March 2023
EPA 820-R-23-003

National Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue

Notice

This report was prepared by the U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology. The results of this pilot study were published in a technical journal article (Ramirez et al., 2009) that was subjected to an external peer review process. The EPA Project Manager for preparation of this document was Leanne Stahl who provided overall project coordination and technical direction. Tetra Tech, Inc. provided primary support for the development of this document under Contract Numbers EP-C-04-030 and EP-C-09-019. Blaine Snyder was the Tetra Tech, Inc. Project Manager. Tetra Tech subcontracted Baylor University's Center for Reservoir and Aquatic Systems Research under Contract Numbers EP-C-04-030 and EP-C-09-019 to conduct the fish tissue analysis for this pilot study. Additional support was provided by Computer Sciences Corporation under Contract Number EP-W-06-046.

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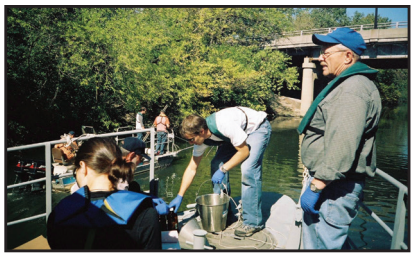

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Acknowledgements

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The principal authors of this report are Leanne Stahl (EPA/OST), Blaine Snyder, John O'Donnell, and Ann Roseberry Lincoln (Tetra Tech, Inc.). Kevin Chambliss and Bryan Brooks (Baylor University) provided all analytical results for this report. David Wells and Ed Partington (EPA/OST) aided in the sampling site selection process. Tetra Tech, Inc. formatted the statistical analysis results, integrated text and graphics developed by the authors, and provided support for final report production.

List of Acronyms and Abbreviations

° C	degrees Centigrade
µL	microliters
BOD5	5-day biological oxygen demand
CAS	Chemical Abstracts Service
CECs	contaminants of emerging concern
CFR	Code of Federal Regulations
cm	centimeter
d	day
ESI	electrospray interface
g	gram
GC	gas chromatography
GC-MS/MS	gas chromatography–tandem mass spectrometry
GLNPO	Great Lakes National Program Office
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
HPLC-MS/MS	high performance liquid chromatography–tandem mass spectrometry
IS	internal standard
L	liter
LC	liquid chromatography
M	molar concentration
MDL	method detection limit
mg	milligram
MGD	million gallons per day
min	minutes
mm	millimeters
MS/MS	tandem mass spectrometry
MS/MSD	matrix spike/matrix spike duplicate
MSTFA	N-methyl-N-trimethylsilyltrifluoracetamide
mTorr	millitorr
N_2	nitrogen gas
NA	not applicable
NCCA	National Coastal Condition Assessment

ng	nanogram
NPDES	National Pollutant Discharge Elimination System
NRSA	National Rivers and Streams Assessment
ORD	Office of Research and Development
OST	Office of Science and Technology
OW	Office of Water
PBDEs	polybrominated diphenyl ethers
PCP	personal care product
PFCs	perfluorinated compounds
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
pH	hydrogen ion concentration, a measure of acidity
ppb	parts per billion
PPCPs	pharmaceuticals and personal care products
ppm	parts per million
psi	pounds per square inch
QAPP	quality assurance project plan
QC	quality control
R^2	correlation coefficient
rpm	revolutions per minute
RRF	relative response factor
RSD	relative standard deviation
s	second
U.S. EPA	United States Environmental Protection Agency
UV	ultraviolet
V	volt
v/v	volume/volume ratio
WWTP	wastewater treatment plant

Executive Summary

Pharmaceuticals and personal care products (PPCPs) are a diverse group of chemicals that have recently received attention as potential environmental pollutants. PPCPs enter the aquatic environment primarily as a result of their persistence through the wastewater treatment process and resulting discharge to surface or ground water. Most existing information on the environmental occurrence of PPCPs focuses on wastewater discharges and surface waters, although an increasing body of literature indicates that certain PPCPs can accumulate in fish. To date, studies of PPCPs in fish tissue generally targeted a specific chemical or chemical class at a single study location. EPA's Office of Science and Technology (OST) responded to this data gap by designing and conducting the National Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue. The specific purpose of the pilot study was to advance the science of detecting PPCPs in the environment by investigating the occurrence of a broad suite of PPCPs in fish collected from several U.S. streams.

