begun in 1997, is devoted to determining status and trends of chemicals across Indiana. As originally planned (Stahl 1997), chemical concentrations in creek chub were to be monitored annually at approximately 80 randomly chosen sites per year. Creek chubs were initially chosen as the biomonitor because they are widely distributed, and they are important links in the food chain for game fish and wildlife. However, by 2000, it was recognized that targeting only one species of fish was too limiting (Stahl 2000). Thus, this
part of the FCMP now attempts to collect a primary target species—creek chubs, bluntnose minnows, stonerollers, or other small forage fish. If available, a secondary target fish, an adult carp, catfish, bass, or sucker, is also collected. The forage fish are analyzed as composites of 3 to 5 similarly sized fish of the same species. Ideally, the secondary fish sample should also be a composite of several fish, but this tissue sample can come from a single fish if conditions warrant. Forage fish are analyzed for lipid, mercury, PCBs. Larger fish are analyzed for these and organochlorine pesticides, cadmium, lead, and mercury. According to Stahl (1997, 2000), the random site selection will enable estimates of chemical levels over entire watersheds and over time.

Trends of four major contaminants (PCB, DDT, dieldrin, and chlordane) have been assessed in adult fish tissue from core stations. Trends are assessed after normalizing to lipids and also by grouping into specific size classes for comparisons. In almost all cases organochlorine contaminants were found to be decreasing. A two-way t-test has been employed for contaminants such as mercury and lead, to compare data sets from different decades. Mercury levels, as evidenced in common carp, largemouth bass, and channel catfish, have not changed over the last two decades, but lead levels in fish have significantly decreased. The organochlorine pesticides have declined to such an extent that they are no longer the cause of any consumption advisories.

**Inter-tribal Fisheries and Assessment Program**

The goal of the Inter-tribal Fisheries and Assessment Program (ITFAP) is to determine whether fish, specifically lake whitefish and lake trout, caught by tribal commercial fishers in the 1836 treaty waters exceed trigger levels for protection of human health provided by the U.S. Food and Drug Administration and the Michigan Department of Community Health (MDCH). A subsidiary goal is to determine if there are temporal or spatial trends in the concentration of these contaminants, and to evaluate the tribal commercial catch for sale within tribal fishers’ hazard analysis critical control point plans. This program has been in existence for about 10 years.

The ITFAP monitors the Great Lakes and its connecting channels (St. Mary’s River). Fish are collected from the tribal commercial catch at specific locations from Lake Huron, Lake Michigan, and Lake Superior every third year on a rotating basis. Lake whitefish and lake trout are caught, and individual skinless fillets are analyzed for contaminants. Contaminants analyzed are PCBs, other pesticides (DDT and metabolites, chlordanes, dioxins and furans), and mercury.

ITFAP’s results are primarily used to formulate fish consumption advisories, but these data are also used for simple trends analyses. ITFAP’s annual data are compared to previous results for each lake by using simple graphs of geometric means of contaminant concentrations. No other statistics have been used.

ITFAP scientists believe lack of standardized analytical methods for organic compounds, including toxaphene and dioxin/furan congeners, hinders trends analyses.
Massachusetts Water Resources Authority

MWRA is responsible for wastewater treatment for the metropolitan Boston area. In September 2000, wastewater discharges into the mouth of Boston Harbor were diverted to a location nine miles east in the deeper waters of Massachusetts Bay. A monitoring program for contaminants in fish, mussels, and lobsters had been in existence for about 10 years, and this program was redesigned to assess any nearshore improvements or impacts offshore due to improvements in sewage treatment and the change in discharge location.

The choice of sampling sites was based upon the proximity to old and new wastewater discharge locations, appropriateness for use as “clean” or “dirty” control, and proximity to the spring feeding grounds of the endangered right whale. This FCMP analyzes the same chemicals as the National Oceanic and Atmospheric Administration Status and Trends Program. The chemicals include polycyclic aromatic hydrocarbons (PAHs), PCBs, and pesticides (DDTs, chlordane, aldrin, dieldrin, endrin, heptachlor epoxide, hexachlorobenzene, lindane, and mirex).

Mussels (*Mytilus edulis*) are collected from a clean site, deployed in cages for 60 days at several locations, retrieved, and whole bodies are analyzed for contaminants. Flounder (*Pseudopleuronectes americanus*) are collected from several locations in Boston Harbor. Chemicals are analyzed separately in flounder edible tissue and livers. In lobster (*Homarus americanus*), the edible tissue and hepatopancreas are analyzed separately for contaminants. For the flounder and lobster, edible tissue is used as a measure of potential human health issues. The liver and hepatopancreas are used because they appear to reflect recent contaminant exposure.

MWRA uses single factor ANOVA to determine if chemical concentrations vary across sites. Data are first evaluated to see if they are appropriate for parametric statistics, and transformed to natural logs if they are not. Following ANOVA, multiple comparisons were conducted with Tukey’s studentized range test. For temporal trends, current concentrations are compared to pre-project concentrations with a t-test, and data from all years are inspected visually for evidence of obvious trends.

MWRA scientists have identified the following problems and issues with sampling and analysis:

- Subtle quantitative changes in chemical analyses have occurred over the lifetime of this FCMP. While the same analytical methods have been used during the 10 years of this program, in the last few years, analysts have become better at separating compounds of interest from co-eluting and interfering compounds. Before temporal trends can be assessed with confidence, some of the samples must be rerun and reanalyzed using gas chromatography/mass spectrometry to quantify the potential impacts due to subtle changes in analytical methods over time.

- Most older analytical methods, even EPA-approved methods, do not have sufficient resolution to rigorously quantify ambient concentrations of many bioaccumulating contaminants of concern (BCCs). The concentrations of
BCCs are either not detected or are over-quantified because interfering or co-eluting compounds are not recognized.

- Consistency in collection date may be very important to avoid biasing the data. For example, lobsters may face different exposure to chemicals during the spring and summer. These differences might affect concentrations in the hepatopancreas, which is thought to reflect recent exposure to chemicals.

- The recent history of animals collected from the wild is always uncertain. Therefore, controlled studies using caged animals might be preferable for some investigations (e.g., impact of new contamination source).

- Lipid-normalization of hydrophobic chemicals concentrations is of dubious utility. Normalization tends to increase the variability of the data and reduce statistical power.

**Michigan**

There are three elements to the MDEQ Surface Water Quality Division (SWQD) analyses of chemicals in fish: an FCMP with caged fish, one based on whole body concentrations, and a third element based on edible portions, usually untrimmed fillets with skins. Each is described in more detail below.

**MDEQ Caged Fish FCMP**

Since 1986, SWQD has used caged fish to evaluate whether existing pollution prevention, regulatory, and remedial programs are effectively reducing chemical contamination in the aquatic environment. The caged fish are used to identify sources of bioaccumulative contaminants and to identify spatial trends in contaminant concentrations. Caged fish studies are conducted in inland rivers, lakes, and reservoirs. MDEQ has the watersheds in the state divided into 5 basin-years, so that watersheds in each of these basins are monitored every 5 years to match the National Pollutant Discharge Elimination System program. Sampling locations for caged fish are also selected based on factors such as assessing the efficacy of remediation, requests from other agencies, and requests from the public.

The caged fish program focuses on chemicals with high bioaccumulation potential in fish tissue such as PCBs, DDTs and other pesticides (chlordanes, toxaphene, dieldrin, endrin, mirex, heptachlor, etc.), and mercury.

Channel catfish purchased from a commercial farm are used as test organisms. In 1999, three watersheds were monitored using caged fish, including a total of 20 sites. Generally, one cage is placed for each site. Control samples are obtained at the beginning of the test period by randomly selecting a subset of channel catfish and combining them into four composite samples. The remaining channel catfish are held in stainless-steel cages at the test site for 28 days. The fish are removed from the cages and divided into four composite samples of whole fish. Each sample has a minimum total weight of 40 g but is ideally 100 g, which
provides the ability to analyze duplicates. The number of fish per composite is a maximum of four samples per cage but is determined by the size of the fish and the number surviving at the end of the 28-day test.

Net uptake of each contaminant is calculated based on the relationship between the concentrations in the control samples and the concentrations in the test samples. Concentrations of organic parameters are lipid normalized by dividing the lipid concentration (percent) by the contaminant concentration. Mercury concentrations are evaluated as wet weights.

Temporal trends are generally based on perusal of data. Differences among stations (i.e., spatial trends) are detected with t-tests and ANOVA. A limitation of these analyses is the assumption that a 28-day period is representative of the annual condition of the site.

**MDEQ Edible Portion**

The primary objective of the edible-portion monitoring program is to develop sport fish consumption advisories and commercial fishing restrictions. The edible portion data are also used to evaluate environmental quality (303(d) list, Remedial Action Plan/Lakewide Management Plan impaired uses, etc.). In a few cases, the data are also used to assess temporal or spatial trends. Although data on chemical concentrations in fish have been collected since the late 1960s, the electronic database contains only data collected since 1980.

Sampling sites on the Great Lakes are selected based on MDCH sport fish consumption advisories and Michigan Department of Natural Resources (MDNR) collection practices. Sampling sites for inland rivers are selected based on barriers to movement (i.e., dams), sources of contaminants, and the ability to obtain samples. Sampling is also often targeted toward sites where there are known or suspected sources of BCC, public access, or sites that are popular with anglers. In most cases no repeat sampling at a site occurs if concentrations are low and no new sources are suspected. However, repeated sampling is conducted if more samples will allow better characterization of the need for an advisory, measurement of the effect of some type of regulatory action or remediation, or if there is an existing advisory for which sufficient time has passed that concentrations are thought to have declined. In other cases, samples have been collected from the same site every year for a 5–6 year period. These are sites that have fish with elevated contaminant concentrations.

Chemicals analyzed are PCBs, DDTs and other pesticides (chlordanes, toxaphene, dieldrin, endrin, mirex, heptachlor, etc.), and mercury. In some cases there is analysis of mercury only, particularly at remote inland lakes or reservoirs with no known source of chlorinated organics (other than atmospheric).

Most of the fish analyzed for the edible portion FCMP are sampled by other agencies. The MDNR Fish Division, tribal organizations, and others (non-MDEQ) collect approximately 80 percent of the edible portion samples. Generally, a predator and a bottom feeder are collected from each site. Over the state, more than 20 species of fish are collected. In general, different species are processed as either skin-on or skin-off fillet, based on surveys of cleaning methods employed by the typical angler. Most predators are processed as fillets with skins, while most bottom feeders are processed as skinned fillets.
The fillet data are used primarily for consumption advisories, but these data are occasionally used for trends analyses. In cases where the data are appropriate, ANCOVA is used to evaluate spatial differences between fish from different water bodies. ANCOVA is used because contaminant concentrations are dependent on fish length, which is especially important for trends analyses with mercury. Alternately, regression is used to estimate concentrations at a “standard length,” and then changes are tracked in these “standardized” concentrations over time.

**MDEQ Whole Fish**

The proximal goal of the FCMP whole-fish monitoring program is to identify spatial differences and temporal trends in the quality of Michigan’s surface waters. The ultimate goal is to evaluate whether existing pollution prevention, regulatory, and remedial programs are effectively reducing chemical contamination in the aquatic environment.

The Michigan whole fish monitoring program has been in existence since 1990. Species and locations were selected to complement and avoid duplication with the EPA/U.S. Geological Survey Great Lakes whole-fish trend monitoring program. A total of 26 locations within the Great Lakes, its connecting channels, rivers, and inland lakes and reservoirs are sampled every two to five years. Consistent size ranges of larger, adult fish of lake trout, walleye, carp, and largemouth bass are targeted, but fish of the targeted size are sometimes not available. Chemical concentrations in individual, whole fish are analyzed.

As with other elements of Michigan’s FCMP, the whole fish monitoring focuses on chemicals with high bioaccumulation potential in fish tissue such as PCBs, DDTs and other pesticides (e.g., chlordanes, toxaphene, dieldrin, endrin, mirex, heptachlor), and mercury.

Regression techniques are used to assess temporal trends. The data are transformed using natural logs of wet weight. Samples are evaluated to confirm that the sizes of the fish do not change over time. If sizes have changed significantly over time, a correction factor is applied or no conclusions about contaminant changes are made from those samples. If significant changes in organic concentrations are detected, the data are lipid-normalized and the change is evaluated again. Spatial trends analyses are limited to general conclusions based on review of graphs (e.g., mercury trends to be higher in inland lake walleye than Great Lakes walleye).

Concerns about the whole fish FCMP program include the following:

- Collecting similar sized fish at some sites, especially smaller lakes and rivers, is sometimes difficult. The resulting variability in fish sizes makes trend analysis difficult to interpret.

- Fish movement, especially by carp and walleye, may significantly influence trends analyses.

- Because MDEQ relies on other agencies to collect fish, no effort is made to control the date of collection from year to year. This may influence contaminant concentrations over time. Growth rates between sexes and
between sites have not been evaluated, and MDEQ is concerned about potential impacts on trends analyses.

**Minnesota**

The program, which began in 1990, has essentially four elements, with different goals and sampling strategy. To determine the spatial variation across the state and across water body types, fish are sampled from various types and sizes of water bodies. Data from this sampling are also used to promulgate contaminant advisories for specific water bodies. Second, to assess temporal trends, a specific list of large lakes and rivers and certain more contaminated water bodies are periodically sampled on a 5–8 year cycle to monitor fish contaminant trends over time. Third, specific lakes are periodically sampled in an effort to better understand mercury cycling. The mercury cycling/mecahnism study started in 1998. Fourth, sampling also investigates local chemical contamination problems on a case by case basis.

Fish are sampled from a variety of inland rivers, lakes, reservoirs, and Lake Superior. The rationale for sampling varies. For temporal trends, the large lakes and rivers are expected to be resampled every five years. Most fish sampling sites on large waterbodies are relatively fixed locations that have been used by fisheries for their standard population assessment surveys over the years. For spatial trends, many of the smaller to medium sized lakes have been sampled only once. Unless unusual levels of contamination are detected, these are not resampled.

Generally, one or two large predators species (northern pike, walleye, bass, or lake trout), one panfish species, and one fatty rough fish species are collected from each system. For both spatial and temporal collections, untrimmed fillets are analyzed for PCBs and mercury.

A formal statistical review of the program was conducted by statisticians from Iowa State University in 1996 (Nusser and Breidt 1996; Wiener et al. 1996; Kaiser et al. 1996; Shierholz et al. 1996). Their analysis concluded that the FCMP data were generally insufficient for deducing temporal and spatial trends. For example, although the data were sufficient to conclude that mercury levels tended to be higher in select species of northeastern Minnesota, there did not appear to be noticeable declines over time in the mercury levels of fish from lakes from this same region (Wiener et al. 1996).

The statistical review made several recommendations based on a combination of statistical and logistical considerations (Nusser and Breidt 1996; Shierholz et al. 1996). The review recommended that sampling of fish should be based on a stratified random design. In general, lakes to be sampled would be chosen randomly from the subset of Minnesota lakes with recreational fisheries and a modicum of limnological information. Lakes were to be chosen randomly from this subset of lakes within constraints imposed by lake location. Imposing constraints in terms of lake location permitted the sampling load to be efficiently allocated among Minnesota Department of Natural Resources offices, which supply samplers and sampling equipment. Consideration of lake location also ensures sampling of different alkalinity regions in the state, which is important in terms of mercury bioaccumulation. Within each lake, six walleye or northern pike of variable size are to be sampled, and analyzed as individual fish.
The small number of fish sampled from each lake represents a compromise between budget and sampling power. Given a limited sampling budget, sampling fewer fish in any one lake increased statistical power because it allowed more lakes to be sampled. This sampling design was considered optimal for detecting spatial and temporal trends of concentrations within broad geographic boundaries. However, higher numbers of fish would need to be sampled to determine trends, either spatial or temporal, between individual lakes.

Some of these recommendations have since been adopted. For example, the Minnesota FCMP now analyzes individual predator fish instead of composites.

**National Fish Tissue Study**

The objective of the National Fish Tissue Study is to estimate the national distribution of the mean levels of selected persistent, bioaccumulative, and toxic (PBT) chemical residues in fish from lakes and reservoirs of the continental United States. This program has been in existence since 1998, when the program’s study design was developed. Field sampling began in the fall of 1999 and will continue through 2003.

The 500 water bodies under study were selected randomly from an estimated 260,000 lakes and reservoirs in the continental United States. Lakes were divided into six size categories, ranging from 2.5 to over 900,000 surface acres, with an equal number of lakes in each category. To be selected for sampling, a lake had to be a permanent body of water with permanent fish populations. The lake had to be at least 2.47 acres in area, with a minimum of 1,000 m² of open water and minimum average depth of 1 m. Ideally, suitable habitats for target species will be determined for each lake, and up to three locations of that habitat will be randomly selected for sampling in each lake.

Target analytes were selected from EPA’s multimedia PBT list of 451 chemicals, and from a list of 130 chemicals taken from several contemporary fish tissue bioaccumulation studies. Target analytes include PCBs, DDTs, mercury, PAHs, dioxins and furans, and other chemicals.

For each sampling location, a representative composite sample for both a predator and a bottom-dwelling species are collected. Species collected are selected because they are commonly consumed, may potentially accumulate high concentrations of chemicals, and have a wide geographic distribution. In total, more than 20 species have been collected. Predator species are analyzed as composites of untrimmed fillets with skins. Whole fish composites are analyzed for bottom feeding fish. Composites for both fillets and whole fish consist of five specimens or more, in order to yield enough tissue for analysis.

Only spatial trends will be assessed with these data. The evaluation of spatial trends of PBTs will be discussed in 2004.
Ohio

Ohio’s FCMP is an interagency endeavor entitled “Long-term Contaminant Monitoring of Ohio’s Sportfish.” Participating agencies are the Ohio Department of Agriculture, Ohio Environmental Protection Agency (OEPA), Ohio Department of Health, and Ohio Department of Natural Resources. The primary goals of the monitoring are to determine what chemicals are present in Ohio sportfish, and to advise the public regarding the risks associated with the consumption of Ohio sportfish. Secondary objectives are determining when to lift advisories, monitoring trends in contaminant levels to assess the success of pollution control efforts, identifying new contaminants being introduced into the environment, and augmenting existing programs that evaluate point and nonpoint sources of pollution. This FCMP was established in 1987.