North Shore Channel, Chicago, Illinois

EPA selected fish sampling sites on five effluent-dominated streams in population centers near wastewater treatment plant (WWTP) discharges based on the assumption that PPCPs were more likely to occur in those areas. These sites included the North Shore Channel in Chicago, Illinois; Trinity River in Dallas, Texas; Little Econlockhatchee River in Orlando, Florida; Salt River in Phoenix, Arizona; and Taylor Run in West Chester, Pennsylvania. EPA also obtained fish from the East Fork Gila River in the Gila River Wilderness Area of New Mexico to represent a reference condition or an area of minimal human influences and impacts. Field crews collected six composites of adult fish of the same resident species from each sampling location during late summer and fall of 2006. Every composite sample contained three or four fish that were individually wrapped as whole-body specimens and collectively bagged as a composite. All fish were frozen on dry ice, shipped to the analytical laboratory at Baylor University, and stored frozen at $\leq -20°$ C prior to preparation of fillet and liver tissue samples for analysis.

Reference site, East Fork Gila River, New Mexico

In the laboratory, technicians removed the entire fillet (including the skin and belly flap) from both sides of each fish in the composite sample, using all available tissue to prepare the fillet composite sample (i.e., the batch method). They homogenized fillet tissue using a high-speed blender and stored homogenate samples in a freezer at ≤ ⁻20° C prior to analysis. Laboratory personnel removed fish livers from each fish by dissection and applied compositing, homogenization, and storage techniques that mirrored those for fillet samples.

Scientists at Baylor University analyzed the fish tissue composites for 36 PPCPs, including 24 pharmaceutical compounds and 12 personal care products. They used a method that applies high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) to analyze fillet samples for 24 pharmaceutical compounds and liver samples for 23 pharmaceuticals, following procedures described by Ramirez et al. (2007). This analytical method provides results for a range of prescription and over-the-counter drugs, including antibiotics, analgesics, antihistamines, along with drugs to treat high blood pressure and high cholesterol, depression, seizures, and fungal infections. Following procedures described by Mottaleb et al. (2009), laboratory staff also extracted and analyzed fillet tissue samples for 12 personal care product (PCP) chemicals using a method that applies gas chromatography with tandem mass spectrometry (GC-MS/MS). This method provides results for a range of chemicals commonly used in personal care products, including a number of fragrances or musks, ultraviolet (UV) light filters, surfactants, antimicrobials, and insect repellents. The laboratory measured lipid content in each of the fish tissue composites using gravimetric (weight-based) methods.

None of the 36 target PPCPs were detected in any of the fillet and liver tissue samples from the Gila River Wilderness reference site. A majority of the 24 pharmaceutical compounds also did not occur in the fish tissue samples from the five effluent-dominated stream sites. Seventeen of the pharmaceutical compounds were not detected in any of the fillet or liver samples, including the six antibiotics (e.g., erythromycin), the three analgesics (e.g., ibuprofen), and three of the four pharmaceuticals used to treat high blood pressure (atenolol, metoprolol, and propranolol). Ten of the 12 personal care product chemicals did not occur above detectable levels in the fillet tissue samples, including triclosan (widely used chemical

in hand soaps), m-toluamide (an insect repellent), and three chemicals that act as ultraviolet (UV) filters in sunscreens (benzophenone, octocrylene, and 4-methylbenzylidene camphor).

Five of the 24 target pharmaceutical compounds were found in the fillet samples from the effluent-dominated stream sites. All of the fillet concentrations for the detected pharmaceutical compounds were measured in the low parts per billion (ppb). The highest fillet concentration reported for any pharmaceutical compound was 19 ng/g of sertraline (antidepressant). In order of decreasing frequency, the pharmaceuticals detected in fillet samples included diphenhydramine (18 of 30 samples), norfluoxetine and sertraline (12 of 30 samples for each), diltiazem (8 of 30 samples), and carbamazepine (6 of 30 samples). There were notable differences in the frequency of detections among sites. For example, none of the pharmaceuticals were detected in fillet samples from Dallas and Orlando. The wastewater treatment plants discharging to streams near the sampling locations in both cities employ advanced treatment technologies that may provide more effective removal of pharmaceuticals from the waste stream. Diphenhydramine occurred in every fillet sample from the remaining three sites. These data suggest widespread discharge of this active ingredient in over-the-counter cold medications into surface waters. In contrast, carbamazepine occurred in all the fillet samples at only a single site (Chicago).

Seven of the target pharmaceutical compounds were detected in the liver samples, including fluoxetine (an antidepressant) and gemfibrozil (a drug used to treat high cholesterol), in addition to the five pharmaceuticals found in the fillet samples (i.e., carbamazepine, diltiazem, diphenhydramine, norfluoxetine, and sertraline). Pharmaceutical compounds occurred more frequently in the liver samples than in the fillet samples. Norfluoxetine and sertraline, two of the antidepressants, were found in liver samples from all five sampling sites. Their detection frequencies were 26 and 23 per 30 samples, respectively. Differences in detection frequencies for pharmaceuticals in the liver samples were also apparent among the sampling sites. The fewest number of detections occurred at Orlando and Dallas, two sites where WWTPs apply advanced treatment technologies before discharging effluents into the streams. The concentrations of pharmaceutical compounds in fish liver samples were greater than those measured in fillets. Differences among their mean concentrations ranged from a factor of nearly three to more than 20. Sertraline had the highest concentrations reported for pharmaceuticals in liver samples with levels as high as 550 ng/g.

Personal care product (PCP) results indicated that two of the 12 target chemicals, galaxolide and tonalide, occurred in fillet samples. Both are fragrances added to common products like cosmetics and detergents, and both PCPs were detected in fillet samples from all five sites. Galaxolide was measured in 29 of the 30 fillet samples, and tonalide was detected in 26 of the 30 fillet samples. Fillet concentrations of galaxolide occurred in the low parts per million range at a majority of the sites, and mean fillet concentrations exceeded 1000 ng/g or 1 part per million (ppm) at three sites. Tonalide concentrations in the fillet samples were about an order of magnitude lower than the galaxolide concentrations with mean concentrations ranging from 55 ng/g to 240 ng/g.

This study supports conclusions from earlier studies and offers new insights, particularly with respect to the benefits of advanced wastewater treatment technologies on the occurrence of PPCPs in fish tissue. Conclusions derive primarily from differences in PPCP detections and concentrations related to type of tissue, geographic location, and level of treatment technologies applied in WWTPs before discharge of effluents into rivers or streams. They include the following:

- Pharmaceutical compounds occurred in greater numbers and at higher detection frequencies and concentrations in liver samples than in fillet samples.
- No significant relationships were observed between lipid content and accumulation of pharmaceuticals in either fillet or liver tissue.
- Differences in wastewater treatment technologies can substantially affect the removal efficiency of pharmaceutical compounds, which affects fish tissue concentrations.
- It appears that the wastewater treatment technologies applied at individual WWTPs is a better predictor of pharmaceutical occurrence than demographics or surrogate data for pharmaceutical use statistics; however, demographics of local populations can influence geographic differences in detections of pharmaceutical compounds in fish tissue.
- The widespread occurrence of norfluoxetine (a metabolite of the antidepressant fluoxetine) in fish tissue samples analyzed for this study provides further evidence of the importance of including metabolic products of target chemicals in future tissue screening studies.

1.0 Introduction

Pharmaceuticals and personal care products (PPCPs) are a diverse group of chemicals that, until recently, have received little attention as potential environmental pollutants. PPCPs include all drugs (both prescription and over-the-counter medications) and non-medicinal consumer chemicals such as fragrances (musks), sunscreens, and soaps. Recent evidence has shown that many PPCPs enter the aquatic environment primarily as a result of their persistence through the wastewater treatment process and resulting discharge to surface or ground water (Daughton and Ternes 1999). The full extent, magnitude, and consequences of their presence in the aquatic environment are largely unknown.