Sampling sites are selected based on several factors. For streams and rivers, preference is given to stream segments that have not been evaluated previously, to impaired segments identified by OEPA, and to stream sections near major urban localities. Sampling of lakes and reservoirs emphasizes those that are publicly owned, greater than five acres in area, and designated for recreational or conservation use. Sites are sampled on a fixed interval, so most sites should be revisited every five years. However, the Ohio River is sampled every year. According to the current sampling plan, a total of 298 inland lakes and reservoirs, including Lake Erie, will be evaluated in a 10-year period.

Chemicals analyzed include PCBs, mercury, DDTs, a variety of other organic pesticides, and some metals such as arsenic, cadmium, lead, and selenium. As the primary intent is protection of fish consumers, the Ohio FCMP samples a variety of species, including predators, panfish, and bottom feeders. Over 50 different species have been sampled across the state. A wide variety of sizes classes are also sampled.

The initial goal of the sampling plan was to geographically cover all water bodies in the state. The next phase will be revisiting areas to monitor for temporal trends. Lake Erie has been sampled over several years. However, trends analyses for the Lake Erie data have been hampered because of sampling inconsistencies within species, as well as variation in the size of fish collected and the locations sampled.

Ontario

The Ontario FCMP, which has been in existence for 25 years, consists of two components, collection of adult fish fillets and whole body YOY fish. Most effort is directed to fillets because the primary objective is to provide fish consumption advice to citizens of Ontario. The results of the monitoring form the basis for “The Guide to Eating Ontario Sport Fish,” which is produced every two years and distributed to the public.

To this end, edible portions (trimmed, skinless fillets) of adult sport fish are collected from major fishing locations around Ontario. Concentrations of hydrophobic PTS in this tissue may be considerably lower than in untrimmed fillets with skins, a point that should be considered when using Ontario data. A wide range of sizes and species, more than 50 over the life of this
FCMP, are collected to provide comprehensive health information. Fish are sampled from the Great Lakes and their connecting channels, and inland rivers and lakes, reservoirs, and impoundments. The choice of sampling sites is reviewed each year and based on several factors. Preference is given to locations where there are known or suspected sources of pollution, and where the site is a major food source for local inhabitants. The latter usually pertains to lakes in the vicinity of Native Reserves. Site selection also depends on whether the site is being considered for recreational development, or if it is part of the monitoring program for long-term studies of contaminants in fish.

Ontario also monitors chemical concentrations in YOY fish. In this program, juvenile fish are collected in nearshore waters of the Great Lakes and its tributary mouths. YOY fish are also collected at some inland locations near known sources of contamination or waters associated with acid rain studies. The YOY fish are analyzed as whole-body composites. The contaminants analyzed include PCBs, DDTs, heavy metals, dioxins and furans, PAHs, chlorinated phenols and benzenes, and other pesticides (chlordanes, toxaphene, dieldrin, endrin, and lindane). Both the food fish and YOY data are used for trends analyses. For the former, statistical evaluation is conducted through the use of regressions to generate a predicted concentration at a standard length. After this, differences in concentrations over time or between sampling locations is estimated with ANOVA and by visual inspection of the data (e.g., see Scheider et al. 1998)

Because age and size are presumably controlled across samples, temporal trends in YOY fish concentrations are based on regression analyses. These analyses suggest that most organochlorines are declining at most Great Lakes sites (e.g., see Suns et al. 1993; Scheider et al. 1998). However, trends analyses with YOY are becoming more difficult as more and more chemicals decline below easily detectable levels. Currently, only PCBs and DDT are detectable at a wide variety of Great Lakes (Suns et al. 1993; Scheider et al. 1998), and concentrations of these chemicals are also becoming harder to detect over time.

Ministry of Ontario Environment (MOE) scientists believe that, ideally, annual monitoring with many replicates is warranted for rapidly changing parameters (e.g., chlorinated organics). Thus, the MOE FCMP samples both fillets and YOY fish “whenever possible.” In practice, many sites are sampled as often as every one to two years (Scheider et al. 1998.)

Pennsylvania

The Pennsylvania Boat and Fish Commission and the Department of Environmental Protection jointly monitor chemicals in fish. The primary purpose of the FCMP is detection and evaluation of fish tissue contamination and development of consumption advisory information for the fish consuming public. Routine monitoring for contaminants in fish tissue, encouraged by EPA, was initiated in 1979 as part of a nationwide network of 1,000 stations. In Pennsylvania, whole fish samples from 36 locations are collected and analyzed to provide a nationwide view of contaminant loads. In 1988, the program began collecting almost exclusively fillet samples to support public health protection. Fillets are sampled at fixed stations in Pennsylvania’s water quality network on a rotating 5-year basis. In two no-kill zones, where fish have high
organochlorine concentrations, concentrations in fillets are monitored every other year. Other locations are selected for sampling each year in order to follow-up on existing advisories.

Species sampled are those commonly taken by anglers for consumption. Sampled fish are, therefore, legal size individuals of the following species: lake, brown, and rainbow trout; coho salmon; walleye; carp; yellow and white perch; smallmouth bass; northern pike; and others. Sampling generally occurs between August and October. Most species are analyzed as skin-on fillets. Channel catfish or bullhead are analyzed as skinless fillets, while tissue samples for American eel are based on skinned and gutted fish. All species are analyzed as composites samples of five similarly-sized fish. Chemicals analyzed are PCBs, mercury, and pesticides (DDT, chlordane, heptachlors, aldrin, dieldrin, and mirex).

The data are not usually used for trends analyses. However, basic trends are sometimes discerned by visual inspection of the data. Regression analysis over time or fish length has also been applied in limited circumstances.

Wisconsin

The state of Wisconsin has monitored chemicals in fish since the 1970s. The objective of the Wisconsin FCMP is primarily to collect data for fish consumption advisories. Thus, the major component of the monitoring program concerns collection and analysis of edible fish tissue.

Selection of sampling sites depends on a number of factors, such as size of the water body, the ease of access, and the amount of fishing activity. The focus of the sampling has changed from a site-by-site selection of water bodies based on suspected problems in the first years, to a basin assessment approach from 1990 to 2000, where the goal was to return to a basin every five years and where specific problem sites within a basin would be targeted for repeated sampling, to the current monitoring program, which is a baseline assessment adopted for fish community, habitat, and macroinvertebrate community monitoring. The goal of this is a statewide coverage, in addition to the sampling of advisory sites occurring every five years.

The variety of chemicals analyzed includes PCB Aroclors®, DDT and its metabolites, mercury, chlordanes, endrin, dieldrin, and dioxin and furans.

The fish species sampled include a variety of more than 20 edible species. In general, untrimmed fillets with skins are analyzed. However, for carp, alewife, lake herring, and gizzard shad whole fish are analyzed, and skin-off fillets are analyzed for catfish, burbot, and bullheads. Headless, whole fish are analyzed for chub and smelt. Fish are analyzed as individual fish and as composites. When composites are analyzed, the composite consists of one species of a limited size range.

Various statistical methods are used based on the quality of the data. Concentration data are generally log transformed to reduce residual spread at higher concentrations. Results are generally not lipid-normalized unless a specific interspecies comparison is warranted. Various statistical techniques have been applied to the data to analyze trends, but the existing data are
often not amenable to these techniques. A specific sampling program needs to be set up to rigorously assess trends.

**New York YOY**

The objective of the New York State YOY FCMP is to evaluate trends in near shore contaminants in young fish from New York’s Great Lakes Basin. This monitoring program has been in existence since 1984. YOY fish are sampled from the major Great Lakes tributaries and in areas near contaminant sources, to adequately represent areas of concern. Samples were taken annually in the first 4 years of the program in order to establish a baseline. Thereafter, samples have been taken every 5 years.

The three most common YOY fish sampled are the bluntnose shiner, spottail shiner, and the emerald shiner. Species are kept separate and are analyzed separately in order to have the flexibility to address changing fish populations over space and time. Whole fish composites are combined according to species, age, collection date, and total mass. The chemicals analyzed include PCB Aroclors®, DDT and its metabolites, mercury, chlordane, and dieldrin.

Temporal and spatial trends analyses are conducted using graphic representation and Kruskal-Wallis tests. The trends suggest that concentrations of PCBs, DDT compounds, and dioxins and furans, where present, are declining over time. Mercury levels appear to be stable over time. Most other analytes are generally not detected in YOY fish.
Table
Table B-1. Summary of survey responses

<table>
<thead>
<tr>
<th>Type of Monitoring</th>
<th>MDEQ Caged Fish</th>
<th>MDEQ Whole Fish</th>
<th>Canadian DFO</th>
<th>GLUCF FCM</th>
<th>Illinois FCM</th>
<th>Indiana FCM</th>
<th>ITFAP FCM</th>
<th>MNDF FCM</th>
<th>Minnesota FCM</th>
<th>National Fish Tissue</th>
<th>Ohio FCM</th>
<th>Ontario FCM</th>
<th>PA FCM</th>
<th>Wisconsin DNR</th>
<th>NY FKM FCM</th>
<th>COUNT</th>
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<td>X</td>
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<td>X (prior to 1994 only)</td>
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<td>X (prior to 1994 only)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X (fish and lobster)</td>
<td>X (limited)</td>
<td>X (prior to 1994 only)</td>
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<td>X</td>
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<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>9</td>
<td></td>
</tr>
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</table>

Explicit Goals

| Data for consumption advisories | X | X | X | X | X | X | X | X | X | X | X | X | 9 |
| Evaluation of trends | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 14 |
| Evaluation of Effectiveness | X | X | X | X | X | X | X | X | X | X | X | X | 11 |
| Evaluation of env. quality | X | X | X | X | X | X | X | X | X | X | X | X | 9 |

Duration of Program

| 18 | 30 | 11 | 24 | 10 | 25 | 25 | 12 | 10 | 11 | 2 | 25 | 25 | 22 | 18 | 18 |

Any Changes to Protocol?

| X | X | X | X | X | X | X | X | X | X | X | X | X | 14 |

What Type of Systems?

| Great Lakes | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 13 |
| Connecting channels | X | X | X | X | X | X | X | X | X | X | X | X | 6 |
| Inland rivers | X | X | X | X | X | X | X | X | X | X | X | X | 10 |
| Inland lakes | X | X | X | X | X | X | X | X | X | X | X | X | 12 |
| Reservoirs/impoundments | X | X | X | X | X | X | X | X | X | X | X | X | 10 |
| Other | X | X | X | X | X | X | X | X | X | X | X | X | 2 |

How Locations Selected?

| Fixed, representative station | X | X | 2 |
| Randomized design | X | X | 1 |
| Several fixed stations | X | X | 8 |
| Case by case | X | X | X | X | X | X | X | X | X | 9 |
| Other | MDCCH, MDNR collections | X | X | (for screening) | 3 |

How Frequently Sampled

| Every year | X | X | X | X | X (Lake Michigan) | X (Lake Superior: 1-time event) | X (Lake Erie: 1-time event) | 5 |
| Fixed intervals | X | X | X | X | X (Lake Michigan) | X (Lake Superior: 1-time event) | X (Lake Erie: 1-time event) | 5 |
| Randomized design | X | X | X | X | X | X (Lake Superior: 1-time event) | X (Lake Erie: 1-time event) | 5 |
| Case by case | X | X | X | X | X | X (Lake Superior: 1-time event) | X (Lake Erie: 1-time event) | 5 |
| Other | MDCCH, MDNR collections | X | X | (for screening) | 3 |

What Information Collected

<p>| Total length | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 15 |
| Standard length | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 15 |
| Fork length | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 15 |
| Weight | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 15 |
| Age | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 15 |
| Gender | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 15 |</p>
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<thead>
<tr>
<th>MDEQ Caged Fish</th>
<th>MDEQ Fillets</th>
<th>Canadian DFO</th>
<th>GLIFWC FCMP</th>
<th>Illinois FCMP</th>
<th>ITFAP FCMP</th>
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<th>National Fish Tissue</th>
<th>Ohio FCMP</th>
<th>Ontario FCMP</th>
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<th>Wisconsin DNR</th>
<th>NY YOY FCMP</th>
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<td>X</td>
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<td>X (for PCB analysis)</td>
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<td>Lipid level or fat content</td>
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<td>Presence of wounds/ disease/tumors</td>
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**What Species**

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<tr>
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<td>Brown trout</td>
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80075681.0011697/app_bta.xls
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### Analytes Measured?

#### Inland Lakes:
- mercury only
- PCBs (Aroclors®)
- PCBs (congeners)
- DDT
- DDE
- DDD
- Mercury
- Chlordane
- γ-Chlordane
- cis-Nonachlor
- trans-Nonachlor
- Oxychlordane
- Tiochlordane
- Dieldrin
- O-DDT
- O-DDE
- O-DDD
- O-DDE
- O-DDT
- O-DDD
- O-DDE
- Dioxin/furans
- Chlordane
- α-Chlordane
- γ-Chlordane
- cis-Nonachlor
- trans-Nonachlor
- Oxychlordane
- Tiochlordane
- Dieldrin
- O-DDT
- O-DDE
- O-DDD
- O-DDE
- Dioxin/furans

### Methods to Control for Confounding Factors

- No evaluation of trends
- Fish collected from the same location
- Same species of fish collected
- Same size of fish collected
- Same age of fish collected
- Same sex of fish collected
- Same collection time
- Lipid normalization
- Other (please elaborate)

### Other Data Collected?

- pH
- Organic carbon
- TOC
- Other

### Chemicals Collected in Other Media?

- Water column
- Sediments
- Prey fish
- Other

### Other (please elaborate)

- Caged - control on movement
- X (food web samples)
- X (water samples)
- X (during screening)
- X (contaminated areas)
- X (limited & dioxins/furans)
- X (by special request)
- X (for some specific studies)
- X (a few weeks)
- X (limited & contaminants)
- X (ad hoc data)
- For specific research questions
- X (during screening)
- X (water chemistry parameters)
- X (column monitoring program)
- X (ad hoc data)
Table B-1. (cont.)

<table>
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<tr>
<th>MDEQ Caged Fish</th>
<th>MDEQ Whole Fish</th>
<th>Canadian DFO</th>
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</tr>
</tbody>
</table>

* Changes to protocols are described in detail in Attachment B-2.
* Details of how locations are selected are described in Attachment B-3.
* See Attachment B-4 for a complete list of species collected.
* All fish are prepared as per the instructions of the "Protocol for a Uniform Great Lakes Fish Consumption Advisory."
* See Attachment B-5 for a complete list of analytes measured.
* Analytical method described in Attachment B-6.
* Huebs et al. (1996).
* Huebs et al. (1997).
* Borgmann and Whittle (1991b).
* Borgmann and Whittle (1992b).
Attachment B-1

Fish Contaminant Trend Monitoring Questionnaire
Background and Instructions:

The intent of this questionnaire is to learn more about your Fish Contaminant Monitoring Program (FCMP) and techniques used to determine spatial and temporal trends of contaminants in fish. The questionnaire is divided into five parts:

I. Program Description
II. Program Logistics
III. Quality Control, Data Analysis, and Interpretation of Trends
IV. Dissemination of Results
V. Conclusion and Summaries

In some cases, the FCMPs include several elements with different goals and designs (e.g., edible portion, young-of-year (YOY), or whole adult fish). If your program has multiple elements, then it would be helpful to the Michigan Department of Environmental Quality (MDEQ) if the questions were answered separately for each element. We have provided three copies of the questionnaire for this purpose. We have also provided the entire questionnaire on disk should you wish to respond electronically. Please feel free to append reports or other written materials when you feel that they will answer questions posed below.

Please send survey responses, reports, or other written materials and fish contaminant data to Bob Day at:

Dayrm@state.mi.us

Or

Michigan Department of Environmental Quality
Water Resources Division
P.O. Box 30458
Lansing, Michigan 48909

If you would like additional information about the questionnaire or the project, please contact Mr. Day at 517-284-5513. We would appreciate receiving your responses by November 26, 2001. Thank you in advance for your assistance.
Fish Contaminant Monitoring Program Questionnaire

Part I. Program Description

1. What type of fish contaminant monitoring do you conduct? (Please check all that apply)
   - Whole adult fish trend monitoring program
   - Young-of-year (YOY) trend monitoring program
   - Caged-organism monitoring program
   - Edible-portion monitoring program
   - Other (please specify)

Please attach a description of each element to this questionnaire. Additionally, if your program includes different elements, please answer questions 2 through 25 for each element.

2. Does your program have a mission statement or equivalent that describes why your program is useful and what those uses are? If so, please describe.

3. What are the explicit goals of your FCMP (or individual program element)?
   - Collection of data for issuing fish consumption advisories
   - Evaluation of trends of chemicals in the environment
   - Evaluation of effectiveness of pollution control activities
   - Evaluation of environmental quality (e.g., attainment of the goals of the Clean Water Act, Remedial Action Plan, or Lakewide Management Plan)
   - Others: Please elaborate
4. How long has your program been in existence? _______

5. Have there been any significant changes in methods (analytical methods, tissue type, labs, etc.) over that period? ___Yes  ___No

If you answered yes, when did these changes occur, what were these changes, and what efforts, if any, were made to make previous data compatible with past? As an example, we have included the following information from MDEQ’s whole-fish trend monitoring program, but feel free to follow a different format if that works better for your program.