Most existing information on the environmental occurrence of PPCPs focuses on wastewater discharges and surface waters. The limited number of studies on PPCPs in fish tissue generally target a specific chemical (or chemical class) at a single study location. As a consequence, there is a need for additional data to provide an understanding of PPCP accumulation in fish at a broad scale that will support the characterization of human health risks associated with PPCPs in the environment. In 2006, OST initially responded to this data gap by designing and conducting a pilot study called the National Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue with support from Baylor University and Tetra Tech, Inc. The purpose of the PPCP Fish Pilot Study was to advance the science related to detecting PPCPs in the environment by investigating the occurrence of a broad suite of PPCPs in the tissue of fish collected from selected U.S. streams.

Reference site – East Fork Gila River, Gila River Wilderness Area, New Mexico

EPA's PPCP Fish Pilot Study is the first study to assess a wide range of PPCPs in fish from sampling locations distributed across the lower 48 states. For this study, EPA selected sites on five effluent-dominated streams

in population centers near wastewater treatment plant (WWTP) discharges based on the assumption that PPCPs were more likely to occur in these areas. The study also included a reference site located on a river in a national wilderness area. Scientists at Baylor University analyzed the fish tissue from these sites for 36 PPCPs, including 24 pharmaceutical compounds and 12 personal care products. Prior to the study, Baylor University researchers had developed analytical methods to analyze fish tissue for this large number of PPCPs. When EPA initiated the pilot study, their laboratory was the only one in the U.S. with this capability. In 2009, EPA, Baylor University, and Tetra Tech collaborated on publication of the study results in the technical journal *Environmental Toxicology and Chemistry* (Ramirez et al. 2009). The journal article reports summary level data for the study. This technical report describes the planning and implementation of the pilot study in greater detail, and appendices to the report provide the complete set of site-specific data generated during this study.

2.0 Study Design and Approach

2.1 Background

The PPCP Fish Pilot Study required four years (2006–2009) for study planning, collection and chemical analysis of the fish samples, review and statistical analysis of the tissue concentration data, and publication of the results. The study team completed site selection, development of Quality Assurance Project Plans (QAPPs) for sample collection and analysis, and fish collection during 2006. It took about a year and a half to homogenize the fish tissue and analyze the fish tissue samples. During this time, Baylor University chemists refined procedures in their analytical method for detecting personal care products in fish tissue to address problems with obtaining reliable results caused by lipid interference. By fall 2008, the tissue data were reviewed and ready to report. Baylor University researchers who participated in the study led the effort to compile and publish the results in a special issue of *Environmental Toxicology and Chemistry* on PPCPs in the environment (Ramirez et al. 2009). The final activities for this study have included production and release of this technical report and a supporting Quality Assurance Report (USEPA 2023).

2.2 Study Design Development

Targeted sampling is an appropriate approach for initial investigation of occurrence of PPCPs in fish. The primary objective of this pilot study was to determine which PPCPs were accumulating in fish, so the study was designed to collect fish from surface waters where PPCPs were most likely to occur. EPA identified a number of criteria that could increase the likelihood of fish being exposed to PPCPs and applied these criteria in selecting sites for the study. EPA adopted the list of target chemicals that Baylor University could detect with their tissue methods for PPCP analysis.

2.2.1 Site Selection

EPA considered a number of factors to identify five sampling locations around the country where PPCPs were more likely to occur and accumulate in fish. The leading factor for site selection was location on an effluent-dominated river or stream just below a WWTP discharge. At three of the five sites, the flow consists of nearly 100% effluent. On average, effluent comprises about two-thirds of the flow at the other two

North Shore Channel, Chicago, Illinois

sites. EPA also assumed locations on streams or rivers running through cities with high population densities in conjunction with higher median incomes and percentages of elderly residents could increase the potential of detecting PPCPs in fish from these areas. EPA applied two other criteria in the site selection process. One was to include areas where WWTP discharges receive different levels of treatment to evaluate the potential impact of treatment technologies on PPCP removal. Another was to target areas where sufficient numbers and sizes of resident fish were available for analysis. The six criteria EPA applied to selection of sampling locations for the pilot study can be summarized as follows:

Salt River, Phoenix, Arizona

Trinity River, Dallas, Texas

- Effluent-dominated river or stream segments below WWTP discharges;
- Urban or suburban areas with high population densities;
- Cities with higher median incomes (used as a surrogate for pharmaceutical sales);
- Geographic areas with a large percentage of residents in the age category of 65 years and older;
- WWTP discharges subject to different levels of treatment; and
- Availability of sufficient numbers and sizes of fish.

Based on these criteria, EPA selected five sampling sites on rivers or streams in the following cities:

- Chicago, Illinois (North Shore Channel)
- Dallas, Texas (Trinity River)
- Orlando, Florida (Little Econlockhatchee River)
- Phoenix, Arizona (Salt River)
- West Chester, Pennsylvania [a suburb of Philadelphia] (Taylor Run)

The study design included identifying and sampling a reference site free from sources of human contamination. The East Fork of the Gila River in the Gila Wilderness Area of southwest New Mexico provided a suitable reference site for the study. The map in Figure 1 displays the sampling locations for the pilot study. Table 1 provides wastewater treatment and discharge information for each facility near the sampling locations, along with population characteristics of the cities associated with each sampling site.

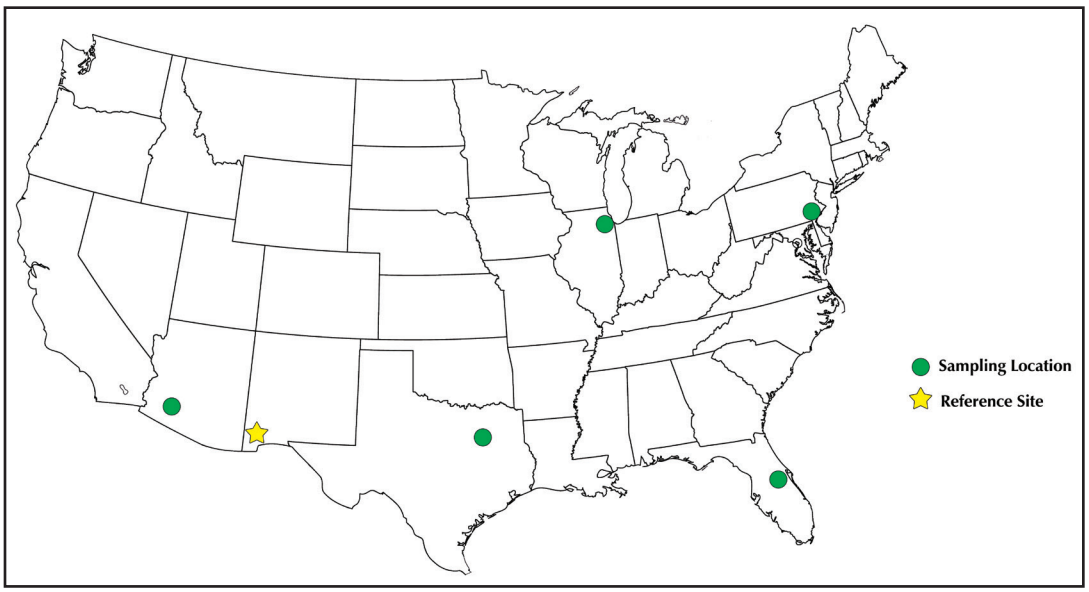


Figure 1. Sampling locations for the PPCP Fish Pilot Study.

Table 1. Wastewater Treatment and Discharge Information for Facilities in the Vicinity of Each Sampling Location and Population Characteristics of Associated Cities.

Location	Treatment	Receiving Water	Population	Design capacity (MGD)[a]	Existing flow (MGD)	Effluent (%)	65 and older (%)	Median income
Phoenix, Arizona	Advanced treatment I with nutrient removal[b]	Salt River	1,418,041	165	153	100	8.1	$41,207
Orlando, Florida	Advanced treatment II with nutrient removal[c]	Little Econlockhatchee River	442,542	40	36	64	11.3	$35,732
Chicago, Illinois	Advanced treatment I with nutrient removal[b]	North Shore Channel	5,376,741	333	234	100[d]	10.3	$38,625
West Chester, Pennsylvania	Advanced treatment I with nutrient removal[b]	Taylor Run	17,701	1.8	1.3	36–86	9.0	$37,803
Dallas, Texas	Advanced treatment II with nutrient removal[c]	Trinity River	3,500,000	175	152	100[d]	8.1	$43,324

[a] Million gallons per day.