<table>
<thead>
<tr>
<th>FCMP Element</th>
<th>Date (Initiation or Change)</th>
<th>Description (brief description of program or change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole fish trend monitoring</td>
<td>1990</td>
<td>Initiated fixed station whole fish trend monitoring program at 27 sites.</td>
</tr>
<tr>
<td>Whole fish trend monitoring</td>
<td>1996</td>
<td>Dropped one station because we had difficulty collecting samples.</td>
</tr>
<tr>
<td>Whole fish trend monitoring</td>
<td>2000</td>
<td>Switched from polychlorinated biphenyl (PCB) Arochlors to PCB congener analyses at all 26 sites. We compared the results of congener and Arochlor analyses in 450 samples in which both congeners and Arochlores were analyzed in order to ensure method comparability.</td>
</tr>
</tbody>
</table>

6. What types of aquatic systems are monitored by your FCMP? (Please check all that apply)

_____ Great Lakes
_____ Great Lakes connecting channels
_____ Inland rivers
_____ Inland lakes
_____ Reservoirs or impoundments
_____ Other (please describe)
7. With respect to large aquatic systems (e.g., a Great Lake or major river), how do you select monitoring locations? (Please check all that apply)

- One fixed station that represents the entire waterbody
- A randomized design to select sites based on stream reach, or grid
- Several fixed stations that represent the waterbody
- Case by case determination (if so, can you describe your criteria)
- Other (please specify)

8. How frequently do you repeat monitoring at sites? (Please check all that apply)

- Every year
- Fixed intervals (please describe)
- Based on a randomized design
- Case by case determination (if so, can you describe criteria)
- Other (please describe)

9. What are the key factors that your agency considers when selecting the waterbodies, fish species, chemicals, and tissue types to be investigated each year? Has this decision-making process been documented in a written format? Please describe how this decision-making process is implemented each year.
Part II. Program Logistics and Mechanics

10. What non-chemical information is collected about the fish? (Please check all that apply)

- Total length
- Standard length
- Fork tail length
- Weight
- Age
- Gender
- Reproductive condition
- Lipid level or fat content
- Other (please describe)?

11. What species of fish are collected? (Please check all that apply)

- Lake Trout
- Sicowet Lake Trout
- Coho Salmon
- Chinook Salmon
- Brown Trout
- Rainbow Trout
- Walleye
- Carp
- Yellow Perch
- Smallmouth Bass
- Largemouth Bass
- White perch
- Smelt
- Alewife
- Northern Pike
- Other? (please list)
12. What tissue is collected? (Please check all that apply)

- Whole fish
- Untrimmed fillet with skins
- Trimmed fillet with skins
- Untrimmed skinless fillets
- Trimmed skinless fillets
- Dorsal plugs (please describe the length, width and weight)

- Other? (please elaborate)

13. Are individual or composite samples analyzed?

- Individual fish or fish tissues are analyzed.
- Tissue from more than one fish is combined into a composite sample.

If composite samples are analyzed, then which of the following factors are considered when combining tissues? (Please check all that apply)

- species
- age
- length
- weight
- collection date
- sample volume
- Other (please describe)
14. What bioaccumulative chemicals of concern (BCC) are analyzed? What are the methods and quantification levels?

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analytical Method</th>
<th>Quantification Level</th>
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<tbody>
<tr>
<td>PCBs</td>
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<tr>
<td>Arochlorls</td>
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<td>Congeners</td>
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<td>DDT</td>
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<tr>
<td>DDD</td>
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<td>g-chlordane</td>
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<tr>
<td>cis-nonachlor</td>
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<td>trans-nonachlor</td>
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<tr>
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<td>Toxaphene</td>
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<td>Dieldrin/Endrin</td>
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<tr>
<td>BHC</td>
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<tr>
<td>Other Chemicals? Please list.</td>
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</table>
Part III. Quality Control, Data Analysis, and Interpretation of Trends

15. Is your program reviewed periodically in terms of goals, efficacy in meeting those goals, cost-effectiveness, or other attributes? If yes, please describe. (Such reviews could include internal reviews and analyses as well as more formal external review.) Have your sampling and analytical programs ever been assessed in terms of their statistical power or ability to discern differences? If so, please describe. If any documents or summaries of these reviews are available, please append them.

16. Are the FCMP data used to evaluate temporal or spatial trends of BCC? If so, what if any methods are used to control for confounding factors that also affect concentrations of chemicals in fish over time and space. (Please check all that apply)
   - [ ] Fish collected from the same location
   - [ ] Same species of fish collected
   - [ ] Same size of fish collected
   - [ ] Same age of fish collected
   - [ ] Same sex of fish collected
   - [ ] Same collection time
   - [ ] Lipid normalization
   - [ ] Others? (please elaborate)
17. Are other data collected to augment or complement the interpretation of BCC in fish? For example, do you collect limnological data such as pH or organic carbon to help describe the bioavailability and bioaccumulation of BCC in fish?

18. Are BCC data collected in other media (e.g., water column, sediments or biota) to complement the fish studies? If so, please describe.

19. Does your program archive tissues or extracts? If so, please describe this aspect of your program.

20. How are temporal trends evaluated, by perusal of data or simply looking at graphs, formal statistical analyses, or some other methods? Please describe in detail. For example, if statistics are used, what statistical methods are used, what null hypotheses are assumed, what if any transformations are performed, etc.
21. If trends analyses have been conducted with your data, what, if any, general conclusions have been drawn? For example, are BCC generally decreasing, increasing, or remaining stable over time? If there are differences among species, chemicals, or systems (e.g., PCBs are declining but mercury is increasing), please elaborate.

22. Please think about the elements of your FCMP with respect to monitoring and detecting trends. What methods have worked well for deducing trends from time to time and space to space? What has not worked as well as expected?

Part IV. Dissemination of Results

23. Are the results of your FCMP disseminated internally or externally on a regular basis? If so, what is the format of those reports? Please include any such reports or memoranda that are available. (Internal memoranda can be kept confidential upon request.) Have your data been used in the peer-reviewed literature? If so, please list examples.
24. Are your fish contaminant data stored in a publicly accessible database such as Storage and Retrieval System or an agency web site? If not, are your raw data available to MDEQ?

Part V. Conclusion and Summary

25. Based on your experience, can you think of any important points or questions concerning interpretation of your fish contaminant data that are not covered by the questions above? Are there areas of uncertainty or factors that you think should be addressed by our analysis? If so, please list them and describe why you think they are important?

Thanks again for your time and effort on these questions.
Attachment B-2

Changes in Protocol
Changes in Protocol

MDEQ Caged Fish

Sampling  None stated

Analytical  In 2000, switched from polychlorinated biphenyl (PCB) Aroclors® to PCB congener analyses. The results of congener and Aroclor® analyses were compared in 450 samples in order to ensure method comparability.

MDEQ Whole Fish

Sampling  In 1990, initiated fixed station whole fish trend monitoring program at 27 sites.

In 1996, dropped one station because there was difficulty in collecting samples.

Analytical  In 2000, switched from PCB Aroclors® to PCB congener analyses. The results of congener and Aroclor® analyses were compared in 450 samples in order to ensure method comparability.

MDEQ Fillets

Sampling  None stated

Analytical  In 1987, switched labs due to concerns about quality assurance at lab used prior to 1987.

In 2000, switched from PCB Aroclors® to PCB congener analyses. The results of congener and Aroclor® analyses were compared in 450 samples in order to ensure method comparability.

Other  Data collected since 1980 are entered in an electronic database.

MA FCMP

Sampling  None stated


Labs were required to use analytical methods used in NOAA Status and Trends Programs, and to participate in the annual NIST/NOAA-NS&T Inter-comparison Exercise Program.

**PA FCMP**

**Sampling**
Sampling of both whole fish (as prescribed in the EPA Core program) and the edible portion (FDA standard fillets) began in 1983 at half of the 36 Core stations.

In 1988 sampling of the edible portion began almost exclusively to determine the need for consumption advisories. In addition, sampling was no longer limited to the original 36 stations. The tissue monitoring is rotated through the entire ambient monitoring network on a 5-year basis, in order to follow up on existing advisories and allow the field biologists to recommend sampling in areas of concern in their geographic area of responsibility.

**Analytical**
None stated

**Indiana FCMP**

**Sampling**
The most significant issue has been in the preparation of the sample. In the past, cross-sectional stakes had been prepared for Lake Michigan salmonids. There was a period in the 1980s where all tissue samples were prepared as skin off fillets. Bullhead were treated as gutted and beheaded “fiddlers.” Sample preparations are clearly marked in the database and one should always take that into consideration when doing an exploratory analysis on the data.

**Analytical**
None stated

**Intertribal Fisheries Assessment Program**

**Sampling**
From 1991 to 1997, the Intertribal Fisheries Assessment Program (ITFAP) analyzed trimmed fillets with skins; however, ITFAP began analyzing skinless fillets in 1998. Comparison of skinless fillets to fillets with skin on for trend analysis is estimated by assuming that 30 percent of contaminants are eliminated by removing skin from samples, based on studies by Zabik et al. (1993), and others.

**Analytical**
The chlordane congeners oxychlordane, *trans*-nonachlor, and *cis*-nonachlor were added in 1997 to compare to Michigan’s FCMP.
GLIFWC FCMP

Sampling
None stated

Analytical
None stated

Minnesota FCMP

Sampling
None stated

Analytical
Changes in Laboratories:

• The majority of data from 1967 to 1989 were produced at the Minnesota Department of Health lab under the Pollution Control Agency program. This included mercury and PCB residue work and also some limited number of other heavy metals and organics analyses.

• The Minnesota DNR laboratory at Carlos Avery also did a fair amount of fish contaminant analysis, predominantly mercury residue work, in the 1970s.

• A local private laboratory (Braun Intertec) produced virtually all contaminant data for DNR from 1990 to 1998.

• The Minnesota Department of Agriculture lab took over all fish contaminant work in 1999 and continues to present.

From 1967–1989, essentially the same analytical method for mercury (cold vapor AA) was used by both state laboratories. Tissue types varied somewhat during those years. For much of the early mercury work in the 1970s muscle plugs without skin were analyzed; from roughly 1980 to the present, edible fillets have been predominantly used. Some attempts were made to compare mercury levels in these two tissue types, with fairly similar results. PCB analytical methods used were similar. Tissue types included both whole fish and edible fillets.

1990 to the present: In 1990, and again in 1998, when lab switches were made, attempts were made to establish data comparability by having both labs run numbers of split samples for mercury and PCBs.

1990: Department of Health laboratory and Braun Intertec laboratory each ran 30–40 split samples for mercury and PCBs. Mercury results compared very well, with somewhat more variation in PCB results. Both labs used essentially the same mercury cold-vapor method. Both labs used Aroclor® method for PCBs, but used a somewhat different set of peaks, which probably accounted for the slight differences in final results.
1998: Braun Intertec laboratory and Department of Agriculture laboratory each ran about 50 split samples for mercury and PCBs. Again, results showed good agreement on mercury levels, but not quite as good agreement on PCB results. Methods for both labs were essentially the same: cold-vapor for mercury, PCBs as Aroclor® (60/40 Aroclor® 1254/1260 mixture as calibration standard).

Canadian DFO

Sampling  None stated

Analytical  Laboratory analytical procedures have been modified over the past 25 years. An inter-comparison of data generated by various analytical methods has been made by the reanalysis of samples used to generate “historical data” (i.e., comparison of total PCB measurements via pack column vs. capillary column GC methods) (see Huestis et al. 1996).

Ohio FCMP

Sampling  Early program was focused on using whole body fish samples to evaluate known or suspected “hot spots.” After 1993 the emphasis shifted to collection of fillet samples for use in the sport fish consumption advisory program.

Analytical  None stated

Illinois FCMP

Sampling  In 1976, the Illinois Department of Conservation (IDOC) expanded fish contaminant sampling to include 40 state lakes, a few public lakes and three U.S. Army Corps of Engineers lakes.

In 1983, a memorandum agreement coordinated sampling with the IDOC/Illinois Environmental Protection Agency (IEPA) Basin Survey program.

In 1989, modifications to routine sampling sites were made.

In 1992, a lack of funding required that the FCMP was reduced to sampling in Lake Michigan and a few specific problem areas only.

Analytical  In 1984, analysis of mercury was suspended based on an analysis of historical data.
In 1985, new procedures for quality assurance in laboratories (IEPA, Illinois Department of Public Health [IDPH], Illinois Department of Agriculture) were required to meet quality assurance and quality control (QA/QC) spiked sample testing supplied through the U.S. Environmental Protection Agency (EPA) and U.S. Food and Drug Administration (FDA) quality assurance programs.

In 1986, the *Work/Quality Assurance Guidance Plan* was revised so that all fish contaminant samples were analyzed by the IEPA labs. In 1992, analysis of mercury was reinstated for all predator samples collected.

In 1997, the *Protocol for a Uniform Great Lakes Sport Fish Advisory on Lake Michigan* was adopted, using the actual levels of contaminants found in a single sample rather than the percentage exceeding the action level for several samples.

*Other*  
In 1977, IDPH, IDOH, FDA, IEPA and IDOC coordinated fish contaminant sampling and established quality control procedures. It was agreed that all data were to be stored in the EPA computer system, STORET, through IEPA.

**Ontario FCMP**

**Sampling**  
None stated

**Analytical**  
In 1997, photomirex was added to the list of contaminants analyzed; in 2000, dioxin-like PCBs were added to the analysis; in 2001, there was a reduction in the toxaphene detection limit; in 2002, additional chlordane congeners were analyzed.

**National Fish Tissue**

**Sampling**  
None stated

**Analytical**  
None stated

**Wisconsin DNR**

**Sampling**  
In the first years, the focus was on industrial rivers and the Great Lakes, then a basin approach, and now a statewide coverage is the focus.

**Analytical**  
Clean-up methods for PCBs and pesticides analyses have been added, and there have been changes in the columns for pesticide analysis.
New York YOY FCMP

Sampling  The number of stations increased with time.

Analytical  The number of chemicals analyzed has changed over time (PAHs were only analyzed in 1992, dioxins/furans were added to the list in 1992, PCB congeners were added in 1997). Once added, the same methods were retained for these analytes if analyzed in the future.
Attachment B-3

Key Factors Determining Sampling (Sampling Location, Choice of Water Bodies, Species Selection, Chemicals Used, Tissue Type)
Key Factors Determining Sampling
(Sampling Location, Choice of Water Bodies, Species Selection, Chemicals Used, Tissue Type)

MDEQ Caged Fish

Sampling Locations
Locations for caged fish samples are selected based on the objectives of the study. For example: Great Lakes tributary mouths are monitored to determine the presence or absence of bioaccumulative contaminants of concern (BCC) and the relative magnitude compared to other Great Lakes tributary mouths. Sites are selected upstream and downstream of a suspected source of BCC. Sites are selected upstream and downstream of a remediated area (e.g., contaminated sediment removal) to assess the effectiveness of the remediation. Sites may be placed at a number of locations, from the source to the mouth of a river, in order to identify source areas of BCC.

MDEQ Whole Fish

Sampling Locations
There is one station within the inland river and connecting channel sites. The Great Lakes sites are located in bays and there may be a need for more than one site per Great Lake (e.g., Little Bay De Noc and Grand Traverse Bay in Lake Michigan or Saginaw Bay and Thunder Bay in Lake Huron).

MDEQ Fillets

Sampling Locations
The Great Lakes sites are selected based on Michigan Department of Community Health sport fish consumption advisories and MDNR collection practices. In some cases a difference in contaminant concentrations along a gradient has been observed (e.g., concentrations in northern Lake Michigan fish are different from concentrations in southern Lake Michigan fish) and both areas are covered. Sites on long rivers are selected based on barriers to movement (i.e., dams), sources of contaminants, and our ability to obtain samples.

Choice of Water Bodies
MDNR Fish Division, tribal organizations and others (non-MDEQ) collect about 80 percent of the edible portion samples. Therefore, there is heavy reliance on the work plans of others. Also, sampling is often targeted toward sites where there are known or suspected sources of BCC, public access, and sites that are popular with anglers. In some cases only mercury will be analyzed, particularly at remote inland lakes or reservoirs with no known source of chlorinated organics (other than atmospheric).
MA FCMP

Sampling Locations
Flounder and lobster collections take place in the immediate vicinity of old and new wastewater discharges and in the Cape Cod Bay. Cape Cod Bay is used because it is a spring feeding area for the endangered right whale. In addition, flounder are collected from two locations between the old and new discharge sites, one in a somewhat contaminated site, the other in a cleaner location. These sites are monitored less frequently. Mussels are collected from clean reference sites and deployed in the zone of initial dilution of the old and new discharges, in Cape Cod Bay, and in a very contaminated location in Boston’s Inner Harbor.

Choice of Water Bodies
Locations chosen based upon: a) proximity to wastewater discharge, b) appropriateness for use as “clean” or “dirty” control, and c) proximity to the spring feeding grounds of the endangered right whale.

Species Selection
Mussels were used because they are commonly used as an indicator of water quality. Lobsters used because of their commercial value in the Massachusetts Bay region. Flounder used because they were an early (1970s) indicator of presumed contamination impacts (high incidence of liver disease) in Boston Harbor and because they have minimal seasonal migrations in and around the harbor/bay region.

Chemicals Used
Based upon consistency with the NOAA Status and Trends Program.

Tissue Type
Edible tissue as a measure of potential human health issues. Liver and hepatopancreas used because they appear to be short-term integrators of recent contaminant exposure.

PA FCMP

Sampling Locations
Chosen case by case with no set criteria. Sampling is accomplished in conjunction with other sampling activities when possible, or the field biologists select locations based on their knowledge of pollution sources as well as logistical concerns.

Choice of Water Bodies
A rotation of fish tissue sampling through the routine is applied, with a fixed-station water quality network on a 5-year basis. Trends have been followed in two no kill zones every other year (alternating). Other locations are selected for sampling each year in order to follow up on existing advisories, or selected by the field biologists in suspected problem areas or popular fishing locations.

Species Selection
Depends on the type of water body (cold water stream, warm water stream, large river, lake, Great Lake), species under advisory, and/or species availability. The general instruction to the collectors is to sample a
recreationally important species of legal size, unless following up on an outstanding advisory for some other species.

**Tissue Type**  
Edible portion (FDA standard fillets) is used. The parameter list is specified in the EPA Core program. This list must be reevaluated, but resource constraints have kept it from happening.

**Indiana FCMP**

**Sample Locations**  
Sites are selected by a stratified probabilistic design of the Watershed Monitoring Program by the U.S. EPA Corvallis Laboratory for fish community assessment. These sites are a subset of a larger site stratified probabilistic (site) draw for surface water chemistry assessment. From the pool of 2001 probabilistic sites, thirty wadable and non-wadable stream sites in the Patoka River and 42 in the West Fork White River basins were selected. Four of the river sites are “core stations.” Core stations are larger river locations where monitoring has occurred periodically for a number of years.

**Choice of Water Bodies**  
Choice is based on accessibility, whether there has been monitoring before, evidence of past problems, etc. A program plan is developed for each sampling year that lists intended target sites. Because “sport” fish are not the only fish consumed by people who fish Indiana’s waters, the program attempts to collect a bottom feeder, a top end predator, and another species representative of the site.