[b] Advanced treatment I. Wastewater discharged after receiving biological treatment, physical or chemical treatment, or both. A wastewater treatment plant with a concentration of biochemical oxygen demand (BOD5; the amount of dissolved oxygen consumed in 5 days by biological processes breaking down organic matter) greater than or equal to 10 mg/L but less than 20 mg/L (based on 30-d average) in its National Pollutant Discharge Elimination System (NPDES) permit is considered to be providing advanced treatment I.

[c] Advanced treatment II. Wastewater discharged after receiving biological treatment, physical or chemical treatment, or both. A wastewater treatment plant with a BOD5 concentration less than 10 mg/L (based on 30-d averages) in its NPDES permit is considered to be providing advanced treatment II. Note that the addition of nutrient removal is considered to be an improvement in effluent quality (e.g., secondary effluent with nutrient removal represents higher quality effluent than secondary effluent without nutrient removal).

[d] Flow is primarily made up of effluent discharged from multiple facilities.

2.2.2 Target Chemicals

Two analytical methods developed by Baylor University specify the target chemicals that apply for this study. Their tissue method for pharmaceutical analysis provides screening data for 24 compounds representing a wide range of medical uses that include antibiotics, analgesics, antidepressants, anti-hypertension drugs, an antihistamine, and an anti-seizure drug. Their other method screened fish tissue for 12 chemicals in personal care products, which consisted primarily of fragrances or musks in lotions and soaps and the ultraviolet filtering chemicals in sunscreen products. Tables 2 and 3 list the names and uses of each pharmaceutical compound and personal care product chemical that these methods could detect in fish tissue, respectively.

Table 2. Target Pharmaceutical Chemicals, Uses, and Chemical Abstracts Service (CAS) Registry Numbers for the PPCP Fish Pilot Study.

Pharmaceuticals Using HPLC-MS/MS Method		
Chemical	**Use**	**CAS Number**
Acetaminophen	Analgesic	103-90-2
Atenolol	Anti-hypertension	29122-68-7
Caffeine	Stimulant	58-08-2
Carbamazepine	Anti-seizure	298-46-4
Cimetidine	Anti-acid reflux	51481-61-9
Codeine	Analgesic	76-57-3
Diltiazem	Anti-hypertension	42399-41-7
1,7-Dimethylxanthine (caffeine metabolite)	Antispasmodic	611-59-6
Diphenhydramine	Antihistamine	58-73-1
Erythromycin	Antibiotic	114-07-8
Fluoxetine	Antidepressant	54910-89-3
Gemfibrozil	Antilipemic	25812-30-0
Ibuprofen	Analgesic	15687-27-1
Lincomycin	Antibiotic	154-21-2
Metoprolol	Anti-hypertension	37350-58-6
Miconazole	Antifungal	22916-47-8
Norfluoxetine (Fluoxetine metabolite)	Antidepressant	54910-89-3
Propranolol	Anti-hypertension	525-66-6
Sertraline	Antidepressant	79617-96-2
Sulfamethoxazole	Antibiotic	723-46-6
Thiabendazole	Antibiotic	148-79-8
Trimethoprim	Antibiotic	738-70-5
Tylosin	Antibiotic	1401-69-0
Warfarin	Anticoagulant	81-81-2

Table 3. Target Personal Care Product Chemicals, Uses, and Chemical Abstracts Service (CAS) Registry Numbers for the PPCP Fish Pilot Study.

Personal Care Products Using GC-MS/MS Method		
Chemical	**Use**	**CAS Number**
Benzophenone	UV filter	119-61-9
Celestolide (ADBI)	Fragrance/Musk	13171-00-1
Galaxolide	Fragrance/Musk	1222-05-5
4-Methylbenzylidene Camphor (4-MBC)	UV Filter	36861-47-9
Musk Ketone	Fragrance/Musk	81-14-1
Musk Xylene	Fragrance/Musk	81-15-2
p-Nonylphenol	Surfactant	104-40-5
Octocrylene	UV Filter	6197-30-4
p-Octylphenol	Surfactant	1806-26-4
m-Toluamide (DEET)	Insecticide	618-47-3
Tonalide	Fragrance/Musk	1506-02-1
Triclosan	Antimicrobial	3380-34-5