**Intertribal Fisheries Assessment Program**

**Sample Locations**  
Fish are collected from tribal commercial catch at specific locations from Lake Huron, Lake Michigan, and Lake Superior every third year.

**Choice of Water Bodies and Species**  
ITFAP’s goal is to represent the tribal commercial catch of lake whitefish and lake trout from the 1836 treaty waters and have the ability to compare those results to the U.S. Food and Drug Administration trigger levels and the state of Michigan’s results.

**GLIFWC FCMP**

**Sample Locations**  
On Lake Superior, sites are chosen relative to their importance as part of tribal harvest. Areas are delineated by Great Lakes Fisheries Commission Lake Trout Management units. So, the tendency is to group contaminant data by these management units, although specific locations of collections are available in lat-long coordinates.
Choice of Water Bodies

Lakes to be sampled will be based on: 1) age of previously collected data (>5 years puts the lake on the priority list); 2) importance to tribal harvest (lakes that make up 90%, or appear to have a rising profile of importance in total tribal harvest); 3) long-term study lakes (twelve long-term study lakes will be sampled every 2 years); 4) temporal fluctuations in mercury data (lakes with higher variability may require more frequent testing).

Minnesota FCMP

Sample Locations

Most fish sampling sites on these large water bodies are relatively fixed locations that have been used by the Fisheries Department for their standard population assessment surveys over the years. For large lakes there are 1–5 standard netting sites, usually depending on the number of distinct bays. Fish for contaminant testing nearly always come from these standard netting activities. Large rivers also have established sampling sites that represent certain reaches. There are always at least two of these standard sites, but can be more depending on the length/nature of the river. To screen for emerging issues or potential problems, samples are taken where the chemical will most likely be detected. Typically, this would be below potential sources (WWTP or industrial discharges) or areas of sediment accumulation (behind dams). If a more comprehensive study is planned, a control (unimpacted) site will be included.

In addition to the fixed sampling sites, a number of other sites are sampled each year for analyses of spatial and temporal trends. Factors considered in selecting sites for spatial trends analyses are 1) whether the site is on Fisheries’ survey plan that year; 2) the importance of the fishery 3) a known or suspected local contamination problem; and 4) lack of contaminant data in the watershed of this lake or river.

Factors considered when selecting sites for temporal trends analysis: 1) whether the site is on Fisheries’ survey plan that year; 2) at least 5–8 years should have elapsed since last sampling.

Species Selection

For spatial trends, usually one or two large predators that are important to the particular fishery, such as northern pike, walleye, bass, or lake trout were targeted. The most important panfish species, and one rough species that contains the highest fat content were also ideally obtained. What is actually collected for testing ultimately comes down to what Fisheries personnel are able to net, which is largely determined by the most common species and to some extent luck.

For temporal trends, fish species similar to those described for spatial trends water bodies are selected, but often the number of species tested expands for these lakes and rivers. This is because more species are often available and important to the fishery. For screening, fish species are selected to provide tissue most likely to accumulate the chemical of interest (i.e., a large, high-fat
fish would be sampled to test for contaminants that accumulate in fat [PBDE, dioxin], while a large predator fish may be sampled to test for a contaminant that binds to protein [mercury, PFOS]). Sometimes this is limited by the fish available for analyses.

**Chemicals Used**

For spatial trends, mercury in all samples is routinely analyzed, since it is ubiquitous, and is the basis of most of the consumption advisories. PCBs are selectively analyzed, either if the area has a history of contamination (Lake Superior, certain large rivers, certain urban lakes), and some limited screening for PCBs in other water bodies is done depending on funding available. One or two samples of higher-fat-content fish may be screened for PCBs in a water body even if it is not expected to have a detection.

For temporal trends, similar procedures to those described above for chemicals analyzed (mercury on all, PCBs selectively) are used. However, there is a tendency to test more for PCBs on the large river samples because they historically have had more of this contamination.

**Tissue Type**

In nearly all cases the edible fillet is analyzed.

**Canadian DFO**

**Choice of Water Bodies**

Monitoring stations located on the lower Great Lakes (Ontario and Erie) are surveyed annually. Designated monitoring stations on the upper Great Lakes (Huron and Superior) are surveyed in a rotating pattern. Two stations on each lake are surveyed each year. Although lake trout may migrate throughout the lake after stocking, DFO samples multiple stations in each Great Lake to insure that the final sample contains a representative mixture of the strains of lake trout in each Lake. An attempt is made to collect fish for two consecutive years at any one station.

**Species Selection**

The program monitors contaminant trends in lake trout (and walleye where appropriate) plus elements of the forage base including smelt, alewife, and sculpins as they are available at any single site. Collections of the invertebrate forage base (Mysis, Diporeia, Plankton) are also made at many of the primary monitoring sites.

**Ohio FCMP**

**Sample Locations**

Known or suspected “hot spots,” an attempt is made to bracket the contamination.
Choice of Water Bodies  Choice is based on the following criteria: 1) water bodies that have never been sampled, 2) water bodies that are due for resampling, 3) emergency response situations, 4) are additional samples needed to resolve possible contamination issues remaining from the previous year’s results, and 5) TMDL sampling requirements. ODNR, OEPA and OPH meet annually just prior to the fishing season to decide on an appropriate sampling schedule.

Illinois FCMP

Choice of Water Bodies  Each year approximately 50 percent of samples are collected from waters with existing advisories (to allow evaluation of any changes that might be needed in advice), 25 percent are collected as part of routine river basin surveys, and 25 percent are collected to follow up on new sample data from the previous 2 years (to allow evaluation of the need for new advisories). In addition, a few samples are often collected based on special requests. None of the samples are collected based on written procedures. IEPA and IDNR staff meet in December and January to identify waters to be sampled in the summer based on reviews of previous data and scheduled surveys.

Ontario FCMP

Choice of Water Bodies  Water bodies are chosen because there is a known or suspected source of pollution nearby; because they are a major food source for local inhabitants (usually lakes in the vicinity of Native Reserves); because they are open for recreational development; because they are part of the monitoring program for long-term studies of contaminants in fish; or because there is angler interest.
### National Fish Tissue

#### Sample Locations
Locations are determined by the field crew at each site. The field objective is for sampling teams to obtain a representative composite sample for both a predator and a bottom-dwelling species from each site. To obtain a representative sample of the targeted species in lakes and reservoirs, field teams consider factors such as availability, suitable habitat, and the presence of contaminant gradients in planning sampling locations for the target lake. Ideally, habitats suitable for target species would be determined for the lake, and up to three locations of that habitat would be randomly selected for sampling in the lake (particularly in large water bodies). If a contamination gradient may be present in the water body, then 3 locations across the gradient should be selected for sampling. The composite is intended to estimate the mean fish tissue contaminant concentration for the lake or reservoir. Given the diversity of lakes and reservoirs in the study, and given the multiple species that must be used, the study must rely on the local knowledge of the field teams in the selection of the representative composite samples.

#### Choice of Water Bodies
The 500 water bodies in this study were randomly selected from the estimated 260,000 lakes and reservoirs in the continental United States. The lakes were divided into 6 size categories, ranging from 2.5 to over 900,000 surface acres, with an equal number of lakes in each category. Before sampling, field teams verified that each lake met the study’s definition of lake. This study defines a lake as a permanent body of water of at least one hectare (2.47 acres) in surface area with a minimum of 1,000 m² of open (unvegetated) water and a minimum average depth of one meter. The lakes in this study must also have a permanent fish population.

#### Species Selection
The primary criteria for selecting target fish species is that they are commonly consumed in the study area, that they may potentially accumulate high concentrations of chemicals, and that they have a wide geographic distribution. Secondarily, the target species should be easy to identify, abundant, easy to capture, and large enough to provide adequate tissue for analysis (i.e., adult specimens that as a five-fish composite will provide at least 560 g of edible tissue for analysis). Two distinct ecological groups of fish, bottom-dwellers and predators, are sampled for this study. This permits monitoring of a wide variety of habitats, feeding strategies, and physiological factors that might result in differences in bioaccumulation of contaminants.

#### Chemicals Used
Candidate target analytes were selected from EPA’s multimedia persistent bioaccumulative toxic (PBT) list of 451 chemicals, and from a list of 130 chemicals taken from several contemporary fish tissue bioaccumulation studies. The list of candidate analytes was discussed by scientists from state, federal, and tribal agencies attending a 1988 Study Design Workshop. Final analyte selection was completed by a team of analytical experts convened in 1999. The analytical expert work group selected 265 target analytes for this study (including breakdown products and PCB congeners) based generally on...
the following criteria: detailed information available; the chemical is of immediate concern and known to accumulate; the chemical is representative of a group or class of compounds; the chemical is considered to be important in one or more EPA programs.

**Tissue Type**
The National Fish Tissue Study includes composite sampling of fish fillets for predator/game fish species and whole fish for bottom-dwelling species for each sample lake. Five individuals per composite are collected, all of which must be large enough to provide sufficient tissue for analysis of the group of target analytes. At least 560 g of edible tissue per predator composite and 560 g of total body tissue per bottom-dweller composite are required to allow for analysis of the complete list of target analytes.

**Wisconsin DNR**

**Sample Locations**
For the Great Lakes, a general area is listed by the fish managers and the grid is left to the collector. For large rivers, a segment is listed on the collection schedule and the collector decides where best to collect the desired species.

**Choice of Water Bodies**
Choice is based on the size of the water body, and access fishing pressure. Early in the program, site by site choices were based on suspected problems. From 1990 to 2000, a basin assessment approach was used, where the goal was to return to a basin every 5 years. Specific problem sites within a basin were targeted for repeated sampling. Now a “baseline” assessment monitoring program has been adopted for fish community, habitat, and macroinvertebrate community monitoring. The goal of this is statewide coverage. So collection of fish for contaminant analysis depends on these guidelines and statewide coverage is a goal, in addition to sampling advisory sites every 5 years.

**New York YOY FCMP**

**Sample Locations**
Based on proximity to significant population centers.

**Choice of Water Bodies**
This program emphasizes major tributaries and water bodies near contaminant sources.
Attachment B-4

List of Fish Species Sampled
List of Fish Species Sampled

MDEQ FCMP

Caged Fish  Young-of-the-year channel catfish
Whole Fish  Lake trout, walleye, carp, largemouth bass
Edible Fish  Lake trout, siscowet lake trout, coho salmon, chinook salmon, brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, white perch, smelt, northern pike, channel catfish, white sucker, bluegill, yellow perch, splake, black crappie, lake sturgeon, rock bass, black bullhead, brown bullhead, sunfish, brook trout, freshwater drum, redhorse sucker, Unionidae, lake herring, muskellunge, gizzard shad, lake whitefish, alewife, chub, longnose sucker, burbot, tiger muskie, minnow, grass pickerel

MA FCMP

Winter flounder, northern lobster, blue mussel

PA FCMP

Lake trout, coho salmon, brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, white perch, northern pike

Indiana FCMP

Coho salmon, chinook salmon, brown trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, northern pike, black crappie, white crappie, white bass, lake trout, rainbow trout, brook trout, blue catfish, flathead catfish, brown bullhead, yellow bullhead, white sucker, spotted sucker, bigmouth buffalo, smallmouth buffalo, sauger, shorthead redhorse, silver redhorse, black redhorse, freshwater drum, spotted bass, quillback, rock bass, rivercommon carpsucker, bluegill

Intertribal Fisheries Assessment Program

Lake trout, whitefish
GLIFWC FCMP

Inland Lake Monitoring  Walleye and muskellunge
Lake Superior Study  Walleye, herring, lean lake trout, siscowet lake trout, lake sturgeon

Minnesota FCMP

Siscowet lake trout, coho salmon, chinook salmon, walleye, carp, yellow perch, smallmouth bass, largemouth bass, northern pike, channel catfish, crappie, white sucker, bullheads, bluegill, buffalo, flathead catfish, sauger, white bass, fresh water drum

For the Screening  Carp, walleye, northern pike

Canadian DFO

Lake trout, walleye, smelt, alewife, sculpin, invertebrates

Ohio FCMP

Brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, white perch, northern pike, channel catfish, crappie, whitefish, bigmouth buffalo, black bullhead, black crappie, black redhorse, blueguill sunfish, brook trout, brown bullhead, burbot, central stoneroller, crank chub, emerald shiner, flathead catfish, freshwater drum, golden redhorse, goldfish, grass pickerel, green sunfish, hog sucker, hybrid striped bass, longear sunfish, muskellunge, northern hogsucker, pumpkinseed sunfish, quillback carpsucker, rodear sunfish, river carpsucker, rock bass, round goby, sauger, saugene, shorthead redhorse, silver redhorse, smallmouth buffalo, spottail shiner, spotted bass, striped bass, white bass, white sucker, yellow bullhead

Illinois FCMP

Lake trout, coho salmon, chinook salmon, brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, smelt, alewife, channel catfish, crappie, flathead catfish, white bass, bullhead species, bluegill, buffalo species, spotted bass, sauger, freshwater drum

Ontario FCMP

Lake trout, siscowet lake trout, coho salmon, chinook salmon, brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, white perch, smelt, alewife, northern pike, channel catfish, crappie, whitefish, white bass, bowfin, Atlantic and pink salmon,
brook trout, splake, cisco, aurora trout, whitefish, humper, herring, chub (cisco species other than *C. artedii*), goldeye, mooneye, longnose sucker, common white sucker, big mouth buffalo, shorthead redhorse, redhorse sucker, goldfish, brown bullhead, American eel, burbot, rock bass, pumpkinseed, bluegill, sauger, freshwater drum, sturgeon

**National Fish Tissue FCMP**

Lake trout, brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, white perch, northern pike, channel catfish, crappie, white bass, brook trout, blue catfish, brown and yellow bullhead, white sucker

**Wisconsin FCMP**

Lake trout, siscowet lake trout, coho salmon, chinook salmon, brown trout, rainbow trout, walleye, carp, yellow perch, smallmouth bass, largemouth bass, white perch, smelt, alewife, northern pike, channel catfish, redhorse, white suckers, blue gill, other species not specified

**New York YOY FCMP**

Bluntnose minnow, spottail shiner, emerald shiner
Attachment B-5

List of Chemicals Analyzed
## List of Chemicals Analyzed

<table>
<thead>
<tr>
<th>Program</th>
<th>Chemicals Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDEQ FCMP</strong></td>
<td>Hexachlorobenzene, γ-BHC (lindane), aldrin, dieldrin, 4,4′-DDE, 4,4′-DDD, 4,4′-DDT, heptachlor epoxide, mercury, oxychlordane, γ-chlordane, trans-nonachlor, α-chlordane, cis-nonachlor, octachlorostyrene, hexachlorostyrene, heptachlorostyrene, pentachlorostyrene, heptachlor, terphenyl, toxaphene, mirex, PBB (FF-1, BP-6), PCBs (Aroclors® 1242, 1248, 1254 and 1260, 74 PCB congeners since 2000), dioxin/furans</td>
</tr>
<tr>
<td><strong>MA FCMP</strong></td>
<td>PCBs (congeners), DDE, DDD, DDT, α-chlordane, trans-nonachlor, heptachlor epoxide, aldrin, hexachlorobenzene, lindane, mirex, PAH</td>
</tr>
<tr>
<td><strong>PA FCMP</strong></td>
<td>PCBs (Aroclors®), DDT, DDE, DDD, mercury, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, dieldrin/endrin, BHC, methoxychlor, chlordane, heptachlor, heptachlor epoxide, aldrin, lindane, kepone, mirex, lead, copper, chromium, cadmium</td>
</tr>
<tr>
<td><strong>Indiana FCMP</strong></td>
<td>PCBs (Aroclors®), PCBs (congeners), DDT, DDE, DDD, mercury, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, BHC, aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, nickel, potassium, selenium, silver, sodium, thallium, vanadium, zinc, cyanide, endosulfan I, endosulfan II, endosulfan sulfate, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide, hexachlorobenzene, methoxychlor, pentachloroanisole</td>
</tr>
<tr>
<td><strong>Intertribal Fisheries</strong></td>
<td>PCBs (congeners), DDT, DDE, DDD, mercury, chlordane, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, dioxin/furan</td>
</tr>
<tr>
<td><strong>Assessment Program</strong></td>
<td><strong>Inland Lakes Study</strong>: mercury only. <strong>Lake Superior Study</strong>: PCBs (Aroclors®), DDT, DDE, DDD, mercury, chlordane, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, lindane, aldrin, heptachlor, endosulfans, methoxychlor, endrin ketone, aldehyde, hexachlorobenzene, pentachloroanisole, mirex</td>
</tr>
<tr>
<td><strong>GLIFWC FCMP</strong></td>
<td>Inland Lakes Study: mercury only. Lake Superior Study: PCBs (Aroclors®), DDT, DDE, DDD, mercury, chlordane, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, lindane, aldrin, heptachlor, endosulfans, methoxychlor, endrin ketone, aldehyde, hexachlorobenzene, pentachloroanisole, mirex</td>
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<tr>
<td><strong>Minnesota FCMP</strong></td>
<td>PCBs (Aroclors®), mercury. <strong>In the screening</strong>: toxaphene, dioxin, polybrominated diphenyl ethers (PBDES), perfluorooctane sulfonate (PFOS), various household/industrial wastewater compounds</td>
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</tbody>
</table>


January 29, 2003

<table>
<thead>
<tr>
<th>Program</th>
<th>Chemicals Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Canadian DFO</strong></td>
<td>PCBs (Aroclors®), DDT, DDE, DDD, mercury, chlordane, (\alpha)-chlordane, (\gamma)-chlordane, dieldrin/endrin, mirex, heptachlor epoxide, lindane, toxaphene (non-routine analyses of total and congener specific), PCB isomers, dioxins/furans (2,3,7,8 substituted congeners). DFO also measures concentrations of several metals (arsenic, copper, cadmium, lead, nickel, selenium, and zinc) in selected forage fish samples.</td>
</tr>
<tr>
<td><strong>Ohio FCMP</strong></td>
<td>PCBs (Aroclors®), DDT, DDE, DDD, mercury, chlordane, (\alpha)-chlordane, (\gamma)-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene (by special request), dieldrin/endrin, BHC, aldrin, endosulfan I, endosulfan II, endosulfan sulfate, heptachlor, heptachlor epoxide, hexachlorobenzene (HCB), methoxychlor, mirex, arsenic, cadmium, lead, nickel, selenium</td>
</tr>
<tr>
<td><strong>Illinois FCMP</strong></td>
<td>PCBs (Aroclors®), DDT, DDE, DDD, mercury, chlordane, (\alpha)-chlordane, (\gamma)-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, lindane, aldrin, heptachlor, heptachlor epoxide, methoxychlor, hexachlorobenzene, mirex</td>
</tr>
<tr>
<td><strong>Ontario FCMP</strong></td>
<td>PCBs (Aroclors®), PCBs (congeners), DDT, DDE, DDD, mercury, chlordane, (\alpha)-chlordane, (\gamma)-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, lindane, heavy metals, dioxins/furans, dioxin-like PCBs, PAHs, chlorinated phenols/benzenes</td>
</tr>
<tr>
<td>National Fish Tissue FCMP</td>
<td>PCBs (Aroclors®), PCBs (congeners), DDT, DDE, DDD, mercury, chlordane, (\alpha)-chlordane, (\gamma)-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, lindane, dioxins/furans, 18 PAHs, total inorganic arsenic, aldrin, dicrofol, endosulfan I and II, endosulfan sulfate, ethalfluralin, heptachlor, heptachlor epoxide, isodrin, kepone, methoxychlor, mirex, octachlorostyrene, pendimethalin, pentachloroanisole, pentachloronitrobenzene, permethrin I and II, trifluralin, chloropyrifos, diazinon, disulfoton, disulfoton sulfone, ethion, ethyl parathion, paraoxon, terbufos, terbufos sulfone, bis[2-ethylhexyl]phthalate, 4-bromophenyl phenyl ether, butyl benzyl phthalate, dibutyl phthalate, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 3,3′-dichlorobenzidine, diethylstibestrol (DES), hexachlorobenzene (HCB), hexachlorobutadiene, 4,4′-methylene bis[2-chloroaniline], naphthalene, nitrobenzene, nonylphenol, pentachlorobenzene, pentachlorophenol (Tier 1), phenol, 2,4,6-tris[1,1-dimethylethyl] phenol, tetrabromobisphenol A, 1,2,4,5-tetrachlorobenzene, 1,2,4-trichlorobenzene (TCB), 2,4,5-trichlorophenol</td>
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<tr>
<td>Program</td>
<td>Chemicals Analyzed</td>
</tr>
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<tr>
<td>Wisconsin DNR FCMP</td>
<td>PCBs (Aroclors®), PCBs (congeners), DDT, DDE, DDD, mercury, chlordane, $\alpha$-chlordane, $\gamma$-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, lindane, dioxin/furans 2,3,7,8-substituted congener analysis</td>
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<tr>
<td>New York YOY FCMP</td>
<td>PCBs (Aroclors®), PCBs (congeners), DDT, DDE, DDD, mercury, chlordane, dieldrin</td>
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</table>
Attachment B-6

Analytical Methods Used
### Analytical Methods Used

<table>
<thead>
<tr>
<th>Program/Analyte</th>
<th>Analytical Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDEQ</strong></td>
<td></td>
</tr>
<tr>
<td>PCBs (Aroclors®), DDT, DDE, DDD, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor oxychlordane, toxaphene, dieldrin/endrin, BHC</td>
<td>Packed column gas chromatography and a method developed by MDCH staff (Price et al. 1986)</td>
</tr>
<tr>
<td>PCBs (congeners)</td>
<td>Gas chromatograph with electron capture device and a modified version of the Mullin et al. (1984) method</td>
</tr>
<tr>
<td>Mercury</td>
<td>Atomic absorption spectrophotometer</td>
</tr>
<tr>
<td><strong>MA FCMP</strong></td>
<td></td>
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<tr>
<td>PCBs (Aroclors®)</td>
<td>GC/ECD and quantified using the second column (dual column confirmation). Analytes include 20 PCB congeners (numbers 8, 18, 28, 44, 52, 66, 77, 101, 105, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206, 209)</td>
</tr>
<tr>
<td>PCBs (congeners)</td>
<td>GC/ECD and quantified using the second column (dual column confirmation); analytes include 2-4'-DDD and 4-4'-DDD</td>
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<tr>
<td>DDT, DDD, DDE, cis-nonachlor, trans-nonachlor, toxaphene, BHC, heptachlor epoxide, aldrin, hexachlorobenzene, lindane mirex</td>
<td>GC/ECD and quantified using the second column (dual column confirmation)</td>
</tr>
<tr>
<td>PAHs</td>
<td>GC/MS; 48 individual compounds are quantified with an average quantification level around 0.5 ng/g dry weight</td>
</tr>
<tr>
<td><strong>PA FCMP</strong></td>
<td></td>
</tr>
<tr>
<td>PCBs (Aroclors®)</td>
<td>Nominal reporting levels for all Aroclors® are 0.050 mg/kg based on wet weight. The methodology employed for extraction is a superficial fluid extraction (SFE) utilizing CO₂, similar to EPA Method 3561, which incorporates a lipid removal process as part of the extraction. This method was detailed in a paper presented at the “Pittsburgh Conference of Analytical Chemistry and Applied Spectroscopy” in March 1995. The determinative steps are modifications of EPA Method 8082.</td>
</tr>
</tbody>
</table>
Nominal reporting levels of 0.004 mg/kg based on wet weight, with the assumption that there are not any PCB interferences. The methodology employed for extraction is an SFE utilizing CO₂, similar to EPA Method 3561, which incorporates a lipid removal process as part of the extraction. This method was detailed in a paper presented at the “Pittsburgh Conference of Analytical Chemistry and Applied Spectroscopy” in March 1995. The determinative steps are modifications of EPA Method 8081.

Mercury
EPA Method 245.1 quantitation limit = 0.02 mg/kg

Lead
EPA Method 200.8, quantitation limit=0.025 mg/kg

Copper
EPA Method 200.8 quantitation limit=0.10 mg/kg

Chromium
EPA Method 200.7, quantitation limit=0.10 mg/kg

Cadmium
EPA Method 200.8, quantitation limit=0.005 mg/kg

**Indiana FCMP**

**PCBs (Aroclors®)**
Method 8082 quantitation limit = NA

**PCBs (congeners)**
Method 8082 quantitation limit = 0.050 mg/kg

**DDT, DDE, DDD, cis-nonachlor, trans-nonachlor, oxychlordane, dieldrin/endrin, endosulfan II, endosulfan sulfate, endrin aldehyde, endrin ketone**
Method 8081A, quantitation limit = 0.005 mg/kg

**α-Chlordane, γ-chlordane, lindane, endosulfan I, heptachlor, heptachlor epoxide, hexachlorobenzene, pentachloroanisole**
Method 8081A, quantitation limit = 0.0025 mg/kg

**Methoxychlor**
Method 8081A, quantitation limit = 0.025 mg/kg

**Toxaphene**
Method 8081A, quantitation limit = 0.25 mg/kg

**Mercury**
Method SW846 6010B (Mercury: SW846 7471A) quantitation limit = 0.050 mg/kg

<table>
<thead>
<tr>
<th>Program/Analyte</th>
<th>Analytical Method</th>
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<td>DDT, DDE, DDD, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, dieldrin/endrin, BHC, and other organic compounds</td>
<td>Nominal reporting levels of 0.004 mg/kg based on wet weight, with the assumption that there are not any PCB interferences. The methodology employed for extraction is an SFE utilizing CO₂, similar to EPA Method 3561, which incorporates a lipid removal process as part of the extraction. This method was detailed in a paper presented at the “Pittsburgh Conference of Analytical Chemistry and Applied Spectroscopy” in March 1995. The determinative steps are modifications of EPA Method 8081.</td>
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### Program/Analyte Analytical Method

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<th>Program/Analyte</th>
<th>Analytical Method</th>
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<tr>
<td><strong>Metals</strong></td>
<td>Method SW846 6010B, with quantitation limit (mg/kg) Al: 5.0, Sb: 2.0, As: 1.0, Ba: 5.0, Be: 0.50, Cd: 0.01, Ca: 500, Cr: 0.1, Co: 5.0, Cu: 0.1, Fe: 5.0, Pb: 0.07, Mg: 500, Mn: 1.5, Ni: 1.0, K: 500, Se: 0.1, Ag: 0.5, Na: 500, Th: 1.0, V: 5.0, Zn: 2.0</td>
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#### Intertribal Fisheries Assessment Program

- **PCBs Aroclors®/congeners, DDT,**
- **DDE, α-chlordane, γ-chlordane,**
- **cis-nonachlor, trans-nonachlor**

- **DDD** CVAAS quantitation limit = 0.01 mg/kg
- **BHC** GC/MS sum of congeners

#### GLIFWC FCMP

- **PCBs (Aroclors®)** Soxhlet Extraction: Method 5520; Lipid determination: Method 5520; Gel permeation: Method 3640A; Silica Gel Cleanup: Method 3630C; GC-ECD Analysis: method 8000B and 8081A. Quantitation Limit = 0.05 mg/kg

- **DDT, DDE, DDD, cis-nonachlor,**
- **trans-nonachlor, oxychlordane,**
- **endrin ketone, endrin aldehyde,**
- **mirex** Same method as PCBs (Aroclors®) with quantitation limit = 0.005 mg/kg

- **α-chlordane, γ-chlordane,**
- **dieldrin/ endrin, BHC, aldrin,**
- **heptachlor, heptachlor epoxide,**
- **endosulfan I and II, endosulfan sulfate,**
- **hexachlorobenzene,**
- **pentachloroanisole** Same method as PCBs (Aroclors®) with quantitation limit = 0.0025 mg/kg

- **Methoxychlor** Same method as PCBs (Aroclors®) with quantitation limit = 0.025 mg/kg

- **Toxaphene** Same method as PCBs (Aroclors®) with quantitation limit = 0.25 mg/kg

- **Mercury** Lake Superior Research Institute’s standard operating procedure—cold vapor mercury analysis; quantitation limit = 0.04 mg/kg
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<thead>
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<th>Program/Analyte</th>
<th>Analytical Method</th>
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</thead>
<tbody>
<tr>
<td><strong>Minnesota FCMP</strong></td>
<td>AOAC Method 970.52 for extraction of PCBs from fish tissue. EPA SW-846, Method 8082, <em>PCBs by Gas Chromatography</em>. 60/40 Aroclor® 1254/1260 mixture as the reference standard.  Quantitation limit: around 0.010 mg/kg wet weight for most fish tissue.</td>
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<tr>
<td>Mercury</td>
<td>EPA Method 7473, <em>Mercury in Solids by Thermal Decomposition Amalgamation and Atomic Absorption Spectrophotometry</em>. Quantitation limit: ~0.010 mg/kg wet weight for most fish tissue.</td>
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<tr>
<td><strong>Canadian DFO</strong></td>
<td>For most analyses, PCBs are quantified using Aroclors® 1254 as a standard. Organochlorines are quantified using methods from Langlois et al. (1964). Quantitation limits are 0.050 for PCBs and 0.005 mg/kg for organochlorines. For analyses requiring lower detection limits, OC and PCB analyses are performed using a Varian 3600 gas chromatograph with dual electron capture detectors (ECD). Analyses of p,p-DDE requires dual channel confirmation. Ocs are quantified against an eight-point calibration curve and final concentrations are corrected for recovery. Total PCBs are quantitated using a standard containing a 1:1:1 mixture of Aroclors® 1248, 1254, and 1260. Quantitation limits: 0.002 mg/kg for PCBs and organochlorines, 0.010 mg/kg for toxaphene. Detailed information on these methods can be found in a number of internal reports available from DFO.</td>
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<td>Method is not reported, 0.050 mg/kg minimum detection limit Method is not reported, 0.01 mg/kg minimum detection limit Method is not reported, 0.02 mg/kg minimum detection limit Method is not reported, 0.02 mg/kg minimum detection limit</td>
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<tr>
<td>PCBs (Aroclors®)</td>
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<td>α-Chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane</td>
<td>Method is not reported, 0.02 mg/kg minimum detection limit Method is not reported, 0.02 mg/kg minimum detection limit Method is not reported, 0.02 mg/kg minimum detection limit Method is not reported, 0.02 mg/kg minimum detection limit</td>
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<td>Program/Analyte</td>
<td>Analytical Method</td>
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<tr>
<td>----------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
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<tr>
<td>Toxaphene</td>
<td>Method is not reported, 0.05 mg/kg minimum detection limit</td>
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<tr>
<td>Arsenic, lead, selenium</td>
<td>Method is not reported, 0.04 mg/kg minimum detection limit</td>
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<tr>
<td>Cadmium</td>
<td>Method is not reported, 0.004 mg/kg minimum detection limit</td>
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<td><strong>Illinois FCMP</strong></td>
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<tr>
<td>PCBs (Aroclors®), DDT, DDE, DDD</td>
<td>Method developed by IEPA Bureau of Laboratories</td>
</tr>
<tr>
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<td>quantitation limit = 0.1 mg/kg</td>
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</table>
| \( \alpha \)-Chlordane, \( \gamma \)-chlordane, cis-
|                                        | nonachlor, trans-nonachlor, oxychlordane                                            |
|                                        | Method developed by IEPA Bureau of Laboratories                                    |
|                                        | quantitation limit = 0.02 mg/kg                                                      |
| Toxaphene                              | Method developed by IEPA Bureau of Laboratories                                    |
|                                        | quantitation limit = 1 mg/kg                                                        |
| Dieldrin/endrin, BHC, and other organic compounds | Method developed by IEPA Bureau of Laboratories                                    |
|                                        | quantitation limit = 0.01 mg/kg                                                      |
| Methoxychlor                           | Method developed by IEPA Bureau of Laboratories                                    |
|                                        | quantitation limit = 1 mg/kg                                                        |
| Mercury                                | Method not reported, quantitation limit = 0.01 mg/kg                                 |
| **Ontario FCMP**                       |                                                                                   |
| PCBs (Aroclors®)                       | GC/ECD analysis (quantitation limit = 0.02 mg/kg)                                   |
| PCBs (congeners)                       | GC/ECD analysis (quantitation limit = 0.001 mg/kg)                                  |
| DDT, DDD                               | GC/ECD analysis (quantitation limit = 0.005 mg/kg)                                  |
| DDE                                    | GC/ECD analysis (quantitation limit = 0.001 mg/kg)                                  |
| Mercury                                | Method not reported, quantitation limit = 0.01 mg/kg                                 |
| \( \alpha \)-Chlordane, \( \gamma \)-chlordane, cis-
|                                        | nonachlor, trans-nonachlor, oxychlordane                                            |
|                                        | GC/ECD analysis (quantitation limit = 0.002 mg/kg)                                  |
| Toxaphene, dieldrin/endrin             | GC/ECD analysis (quantitation limit = 0.05 mg/kg)                                  |
| Lindane (BHC)                          | GC/ECD analysis (quantitation limit = 0.001 mg/kg)                                  |
### National Fish Tissue

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<td>Method 1656A</td>
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<tr>
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<td>PAH, benzene compounds</td>
<td>Method 1625C</td>
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<tr>
<td>Dioxin/furans</td>
<td>Method 1613B</td>
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<td>Arsenic</td>
<td>Method 1632A</td>
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### Wisconsin DNR

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<td>PCBs (Aroclors®), PCBs (congeners), DDT, DDE, DDD, α-chlordane, γ-chlordane, <em>cis</em>-nonachlor, <em>trans</em>-nonachlor, oxychlordane, toxaphene, dieldrin/endrin, BHC, and other organic chemicals</td>
<td>Analytical method and quantitation levels change over time</td>
</tr>
<tr>
<td>Mercury</td>
<td>Low level mercury atomic fluorescence ( \geq 0.005 \text{ mg/kg} ) LOD, mercury cold vapor atomic absorption (Sullivan and Delfino 1982) ( \geq 0.004 \mu g/g ) LOD</td>
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### NY YOY FCMP

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<td>PCBs (Aroclors®)</td>
<td>Modified EPA 8080 (quantitation limit = 0.05 mg/kg)</td>
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<tr>
<td>PCBs (congeners)</td>
<td>ITS Environmental SOP</td>
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<td>PAHs</td>
<td>Modified EPA 8310 (quantitation limit = 0.05 mg/kg)</td>
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<td>Chlorinated Dioxins/Furans</td>
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<td>Mercury</td>
<td>Modified EPA 7470 (quantitation limit = 0.05 mg/kg)</td>
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<td>Cadmium</td>
<td>Modified EPA 7131 (quantitation limit = 0.05 mg/kg)</td>
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<tr>
<td>Lipid</td>
<td>En Chem SOP (quantitation limit = 0.01 percent)</td>
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Appendix C

Detailed Description of How to Calculate Power and Minimum Detectable Trend
Detailed Description of How to Calculate Power and Minimum Detectable Trend

In response to the “Power to Detect Trends” comment this appendix describes in detail the process for calculating the power of a simple linear regression model to detect a trend over time. This appendix also describes in detail the process for calculating the minimum detectable trend level. This method is used to calculate how large the trend would have to be in order to be detected.

Power of a Regression Model

The power of a simple linear regression model is the ability of the model to detect a significant slope given the variability and amount of data. A simple linear regression model provides an estimate of the relationship or correlation between the independent and dependent variables. The square root of the $R^2$ value of the fitted regression model is exactly the magnitude of the Pearson correlation coefficient for the samples.

The power for a Pearson correlation can be calculated using the method detailed in Zar (1974). This method uses Fisher’s $z$-transformation of the sample correlation coefficient and the appropriate critical value of the correlation coefficient. Table C-1 (reproduced from Zar 1974) provides the critical values for correlation coefficients. The critical value is found from the table using the degrees of freedom ($\nu$; the sample size minus 2) and the desired significance level (usually 0.05). This calculation assumes a two-tailed test. Fisher’s $z$-transformation is calculated using the sample correlation coefficient and critical value in the following formula, where $r$ is the correlation or critical value.

$$Fisher's\ z\ transform = 0.5 \ln \left( \frac{1 + r}{1 - r} \right)$$

Using these two calculated $z$ transformations, $z_s$ for the sample correlation and $z_c$ for the critical value, the standard normal value, $Z(\beta)$, associated with the level of power can be calculated using the following formula.

$$Z(\beta) = (z_s - z_c)\sqrt{n-3}$$

The probability of a standard normal variate being less than this value, $P(Z \leq Z(\beta))$, is the power to detect the correlation, or the power of the simple linear regression model to detect a trend. Attachment C1 shows the power calculated for the South Manistique whole walleye mercury regression model fit in Appendix E (model 2a in the attachment).
Minimum Detectable Trend

When a regression model fails to detect a significant trend, a logical question to ask is how large must the trend be to be able to detect it, or what is the minimum trend that would be detectable. The significance of the slope or trend in a regression model is calculated using a t-test for whether the slope is significantly different from zero. This test can be rearranged to calculate the minimum value such that the p-value or significance level is equal to the desired significance level, usually 0.05. Standard regression output provides the coefficient value and its associated standard error.

First identify the critical t-value based on the significance or alpha level and the degrees of freedom, for a two-tailed test. A table of critical t-values (Table C-2, reproduced from Zar 1974) is attached. For the Lake Gogebic whole walleye PCB model (model 8a in the attachment to Appendix E), the critical t-value for alpha = 0.05 and 25 degrees of freedom is 2.0595. Multiply this value by the standard error of the regression coefficient (slope), which for this model is 0.0272. The resulting value is the minimum detectable trend; for model 8a, this value is 0.056 in ln-space, or 5.4 percent decrease (or increase) per year. There was no significant trend over time detectable in these data, thus the smallest model (only significant variables plus year) must be used to obtain the estimate of the standard error of the year coefficient. The output for this model is included here as Attachment C2 for convenience along with these calculations.

Reference

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Figure D-1. Power curves for carp from Detroit River

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80% power: -7.6 -11 -10
Figure D-3. Power curves for walleye from Lake Gogebic
Figure D-4. Power curves for largemouth bass from Gull Lake
Figure D-5. Power curves for largemouth bass from Gun Lake

LEGEND

- Mercury
- PCB
- PCB-lipid

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80% power -3.3 -9.1 -11
Figure D-6. Power curves for walleye from Lake Huron

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80% power -7.9 -7.4 -7.4
### Table D-7. Average Trend and Power for Mercury, PCB, and PCB-lipid

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**Legend:**
- Mercury
- PCB
- PCB-lipid

*Figure D-7. Power curves for carp from Kalamazoo River*
Figure D-8. Power curves for lake trout from Lake Michigan
Figure D-9. Power curves for carp from Muskegon River
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Figure E-27.  PCB concentrations vs. year for walleye from Upper Peninsula Inland Lakes

Figure E-28.  PCB concentrations vs. age for walleye from Upper Peninsula Inland Lakes

Figure E-29.  PCB concentrations vs. lipid content for walleye from Upper Peninsula Inland Lakes

Figure E-30.  PCB concentrations vs. length for walleye from Upper Peninsula Inland Lakes

Figure E-31.  PCB concentrations vs. weight for walleye from Upper Peninsula Inland Lakes

Figure E-32.  PCB concentrations vs. pH for walleye from Upper Peninsula Inland Lakes

Figure E-33.  PCB concentrations vs. conductivity for walleye from Upper Peninsula Inland Lakes

Figure E-34.  Predicted PCB concentrations over time for whole walleye samples from Lake Gogebic
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Table E-2. Summary of available data for Upper Peninsula Inland Lakes walleye
Detailed Trend Analysis of Mercury and PCB Concentrations in Walleye from South Manistique Lake and Upper Peninsula Inland Lakes

Introduction

MDEQ requested that Exponent demonstrate how to estimate trends in chemical concentrations in fish tissue over time, including the possible impact of age, lipid content, length, sample type, pH, and conductivity. A dataset was furnished by MDEQ, which contained walleye data from two sites, South Manistique Lake and Upper Peninsula inland lakes, represented by many lakes. Specifically, MDEQ requested to see the process of evaluating the trends over time in mercury and PCB concentrations in walleye samples from South Manistique Lake and Upper Peninsula inland lakes with consideration of age, lipid content, sample type, and length, and consideration of pH and conductivity for the Upper Peninsula inland lakes. This appendix provides a detailed analysis, showing all intermediate steps, to illustrate how this kind of analysis would be done.

Methods

Multiple linear regression was used to relate tissue concentrations of chemicals to the potential explanatory variables. This technique provides a measure of the statistical significance of any apparent trend (the $p$ value), a measure of the amount of overall variability attributable to the explanatory variables (the $R^2$ value), and, if there is a significant trend, an estimate of the rate of change of chemical concentrations over time. Explanatory variables used in the regression analyses were year, length, weight, age, lipid content, pH, and conductivity. The data set included data for both whole fish and for fillets, and combined regression analyses were performed where possible. Prior to conducting regression analyses, concentrations were plotted against each possible explanatory variable for each tissue type. The plots show possible relationships between each variable and concentration. Variables that show strong correlations with concentration are usually the variables significant in the multiple regression model used to assess trends.

Several multiple linear regression models were fit to predict concentration from year, sample type, lipid content, age, and length or weight for varying subsets of samples. Multiple linear regression requires that all variables be measured for all cases (e.g., fish) included in a regression model, whereas the example data set included several variables that were not measured in all fish. Different subsets of the data were therefore used to evaluate the effects of different potential explanatory variables. The models were fit using either weight or length because inclusion of both could create spurious results because of the high correlation between these variables. The length or weight model with the highest $R^2$ value was used as the starting point for assessing all of the variables. The $R^2$ value is the percent of the total variability explained by the model. In most instances only a subset of samples had age data, therefore age was assessed using separate models based on only these samples. Ln-transformed
concentrations were used in order to fit an exponential model using linear regression. Residuals from each regression model were plotted against the predicted concentrations to assess whether the ln-transformation achieved uniform variability over the range of predicted values. A final regression model for each subset of data was arrived at by starting with a model that included all potential explanatory variables, and then successively eliminating those variables that did not have a statistically significant relationship ($p$ value < 0.05) to chemical concentrations in tissue. The final regression model was used to estimate the trend in concentration over time, dependent on any other variables when necessary.

South Manistique Lake Walleye

Available Data

Chemistry data are available for walleye from South Manistique Lake for 6 years, specifically 1988, 1991, 1993, 1995, 1998, and 2001. Whole fish were measured in all years except 1988, when fillets were measured instead. A total of 59 samples were collected over the entire period, including 49 whole fish samples and 10 fillet samples. Length and weight were measured for all fish samples. Age data are available for 30 samples—all years except 1988 and 2001, representing 30 out of 59 samples or 51 percent. Lipid content and mercury concentration were measured in all fish. Total polychlorinated biphenyl (PCB) Aroclor® concentrations were measured in all years except 1988 and 2001 (39 samples). Total PCB congener concentrations were measured in 1988 and 2001 only (20 samples). These total PCB concentrations were not combined to span the entire time interval, but rather only total PCB Aroclor® concentrations were analyzed. Table E-1 provides a summary of the data available from South Manistique Lake.

Trend Analysis—Mercury

Mercury concentrations were plotted against year, age, lipid content, length, and weight (Figures E-1 through E-5). None of these relationships is very strong, but the plots show a possible decline in concentration with year, and an increase with age, length, and weight. Lipid content does not appear to be related to mercury concentration. Length and weight are strongly correlated with each other (Figure E-6), and the correlations of mercury with these variables is a reflection of increasing concentrations with age.

Several multiple linear regression models were used to predict mercury concentration based on the following different subsets of samples:

- All whole fish samples—the most complete dataset
- Whole and fillet samples combined—to assess whether the single year of fillet samples is consistent with the whole samples
- Whole samples with age data—to assess the impact of age on concentrations.
The residual plots from the regression models using ln-transformed concentrations showed uniform variability over the range of data. Length was a better predictor than weight based on $R^2$ values for each of initial models for these datasets. Iteratively, each non-significant variable was removed from the regression model until only significant ($p$-value < 0.05) variables remained.

The final reduced model, using all of the whole walleye samples (49 samples total), includes only year. The $R^2$ value is only 26 percent. The annual trend in mercury concentration is a decline of 4.9 percent per year (Figure E-7).

The final reduced model using the combined whole fish and fillet sample data includes year and length. There is only one year of fillet data, so separate trends could not be estimated. The only assessment of whether the fillet concentrations are consistent with whole fish concentrations is to compare the trend in concentration using both sample types to the trend using only whole fish samples. The estimated annual trend in mercury concentrations is a decline of 2.6 percent per year based on 59 fillet and whole samples (Figure E-8). The $R^2$ value of this model is 27 percent. Inclusion of length in this model and not in the model using only whole samples shows that fillet mercury concentrations are related to fish length. The different magnitudes of declining trends from the two models, 4.9 and 2.6, are not statistically significantly different from each other at alpha of 0.05, but the difference suggests that the fillet concentrations are not entirely consistent with the whole fish trends. Based on the combined data, mercury concentrations increase 7.0 percent per inch length of fish.

The final reduced model using only samples with age recorded includes only age as a predictor of mercury concentration. Age was assessed separately because it was measured for only 30 samples. Including age in a model excludes all samples without an age measurement. This estimate is based on whole fish mercury concentrations from 1991, 1993, and 1998. The $R^2$ value is 24 percent. Mercury concentration increases 11 percent per year of age of the fish (Figure E-9).

**Trend Analysis—PCB Concentrations**

Total PCB concentrations were plotted against year, age, lipid content, length, and weight (Figures E-10 through E-14). The plots show increasing concentration with lipid content but also increasing variability. Concentrations decline with age, length, and weight. The increasing variability with lipid content suggests the concentrations will need to be log-transformed in the regression model to meet the homogeneity of variance assumption.

A multiple linear regression model was fit to predict PCB concentration from year, lipid content, and weight. Weight was a better predictor than length based on $R^2$ values of the initial models. Age was assessed separately because it was not measured in all of the samples. Sample type was not assessed because total PCB concentrations were only measured in whole fish samples. The residuals from this regression model were non-uniform, and thus the analysis was conducted using ln-transformed PCB concentrations. The residuals from the ln-transformed concentration regression model were more uniform. Iteratively, each non-significant variable was removed from each regression model until only significant ($p$-value < 0.05) variables remained.
The final model based on all of the whole fish samples (39 samples total) includes lipid content and weight (Figure E-15). The $R^2$ value is 48 percent. There is no significant trend in PCB concentration over time because year is not a significant variable in the final model. The PCB concentration increases 18 percent with each percent increase in lipid content and increases 16 percent with each pound decrease in fish weight.

The multiple linear regression model fit to the subset of samples with age recorded did not support different conclusions. Age was not a significant factor in explaining PCB concentrations.

**Upper Peninsula Inland Lakes Walleye**

**Available Data**

Chemistry data are available for walleye from 24 inland lakes from the Upper Peninsula spanning years 1985 through 2001, excluding 1996. Fillet samples were measured in all 24 lakes and all years. Whole fish were measured only in Lake Gogebic in years 1992, 1994, 1997, and 2000. A total of 337 samples were analyzed for mercury, including 40 whole fish and 297 fillet samples. Fifty-four samples were analyzed for total PCB Aroclor® concentrations, including 30 whole fish and 24 fillet samples. The fillet samples with PCB concentrations measured were sampled in years 1989 and 1991 and the whole fish were sampled in 1992, 1994, and 1997. Length was measured for all samples and weight was measured for 277 (82 percent) of the samples. Age was recorded for all the whole fish samples except in 2000 (29 samples) as well as fillet samples in 1999 (43 samples). Lipid content was measured in all fish samples analyzed for total PCB Aroclor® concentration plus fillet samples from 1986 and 2000 and whole fish collected in 2000. PH and conductivity were measured only once at each fillet sampling event. The total PCB congener concentrations were measured only in 2000 and were not combined with the PCB Aroclor® concentrations; only PCB Aroclor® concentrations were analyzed. Chlordane and DDT were measured on the same samples as total PCBs except for 1986, when they were not measured. Table E-2 provides a summary of the data available from the Upper Peninsula inland lakes.

**Trend Analysis—Mercury**

Mercury concentrations were plotted against year, age, lipid content, length, weight, pH, and conductivity (Figures E-16 through E-23). There does not appear to be an obvious trend over time based on the plot of concentration versus year. Lipid content, pH, and conductivity do not appear related to mercury concentration. Mercury concentration increases with age, length, and weight. Age appears positively correlated with concentration beyond an age of 6 years. Length seems more closely related to mercury concentration than weight.

Multiple linear regression models were fit to the fillet and whole-fish datasets separately because all of the whole-fish data are from a single lake, whereas the fillet data consist of one to three years of data from each of many lakes. The model fit to the fillet data estimated trends for general Upper Peninsula inland lakes because the specific lakes included in the
analysis are assumed to represent a random sample of such lakes. The whole fish analysis will estimate trends specific to Lake Gogebic because that is the only lake in which whole fish were measured. Age was assessed from a separate regression analysis based on only the whole fish samples with age recorded. All models had larger R² values with length than weight, similar to the South Manistique Lake mercury regression models. The plots of residuals versus predicted concentrations from the ln-transformed models showed uniform variability over the range of concentrations. Iteratively, each non-significant variable was removed from each regression model until only significant (p-value < 0.05) variables remained.

The multiple linear regression model fit to the fillet data initially included year, pH, conductivity and length. pH and conductivity were not significant contributors to the model and were removed. The remaining model fitting was conducted using all of the fillet samples rather than only those from locations where pH and conductivity were measured. Lipid content and age were not measured for most of the fillet samples (these data were measured for only four years and two years, respectively), so were not evaluated. The final model included only length, and had an R² value of 35 percent (Figure E-24). This indicates there is no significant trend over time, because year was not a significant factor (p-value = 0.14). Mercury concentrations increase 14 percent per inch increase in length of fish.

The initial model based on the whole fish samples included year, lipid content, and length. Age was not included because it was not measured for the year-2000 samples. The final whole fish trend model includes year and lipid content with an R² value of 69 percent (Figure E-25). Mercury concentration is predicted to decline 7.5 percent per year and 13 percent per percent decline in lipid content.

A third analysis was conducted to assess the impact of age on mercury concentrations. This analysis used only the whole fish samples collected in 1992, 1994, and 1997 from Lake Gogebic. Including age forced exclusion of the 2000 whole fish data because age was not measured in 2000. The final regression model predicts ln PCB concentration from year, age, and lipid content (Figure E-26). The R² value is 76 percent. Mercury concentration declines 7.0 percent per year and 8.8 percent per percent increase in lipid content. Mercury concentration increases 7.5 percent with each additional year of age of fish.

**Trend Analysis—PCB Concentrations**

Total PCB concentrations were plotted against year, age, lipid content, length, weight, pH, and conductivity (Figures E-27 through E-33). The PCB concentrations for fillet samples are all undetected at the given detection limit, except for one detected result in 1991. Because of the lack of variability in the concentrations, the fillet data is unusable for determining trends. No relationship is apparent between PCB concentration and year, lipid, length, or weight, based on the plots of whole fish samples. The plot of concentration in whole fish versus age shows some positive correlation.

A multiple linear regression model was fit to predict PCB concentration in whole fish samples (29 samples total). The plot of residuals versus predicted concentration from the ln-transformed concentration model meets the uniform variability assumption except in the lower left quadrant, because concentrations are censored at a single detection limit value. The final model included
only age and length, and has an $R^2$ value of 33 percent (Figure E-34). This indicates there is no significant trend over time because year was not a significant factor ($p$-value = 0.52). PCB concentration declines 6.5 percent per inch of length increase and increases 7.8 percent per year of age.
Figures
Figure E-1. Mercury concentrations vs. year in walleye from South Manistique Lake

LEGEND
○ Whole fish
△ Fillet fish
Figure E-2. Mercury concentrations vs. age in walleye from South Manistique Lake
Figure E-3. Mercury concentrations vs. lipid content in walleye from South Manistique Lake.
Figure E-4. Mercury concentrations vs. length in walleye from South Manistique Lake
Figure E-5. Mercury concentrations vs. weight in walleye from South Manistique Lake
Figure E-6. Weight vs. length in walleye from South Manistique Lake

LEGEND
○ Whole fish
△ Fillet fish
Figure E-7. Predicted mercury concentrations over time for whole walleye samples from South Manistique Lake
Figure E-8. Predicted mercury concentrations over time for whole and fillet walleye samples from South Manistique Lake
Figure E-9. Predicted mercury concentrations over time for whole walleye samples with age measurements from South Manistique Lake
Figure E-10. PCB concentrations vs. year for walleye from South Manistique Lake
Figure E-11. PCB concentrations vs. age for walleye from South Manistique Lake
Figure E-12. PCB concentrations vs. lipid content for walleye from South Manistique Lake.
Figure E-13. PCB concentrations vs. length for walleye from South Manistique Lake

LEGEND
○ Whole fish
△ Fillet fish
Figure E-14. PCB concentrations vs. weight for walleye from South Manistique Lake
Figure E-15. Predicted PCB concentrations over time for whole walleye samples from South Manistique Lake

LEGEND
○ Whole fish
△ Fillet fish
Figure E-16. Mercury concentration vs. year for walleye from Upper Peninsula Inland Lakes
Figure E-17. Mercury concentrations vs. age for walleye from Upper Peninsula Inland Lakes

**LEGEND**
- ○ Whole fish
- △ Fillet fish
Figure E-18. Mercury concentrations vs. lipid content for walleye from Upper Peninsula Inland Lakes
Figure E-19. Mercury concentrations vs. length for walleye from Upper Peninsula Inland Lakes.
Figure E-20. Mercury concentrations vs. weight for walleye from Upper Peninsula Inland Lakes
Figure E-21. Weight vs. length for walleye from Upper Peninsula Inland Lakes
Figure E-22. Mercury concentrations vs. pH for walleye from Upper Peninsula Inland Lakes

LEGEND
- Whole fish
- Fillet fish

MERCURY (mg/kg wet)

pH
Figure E-23. Mercury concentrations vs. conductivity for walleye from Upper Peninsula Inland Lakes
Figure E-24. Predicted mercury concentrations over time for fillet walleye samples from Upper Peninsula Inland Lakes
Figure E-25. Predicted mercury concentrations over time for whole walleye samples from Lake Gogebic

LEGEND
○ Whole fish
△ Fillet fish
Figure E-26. Predicted mercury concentrations over time for whole walleye samples with age measurements from Lake Gogebic.
Figure E-27. PCB concentrations vs. year for walleye from Upper Peninsula Inland Lakes

LEGEND
○ Whole fish
△ Fillet fish

PCB (Aroclor®) (mg/kg wet) vs. Year

YEAR

Figure E-28. PCB concentrations vs. age for walleye from Upper Peninsula Inland Lakes
Figure E-29. PCB concentrations vs. lipid content for walleye from Upper Peninsula Inland Lakes.
Figure E-30. PCB concentrations vs. length for walleye from Upper Peninsula Inland Lakes.
Figure E-31. PCB concentrations vs. weight for walleye from Upper Peninsula Inland Lakes
Figure E-32. PCB concentrations vs. pH for walleye from Upper Peninsula Inland Lakes
Figure E-33. PCB concentrations vs. conductivity for walleye from Upper Peninsula Inland Lakes
Figure E-34. Predicted PCB concentrations over time for whole walleye samples from Lake Gogebic
Tables
Table E-1. Summary of available data for South Manistique Lake walleye

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**Note:** -- = no samples were measured
Attachment E1

South Manistique Lake
Mercury Regression Model
Output
Model 2a. Mercury - All Whole Fish Samples

*** Call: lm(formula = log(HG) ~ Year + Lipid + Weight, subset = SampType == "W")
Compare all variable model with Weight and with Length. Weight model has lower R-square value than Length model so begin analysis with Length Model.

Residuals:

   Min 1Q Median 3Q Max
-0.6316 -0.2088 0.008532 0.2393 0.62

Coefficients:

                         Value Std. Error   t value Pr(>|t|)
(Intercept)     88.5483  26.9725     3.2829   0.0020
Year             -0.0450   0.0135    -3.3249   0.0018
Lipid            -0.0317   0.0317    -1.0001   0.3226
Weight            0.0076   0.0689     0.1109   0.9122

Residual standard error: 0.3154 on 45 degrees of freedom
Multiple R-Squared: 0.2777
F-statistic: 5.766 on 3 and 45 degrees of freedom, the p-value is 0.002002

Correlation of Coefficients:

          (Intercept)    Year   Lipid
Year -1.0000
Lipid  0.3515     -0.3573
Weight -0.0274      0.0203  0.2537

*** Call: lm(formula = log(HG) ~ Year + Lipid + Length, subset = SampType == "W")
All variable model including Length. This model has higher R-square value than Weight model above. Length is not significant (p-value>0.05) so remove from the model and refit.

Residuals:

   Min 1Q Median 3Q Max
-0.6322 -0.2088 0.01075 0.2377 0.6188

Coefficients:

                         Value Std. Error   t value Pr(>|t|)
(Intercept)     88.4098  27.2409     3.2455   0.0022
Year             -0.0449   0.0136    -3.2964   0.0019
Lipid            -0.0319   0.0331    -0.9629   0.3407
Length            0.0016   0.0282     0.0570   0.9548

Residual standard error: 0.3154 on 45 degrees of freedom
Multiple R-Squared: 0.2775
F-statistic: 5.761 on 3 and 45 degrees of freedom, the p-value is 0.002011

Correlation of Coefficients:

          (Intercept)    Year   Lipid
Year -1.0000
Lipid  0.2864     -0.2992
Length -0.1420      0.1206  0.3770

*** Call: lm(formula = log(HG) ~ Year + Lipid, subset = SampType == "W")
Refit model excluding Length. Lipid is not significant (p-value>0.05) so remove it and refit model.

Residuals:

   Min 1Q Median 3Q Max
-0.6298 -0.2079 0.01271 0.2371 0.6173
Coefficients:

|            | Value  | Std. Error | t value | Pr(>|t|) |
|------------|--------|------------|---------|----------|
| (Intercept)| 88.6304| 26.6713    | 3.3231  | 0.0018   |
| Year       | -0.0450| 0.0134     | -3.3642 | 0.0016   |
| Lipid      | -0.0326| 0.0304     | -1.0746 | 0.2882   |

Residual standard error: 0.312 on 46 degrees of freedom
Multiple R-Squared: 0.2775
F-statistic: 8.832 on 2 and 46 degrees of freedom, the p-value is 0.0005674

Correlation of Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>(Intercept)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-1.0000</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>0.3707</td>
<td>-0.3748</td>
</tr>
</tbody>
</table>

*** Call: lm(formula = log(HG) ~ Year, subset = SampType == "W")
All model components are significant (p-value<0.05) so this is the final model.

Residuals:

<table>
<thead>
<tr>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.6076</td>
<td>-0.1687</td>
<td>0.003194</td>
<td>0.2662</td>
<td>0.6963</td>
</tr>
</tbody>
</table>

Coefficients:

|            | Value  | Std. Error | t value | Pr(>|t|) |
|------------|--------|------------|---------|----------|
| (Intercept)| 99.2553| 24.8116    | 4.0004  | 0.0002   |
| Year       | -0.0504| 0.0124     | -4.0565 | 0.0002   |

Residual standard error: 0.3125 on 47 degrees of freedom
Multiple R-Squared: 0.2593
F-statistic: 16.46 on 1 and 47 degrees of freedom, the p-value is 0.0001864

Correlation of Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>(Intercept)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-1</td>
</tr>
</tbody>
</table>

Model 2b. Mercury - All Fillet and Whole Fish Samples

*** Call: lm(formula = log(HG) ~ Year + SampType + Lipid + Weight)
Compare all variable model with Weight and with Length. Weight model has lower R-square value than Length model so begin analysis with Length Model.

Residuals:

<table>
<thead>
<tr>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.7077</td>
<td>-0.1976</td>
<td>-0.01497</td>
<td>0.2506</td>
<td>0.6729</td>
</tr>
</tbody>
</table>

Coefficients:

|            | Value  | Std. Error | t value | Pr(>|t|) |
|------------|--------|------------|---------|----------|
| (Intercept)| 87.1022| 28.0210    | 3.1085  | 0.0030   |
| Year       | -0.0446| 0.0141     | -3.1659 | 0.0025   |
| SampType   | 0.1895 | 0.0821     | 2.3089  | 0.0248   |
| Lipid      | -0.0143| 0.0323     | -0.4431 | 0.6595   |
| Weight     | 0.1497 | 0.0538     | 2.7833  | 0.0074   |

Residual standard error: 0.3282 on 54 degrees of freedom
Multiple R-Squared: 0.304
F-statistic: 5.895 on 4 and 54 degrees of freedom, the p-value is 0.000519

Correlation of Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>(Intercept)</th>
<th>Year</th>
<th>SampType</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SampType</td>
<td>0.4247</td>
<td>-0.4236</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>0.3603</td>
<td>-0.3637</td>
<td>-0.3883</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>-0.0268</td>
<td>0.0218</td>
<td>-0.1062</td>
<td>0.1768</td>
</tr>
</tbody>
</table>
*** Call: `lm(formula = log(HG) ~ Year + SampType + Lipid + Length)`
All variable model including Length. This model has higher R-square value than
Weight model above. Lipid is not significant (p-value>0.05) so remove from the
model and refit.
Residuals:
   Min  1Q Median  3Q Max
-0.719 -0.173 -0.007521 0.2294 0.6757

Coefficients:

|            | Value | Std. Error | t value | Pr(>|t|) |
|------------|-------|------------|---------|----------|
| (Intercept)| 80.5125 | 28.0660 | 2.8687 | 0.0059 |
| Year       | -0.0417 | 0.0141 | -2.9589 | 0.0046 |
| SampType   | 0.1462 | 0.0847 | 1.7277 | 0.0898 |
| Lipid      | -0.0050 | 0.0329 | -0.1531 | 0.8789 |
| Length     | 0.0602 | 0.0210 | 2.8685 | 0.0059 |

Residual standard error: 0.3269 on 54 degrees of freedom
Multiple R-Squared: 0.3093
F-statistic: 6.047 on 4 and 54 degrees of freedom, the p-value is 0.0004275

Correlation of Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>(Intercept)</th>
<th>Year</th>
<th>SampType</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-1.0000</td>
<td>-0.4312</td>
<td></td>
</tr>
<tr>
<td>SampType</td>
<td>0.4353</td>
<td>-0.4312</td>
<td>-0.3336</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.3267</td>
<td>-0.3336</td>
<td>-0.4238</td>
</tr>
<tr>
<td>Length</td>
<td>-0.1078</td>
<td>0.0931</td>
<td>-0.2782</td>
</tr>
</tbody>
</table>

*** Call: `lm(formula = log(HG) ~ Year + SampType + Length)`
Refit model excluding Lipid. Sample Type is not significant (p-value<0.05) so remove
it from the model and refit.
Residuals:
   Min  1Q Median  3Q Max
-0.7171 -0.1706 -0.007614 0.2244 0.6878

Coefficients:

|            | Value | Std. Error | t value | Pr(>|t|) |
|------------|-------|------------|---------|----------|
| (Intercept)| 81.9164 | 26.2897 | 3.1159 | 0.0029 |
| Year       | -0.0424 | 0.0132 | -3.2215 | 0.0021 |
| SampType   | 0.1408 | 0.0760 | 1.8523 | 0.0694 |
| Length     | 0.0611 | 0.0200 | 3.0463 | 0.0036 |

Residual standard error: 0.324 on 55 degrees of freedom
Multiple R-Squared: 0.309
F-statistic: 8.2 on 3 and 55 degrees of freedom, the p-value is 0.000133

Correlation of Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>(Intercept)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-1.0000</td>
<td>-0.6705</td>
</tr>
<tr>
<td>SampType</td>
<td>0.6702</td>
<td>-0.6705</td>
</tr>
<tr>
<td>Length</td>
<td>-0.2142</td>
<td>0.2005</td>
</tr>
</tbody>
</table>

*** Call: `lm(formula = log(HG) ~ Year + Length)`
Refit model excluding Sample Type. Year and Length are both significant so this is
the final model.
Residuals:
   Min  1Q Median  3Q Max
-0.7404 -0.2093 0.0299 0.2583 0.6298
Coefficients:  
|                | Value  | Std. Error | t value | Pr(>|t|) |
|----------------|--------|------------|---------|----------|
| (Intercept)    | 49.2805| 19.9308    | 2.4726  | 0.0165   |
| Year           | -0.0261| 0.0100     | -2.6122 | 0.0115   |
| Length         | 0.0681 | 0.0201     | 3.3860  | 0.0013   |

Residual standard error: 0.331 on 56 degrees of freedom  
Multiple R-Squared: 0.2659  
F-statistic: 10.14 on 2 and 56 degrees of freedom, the p-value is 0.0001739

Correlation of Coefficients:  
<table>
<thead>
<tr>
<th></th>
<th>Year</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>-0.9998</td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>0.1201</td>
<td>0.1013</td>
</tr>
</tbody>
</table>

Model 2c. Mercury - All Whole Fish Samples with Age  
*** Call: lm(formula = log(HG) ~ Year + Age + Lipid + Weight, subset = SampType == "W")  
Compare all variable model with Weight and with Length. Weight model has lower R-square value than Length model so begin analysis with Length Model.

Residuals:  
<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.6064</td>
<td>-0.1252</td>
<td>0.02914</td>
<td>0.1731</td>
<td>0.4939</td>
</tr>
</tbody>
</table>

Coefficients:  
|                | Value  | Std. Error | t value | Pr(>|t|) |
|----------------|--------|------------|---------|----------|
| (Intercept)    | -10.0550 | 54.6710  | -0.1839 | 0.8556   |
| Year           | 0.0043  | 0.0274     | 0.1580  | 0.8757   |
| Age            | 0.0928  | 0.0540     | 1.7172  | 0.0983   |
| Lipid          | -0.0825 | 0.0565     | -1.4605 | 0.1566   |
| Weight         | -0.0940 | 0.0859     | -1.0934 | 0.2846   |

Residual standard error: 0.2852 on 25 degrees of freedom  
Multiple R-Squared: 0.3435  
F-statistic: 3.27 on 4 and 25 degrees of freedom, the p-value is 0.02749  
19 observations deleted due to missing values

Correlation of Coefficients:  
<table>
<thead>
<tr>
<th></th>
<th>Year</th>
<th>Age</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>-1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.6810</td>
<td>0.6769</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>0.1787</td>
<td>-0.1843</td>
<td>0.2232</td>
</tr>
<tr>
<td>Weight</td>
<td>0.4554</td>
<td>-0.4566</td>
<td>-0.5325</td>
</tr>
</tbody>
</table>

*** Call: lm(formula = log(HG) ~ Year + Age + Lipid + Length, subset = SampType == "W")  
All variable model including Length. This model has higher R-square value than Weight model above. Year is not significant (p-value>0.05) so remove from the model and refit.

Residuals:  
<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.5768</td>
<td>-0.1614</td>
<td>0.04622</td>
<td>0.1799</td>
<td>0.5398</td>
</tr>
</tbody>
</table>

Coefficients:  
|                | Value   | Std. Error | t value | Pr(>|t|) |
|----------------|---------|------------|---------|----------|
| (Intercept)    | -15.2139 | 54.0075   | -0.2817 | 0.7805   |
| Year           | 0.0073   | 0.0272     | 0.2680  | 0.7909   |
| Age            | 0.0963   | 0.0524     | 1.8384  | 0.0779   |
Lipid  -0.1016   0.0574    -1.7694   0.0890  
Length  -0.0468   0.0353    -1.3249   0.1972  

Residual standard error: 0.2822 on 25 degrees of freedom  
Multiple R-Squared:  0.3572  
F-statistic:  3.474 on 4 and 25 degrees of freedom, the p-value is 0.02179  
19 observations deleted due to missing values

Correlation of Coefficients:
(Intercept) Year Age Lipid
Year  -0.9999
Age   -0.6763   0.6741
Lipid  0.2898    -0.2965  0.0919
Length 0.4525    -0.4618 -0.5044  0.2316

*** Call:  lm(formula = log(HG) ~ Age + Lipid + Length, subset = SampType == "W")
Refit model excluding Year. Length is not significant (p-value>0.05) so remove it and refit model.

Residuals:
Min 1Q Median 3Q Max
-0.5698 -0.163 0.05002 0.1682 0.5426

Coefficients:
Value Std. Error t value  Pr(>|t|)
(Intercept) -0.7405  0.6741    -1.0986  0.2820
Age  0.0869  0.0380     2.2855  0.0307
Lipid -0.0970  0.0538    -1.8020  0.0831
Length -0.0425  0.0308    -1.3790  0.1796

Residual standard error: 0.2771 on 26 degrees of freedom  
Multiple R-Squared:  0.3554  
F-statistic:  4.778 on 3 and 26 degrees of freedom, the p-value is 0.008785  
19 observations deleted due to missing values

Correlation of Coefficients:
(Intercept) Age Lipid
Age  -0.2451
Lipid  -0.5546   0.4136
Length 0.8182    -0.2947  0.1117

*** Call:  lm(formula = log(HG) ~ Age + Lipid, subset = SampType == "W")
Refit model excluding Length. Lipid is not significant (p-value>0.05) so exclude it and refit model.

Residuals:
Min 1Q Median 3Q Max
-0.6302 -0.06779 0.02488 0.2048 0.5015

Coefficients:
Value Std. Error t value  Pr(>|t|)
(Intercept) -1.5011  0.3940    -3.8103  0.0007
Age  0.0714  0.0369     1.9344  0.0636
Lipid -0.0887  0.0544    -1.6313  0.1144

Residual standard error: 0.2817 on 27 degrees of freedom  
Multiple R-Squared:  0.3082  
F-statistic:  6.016 on 2 and 27 degrees of freedom, the p-value is 0.006908  
19 observations deleted due to missing values

Correlation of Coefficients:
(Intercept)  Age
Age -0.8849
Lipid -0.8107  0.4702

*** Call: lm(formula = log(HG) ~ Age, subset = SampType == "W")
Refit model excluding Lipid. Age is significant so this is the final model.
Residuals:
  Min 1Q Median 3Q Max
-0.5969 -0.1208 0.04139 0.2149 0.3839

Coefficients:
  Value Std. Error t value Pr(>|t|)
(Intercept) -2.0221  0.2374 -8.5171  0.0000
  Age  0.0998  0.0335  2.9741  0.0060

Residual standard error: 0.2899 on 28 degrees of freedom
Multiple R-Squared: 0.2401
F-statistic: 8.845 on 1 and 28 degrees of freedom, the p-value is 0.005989
19 observations deleted due to missing values

Correlation of Coefficients:
  (Intercept)
  Age -0.9748
South Manistique Lake PCB(aroclor) Regression Model Output

Model 4a. PCB(aroclor) (log-transformed) - All Whole Fish Samples
*** Call: lm(formula = log(PCBaroclor) ~ Year + Age + Lipid + Length)
Compare all variable model with Weight and with Length. Length model has lower R-square value than Weight model so begin analysis with Weight Model.

Residuals:
Min 1Q Median 3Q Max
-0.6195 -0.2549 0.0102 0.1933 0.7874

Coefficients:

|                | Value  | Std. Error | t value | Pr(>|t|) |
|----------------|--------|------------|---------|----------|
| (Intercept)    | -113.9691 | 67.0654   | -1.6994 | 0.1017   |
| Year           | 0.0561 | 0.0337     | 1.6646 | 0.1085   |
| Age            | 0.0547 | 0.0651     | 0.8410 | 0.4083   |
| Lipid          | 0.1532 | 0.0713     | 2.1491 | 0.0415   |
| Length         | -0.0954 | 0.0439    | -2.1742 | 0.0394   |

Residual standard error: 0.3504 on 25 degrees of freedom
Multiple R-Squared: 0.4534
F-statistic: 5.184 on 4 and 25 degrees of freedom, the p-value is 0.003508
29 observations deleted due to missing values

Correlation of Coefficients:

<table>
<thead>
<tr>
<th>(Intercept)</th>
<th>Year</th>
<th>Age</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-0.9999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.6763</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>0.2898</td>
<td>0.6741</td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>0.4525</td>
<td>-0.4618</td>
<td>0.2316</td>
</tr>
</tbody>
</table>

*** Call: lm(formula = log(PCBaroclor) ~ Year + Age + Lipid + Weight)
All variable model including Weight. This model has higher R-square value than Length model above. Age is not significant (p-value>0.05) so remove from the model and refit.

Residuals:
Min 1Q Median 3Q Max
-0.6419 -0.2357 0.003503 0.1865 0.7999

Coefficients:

|                | Value  | Std. Error | t value | Pr(>|t|) |
|----------------|--------|------------|---------|----------|
| (Intercept)    | -127.0076 | 64.5080   | -1.9689 | 0.0601   |
| Year           | 0.0619 | 0.0323     | 1.9183 | 0.0666   |
| Age            | 0.0747 | 0.0638     | 1.1714 | 0.2525   |
| Lipid          | 0.1935 | 0.0666     | 2.9043 | 0.0076   |
| Weight         | -0.2728 | 0.1014    | -2.6900 | 0.0125   |

Residual standard error: 0.3365 on 25 degrees of freedom
Multiple R-Squared: 0.4959
F-statistic: 6.149 on 4 and 25 degrees of freedom, the p-value is 0.001376
29 observations deleted due to missing values

Correlation of Coefficients:

<table>
<thead>
<tr>
<th>(Intercept)</th>
<th>Year</th>
<th>Age</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>-1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.6810</td>
<td>0.6769</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>0.1787</td>
<td>-0.1843</td>
<td>0.2232</td>
</tr>
<tr>
<td>Weight</td>
<td>0.4554</td>
<td>-0.4566</td>
<td>-0.5325</td>
</tr>
</tbody>
</table>
*** Call: lm(formula = log(PCBaroclor) ~ Year + Lipid + Weight)
Refit excluding Age. Year is not significant (p-value>0.05) so remove it and refit model.
Residuals:
     Min 1Q Median 3Q Max
-0.5237 -0.2519 -0.03701 0.1768 0.8961
Coefficients:
            Value Std. Error t value Pr(>|t|)
(Intercept) -82.4371  43.5853 -1.8914   0.0669
Year         0.0398   0.0219  1.8188   0.0775
Lipid       0.1423   0.0380  3.7427   0.0007
Weight      -0.1935   0.0773 -2.5043   0.0171
Residual standard error: 0.3347 on 35 degrees of freedom
Multiple R-Squared: 0.524
F-statistic: 12.84 on 3 and 35 degrees of freedom, the p-value is 8.149e-006
20 observations deleted due to missing values
Correlation of Coefficients:
     (Intercept) Year Lipid
Year -1.0000
Lipid  0.3480 -0.3521
Weight  0.1207 -0.1257  0.2872

*** Call: lm(formula = log(PCBaroclor) ~ Lipid + Weight)
Refit model excluding Year. Lipid and Weight are significant (p-value<0.05) so this is the final model.
Residuals:
     Min 1Q Median 3Q Max
-0.5481 -0.2643 -0.00837 0.2217 0.8696
Coefficients:
            Value Std. Error t value Pr(>|t|)
(Intercept)  -3.1667   0.2711 -11.6806   0.0000
Lipid        0.1666   0.0367  4.5397   0.0001
Weight      -0.1759   0.0791 -2.2237   0.0325
Residual standard error: 0.3452 on 36 degrees of freedom
Multiple R-Squared: 0.479
F-statistic: 16.55 on 2 and 36 degrees of freedom, the p-value is 7.995e-006
20 observations deleted due to missing values
Correlation of Coefficients:
     (Intercept) Lipid
Lipid -0.7197
Weight -0.8288   0.2616
Attachment E2

Upper Peninsula Inland Lakes Mercury Regression Model Output
Upper Peninsula Inland Lakes Mercury Regression Model Output

Model 6a. Mercury (log transformed) - All Fillet Samples

*** Call: lm(formula = log(HG) ~ Year + Weight, subset = SampType == "F")
Compare all variable model with Weight and with Length. Weight model has lower R-
square value than Length model so begin analysis with Length Model.

Residuals:
  Min 1Q Median   3Q   Max
-1.248 -0.4134 0.02497 0.3615 1.155

Coefficients:
  Value Std. Error t value  Pr(>|t|)
(Intercept) -46.7339   12.7267   -3.6721  0.0003
Year        0.0228    0.0064     3.5622  0.0005
Weight      0.2884    0.0344     8.3931  0.0000

Residual standard error: 0.5182 on 222 degrees of freedom
Multiple R-Squared: 0.2871
F-statistic: 44.7 on 2 and 222 degrees of freedom, the p-value is 0
60 observations deleted due to missing values

Correlation of Coefficients:
  (Intercept)    Year
Year -1.0000
Weight  0.0863    -0.0921

*** Call: lm(formula = log(HG) ~ Year + Length, subset = SampType == "F")
All variable model including Length. This model has higher R-square value than
Weight model above. Year is not significant (p-value>0.05) so remove from the
model and refit.

Residuals:
  Min 1Q Median   3Q   Max
-1.211 -0.382 -0.02913 0.3323 1.272

Coefficients:
  Value Std. Error t value  Pr(>|t|)
(Intercept) -18.4337   10.2641   -1.7959  0.0736
Year        0.0077    0.0052     1.4864  0.1383
Length      0.1308    0.0108    12.1317  0.0000

Residual standard error: 0.4843 on 282 degrees of freedom
Multiple R-Squared: 0.3507
F-statistic: 76.16 on 2 and 282 degrees of freedom, the p-value is 0

Correlation of Coefficients:
  (Intercept)    Year
Year -0.9998
Length  0.0447    -0.0639

*** Call: lm(formula = log(HG) ~ Length, subset = SampType == "F")
Refit excluding Year. Length is significant (p-value<0.05) so this is the final
model

Residuals:
  Min 1Q Median   3Q   Max
-1.161 -0.3794 -0.03176 0.3375 1.333

Coefficients:
  Value Std. Error t value  Pr(>|t|)

8601969.001 0501 0103 BH02
g:\1900\8601969.001 0501 0103 BH02.doc
(Intercept) -3.1800  0.2001   -15.8918   0.0000  
Length   0.1319  0.0108    12.2257   0.0000

Residual standard error: 0.4854 on 283 degrees of freedom
Multiple R-Squared: 0.3456  
F-statistic: 149.5 on 1 and 283 degrees of freedom, the p-value is 0

Correlation of Coefficients:
(Intercept) Length  -0.9896

*** Call: lm(formula = log(HG) ~ Year + pH + Conduct + Length, subset = SampType == "F")
All variable model including Length with pH and Conductivity. PH and Conductivity were not significant for the subset of data and were not evaluated further.

Residuals:
 Min      1Q   Median    3Q  Max
-1.098 -0.3436 -0.07015  0.307 1.46

Coefficients:
 Value Std. Error  t value Pr(>|t|)
(Intercept)   2.6745  15.3946     0.1737   0.8623
Year  -0.0027   0.0077    -0.3549   0.7231
pH  -0.0618   0.0515    -1.2001   0.2317
Conduct   0.3175   0.1639     1.9368   0.0544
Length   0.1266   0.0119    10.6406   0.0000

Residual standard error: 0.4752 on 179 degrees of freedom
Multiple R-Squared: 0.3941
F-statistic: 29.1 on 4 and 179 degrees of freedom, the p-value is 0
101 observations deleted due to missing values

Correlation of Coefficients:
(Intercept)    Year      pH Conduct
Year -0.9996
pH -0.0909      0.0672
Conduct -0.3945      0.3993 -0.2653
Length  0.0466     -0.0590 -0.0742  0.0202

Model 6b. Mercury (log transformed) - All Whole Fish Samples
*** Call: lm(formula = log(HG) ~ Year + Lipid + Weight, subset = SampType == "W")
Weight model has higher R-square than Length model, Weight not significant

Residuals:
 Min      1Q    Median    3Q    Max
-0.4592 -0.1334 0.0001163 0.1368 0.4967

Coefficients:
 Value Std. Error  t value Pr(>|t|)
(Intercept)  152.7590   27.7589     5.5031    0.0000
Year   -0.0770    0.0139    -5.5272    0.0000
Lipid   -0.1402    0.0331    -4.2383    0.0001
Weight    0.0416    0.0667     0.6237    0.5367

Residual standard error: 0.245 on 36 degrees of freedom
Multiple R-Squared: 0.689
F-statistic: 26.59 on 3 and 36 degrees of freedom, the p-value is 3.031e-009

Correlation of Coefficients:
(Intercept)    Year  Lipid
Year -1.0000
Lipid  0.3950     -0.3973  
Weight -0.0889      0.0844 -0.2101

*** Call: lm(formula = log(HG) ~ Year + Lipid + Length, subset = SampType == "W")
Length model has lower R-square than Weight model, Length not significant
Residuals:
Min      1Q   Median     3Q    Max
-0.469 -0.1177 -0.01099 0.1417 0.4932
Coefficients:

Value Std. Error  t value  Pr(>|t|)
(Intercept)  153.8402   27.8108     5.5317    0.0000 
Year  -0.0775    0.0139    -5.5598    0.0000 
Lipid    -0.1376    0.0330    -4.1656    0.0002 
Length    0.0071    0.0244     0.2894    0.7739

Residual standard error: 0.246 on 36 degrees of freedom  
Multiple R-Squared: 0.6864  
F-statistic: 26.27 on 3 and 36 degrees of freedom, the p-value is 3.521e-009

Correlation of Coefficients:
(Intercept)    Year   Lipid
Year -0.9999
Lipid  0.3897     -0.3902
Length -0.0568      0.0411 -0.1830

*** Call: lm(formula = log(HG) ~ Year + Lipid, subset = SampType == "W")
Weight removed from model because not significant, Year and Lipid both significant
Residuals:
Min      1Q   Median     3Q    Max
-0.4738 -0.1176 -0.008694 0.1537 0.4921 
Coefficients:

Value Std. Error  t value  Pr(>|t|)
(Intercept)  154.2977   27.4199     5.6272    0.0000 
Year  -0.0777    0.0138    -5.6467    0.0000 
Lipid    -0.1358    0.0321    -4.2361    0.0001

Residual standard error: 0.2429 on 37 degrees of freedom  
Multiple R-Squared: 0.6857  
F-statistic: 40.36 on 2 and 37 degrees of freedom, the p-value is 5.025e-010

Correlation of Coefficients:
(Intercept)    Year
Year -1.0000
Lipid  0.3865     -0.3896

Model 6c. Mercury (log transformed) - All Whole Fish Samples with Age
*** Call: lm(formula = log(HG) ~ Year + Age + Lipid + Weight, subset = SampType == "W")
Weight model has lower R-square value than Length model, Weight not significant
Residuals:
Min      1Q   Median     3Q    Max
-0.3184 -0.1261 -0.02344 0.1442 0.3203
Coefficients:
Value Std. Error   t value  Pr(>|t|)
(Intercept) 135.8816   38.5022     3.5292    0.0017
Year   -0.0688    0.0193    -3.5638    0.0016
Age    0.0795    0.0286     2.7819    0.0104
Lipid   -0.0837    0.0360    -2.3230    0.0290
Weight  -0.0395    0.0642    -0.6153    0.5441

Residual standard error: 0.1914 on 24 degrees of freedom
Multiple R-Squared: 0.759
F-statistic: 18.89 on 4 and 24 degrees of freedom, the p-value is 3.886e-007
11 observations deleted due to missing values

Correlation of Coefficients:
    (Intercept)    Year     Age   Lipid
    Year -1.0000
    Age -0.1296      0.1225
    Lipid 0.0969     -0.1026  0.6666
    Weight 0.3143     -0.3140 -0.4049 -0.3854

*** Call: lm(formula = log(HG) ~ Year + Age + Lipid + Length, subset = SampType == "W")
Length model has higher R-square value than Weight model, Length not significant
Residuals:
    Min     1Q  Median     3Q    Max
  -0.3066 -0.1399 -0.01389 0.1523 0.2996

Coefficients:
Value Std. Error   t value  Pr(>|t|)
(Intercept) 125.3991   39.2007     3.1989    0.0039
Year   -0.0634    0.0197    -3.2126    0.0037
Age    0.0827    0.0272     3.0389    0.0057
Lipid   -0.0801    0.0344    -2.3316    0.0284
Length  -0.0266    0.0234    -1.1364    0.2670

Residual standard error: 0.1879 on 24 degrees of freedom
Multiple R-Squared: 0.7677
F-statistic: 19.83 on 4 and 24 degrees of freedom, the p-value is 2.526e-007
11 observations deleted due to missing values

Correlation of Coefficients:
    (Intercept)    Year     Age   Lipid
    Year -1.0000
    Age -0.1370      0.1323
    Lipid 0.0912     -0.0939  0.6461
    Length 0.4025     -0.4093 -0.3345 -0.3114

*** Call: lm(formula = log(HG) ~ Year + Age + Lipid, subset = SampType == "W")
Length removed from model, Year, Age, and Lipid all significant
Residuals:
    Min     1Q  Median     3Q    Max
  -0.318 -0.09747 -0.02271 0.1208 0.3318

Coefficients:
Value Std. Error   t value  Pr(>|t|)
(Intercept) 143.3278   36.0941     3.9709    0.0005
Year   -0.0726    0.0181    -4.0074    0.0005
Age    0.0724    0.0258     2.8050    0.0096
Lipid   -0.0922    0.0328    -2.8096    0.0095
Residual standard error: 0.189 on 25 degrees of freedom
Multiple R-Squared: 0.7552
F-statistic: 25.7 on 3 and 25 degrees of freedom, the p-value is 8.291e-008
11 observations deleted due to missing values

<table>
<thead>
<tr>
<th>Correlation of Coefficients:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Lipid</td>
</tr>
</tbody>
</table>
### Upper Peninsula Inland Lakes PCB(aroclor) Regression Model Output

#### Model 8a. PCB(aroclor) - All Whole Fish Samples

*** Call: lm(formula = log(PCB$aroclor) ~ Year + Age + Lipid + Weight, subset = SampType == "W")

*Weight model has lower R-square value, Lipid not significant*

Residuals:

<table>
<thead>
<tr>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.4093</td>
<td>-0.1565</td>
<td>-0.005533</td>
<td>0.1677</td>
<td>0.666</td>
</tr>
</tbody>
</table>

Coefficients:

| Value     | Std. Error | t value | Pr(>|t|) |
|-----------|------------|---------|---------|
| (Intercept) | 48.7756    | 54.2421 | 0.8992  | 0.3775 |
| Year      | -0.0264    | 0.0272  | -0.9700 | 0.3417 |
| Age       | 0.0850     | 0.0402  | 2.1128  | 0.0452 |
| Lipid     | 0.0275     | 0.0508  | 0.5414  | 0.5932 |
| Weight    | -0.1483    | 0.0904  | -1.6404 | 0.1140 |

Residual standard error: 0.2696 on 24 degrees of freedom

Multiple R-Squared: 0.3202

F-statistic: 2.826 on 4 and 24 degrees of freedom, the p-value is 0.04715

11 observations deleted due to missing values

<table>
<thead>
<tr>
<th>Correlation of Coefficients:</th>
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</thead>
<tbody>
<tr>
<td>(Intercept)</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Lipid</td>
</tr>
<tr>
<td>Weight</td>
</tr>
</tbody>
</table>

*** Call: lm(formula = log(PCB$aroclor) ~ Year + Age + Lipid + Length, subset = SampType == "W")

*Length model has higher R-square value, Lipid not significant*

Residuals:

<table>
<thead>
<tr>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.3919</td>
<td>-0.1784</td>
<td>-0.02693</td>
<td>0.1829</td>
<td>0.6316</td>
</tr>
</tbody>
</table>

Coefficients:

| Value     | Std. Error | t value | Pr(>|t|) |
|-----------|------------|---------|---------|
| (Intercept) | 33.5655    | 55.1306 | 0.6088 | 0.5484 |
| Year      | -0.0183    | 0.0278  | -0.6599 | 0.5156 |
| Age       | 0.0832     | 0.0383  | 2.1743  | 0.0398 |
| Lipid     | 0.0247     | 0.0483  | 0.5104  | 0.6144 |
| Length    | -0.0640    | 0.0329  | -1.9459 | 0.0635 |

Residual standard error: 0.2642 on 24 degrees of freedom

Multiple R-Squared: 0.3202

F-statistic: 3.189 on 4 and 24 degrees of freedom, the p-value is 0.03103

11 observations deleted due to missing values

<table>
<thead>
<tr>
<th>Correlation of Coefficients:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Lipid</td>
</tr>
<tr>
<td>Length</td>
</tr>
</tbody>
</table>
*** Call: lm(formula = log(PCBaroclor) ~ Year + Age + Length, subset = SampType == "W")

Year not significant

Residuals:
  Min     1Q Median     3Q    Max
-0.3804 -0.1742 -0.001874 0.1741 0.641

Coefficients:
            Value Std. Error t value Pr(>|t|)
(Intercept) 30.9995  54.0828     0.5732   0.5716
Year   -0.0170   0.0272    -0.6240   0.5383
Age     0.0706   0.0288     2.4531   0.0215
Length  -0.0587   0.0308    -1.9089   0.0678

Residual standard error: 0.2603 on 25 degrees of freedom
Multiple R-Squared: 0.3399
F-statistic: 4.292 on 3 and 25 degrees of freedom, the p-value is 0.01421
11 observations deleted due to missing values

Correlation of Coefficients:

(Intercept) Year Age
Year -0.9999
Age -0.2577  0.2540
Length 0.4553 -0.4635 -0.1838

*** Call: lm(formula = log(PCBaroclor) ~ Age + Length, subset = SampType == "W")

Age and Length are both significant

Residuals:
  Min     1Q Median     3Q    Max
-0.3681 -0.2003 -0.02988 0.1608 0.6424

Coefficients:
            Value Std. Error t value Pr(>|t|)
(Intercept) -2.7457  0.5529    -4.9660  0.0000
Age     0.0752   0.0275     2.7324  0.0112
Length  -0.0676   0.0269    -2.5103  0.0186

Residual standard error: 0.2572 on 26 degrees of freedom
Multiple R-Squared: 0.3296
F-statistic: 6.393 on 2 and 26 degrees of freedom, the p-value is 0.00552
11 observations deleted due to missing values

Correlation of Coefficients:

(Intercept) Age
Age -0.3763
Length -0.8907 -0.0771