2.3 Mobilization

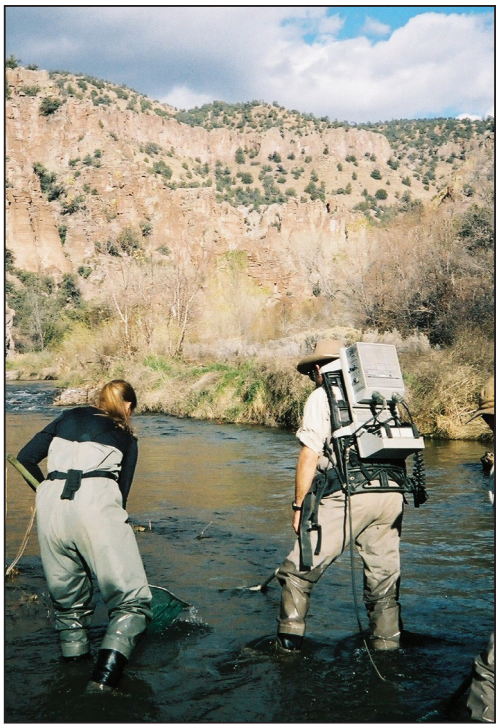
North Shore Channel, Chicago, Illinois

Prior to beginning field sampling, EPA completed some key activities to mobilize for the study. This included preparation of Quality Assurance Project Plans for sample collection and analysis. Copies of the Sample Collection Activities QAPP (USEPA 2006a) and the Laboratory Sample Preparation and Analysis Activities QAPP (USEPA 2006b) are available online at *https://www.epa.gov/fish-tech/pilot-study-pharmaceuticals-and-personal-care-products-fish-tissue*. EPA also formed partnerships for project coordination and sampling assistance in Chicago and for sampling support at the reference site in New Mexico. OST coordinated with EPA's Great Lakes National Program Office (GLNPO) to participate in a broader study of PPCPs in Chicago's North Shore Channel and vicinity (Barber et al., 2011). GLNPO staff participated in the Chicago sampling effort and arranged for the Metropolitan Water Reclamation District of Greater Chicago to provide vessels, equipment, and biologists to assist with fish collection in Chicago. In New Mexico, fisheries biologists from the New Mexico Environment Department provided support for identifying and sampling the reference site on the East Fork of the Gila River in southwest New Mexico.

2.4 Sample Collection

Field crews collected a total of 17 to 24 adult fish of the same resident species from each sampling location during late summer and fall of 2006 (Table 4). This corresponds to the period when lipid content in the fish is usually highest and water levels in the rivers or streams are lowest. Both conditions may increase the likelihood of detecting PPCPs in fish.

The field sampling teams used portable backpack or boat-mounted electrofishing systems to obtain species that are typically consumed by humans and wildlife. At each sampling site, they retained individual fish of a single species that were similar in length (i.e., the smallest fish in the sample was no less than 75% of the length of the largest fish) consistent with the recommendations in U.S. EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis, Third Edition* (USEPA 2000).



Electrofishing at the East Fork Gila River reference site.

The field team recorded the weight (total body mass in grams, wet weight) and length (total length in millimeters) of each fish before dividing the fish into six composite samples. Each composite sample contained three or four fish individually wrapped in solvent-rinsed aluminum foil and secured together in a food-grade polyethylene bag. The fish samples were frozen on dry ice, shipped in coolers to the analytical laboratory at Baylor University via next-day air delivery, and stored in a freezer at ≤ -20° C prior to preparation of fillet and liver tissue samples for analysis. A detailed description of the sampling protocols is available in the *Quality Assurance Project Plan (QAPP) for Sample Collection Activities for a Pilot Study to Investigate the Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue* (USEPA 2006a). This document is available online at ***https://www.epa.gov/fish-tech/pilot-study-pharmaceuticals-and-personal-care-products-fish-tissue***.

Table 4. Fish Collected for the PPCP Fish Pilot Study.

State	Sampling Location	Date	Species	Number of Fish
AZ	Salt River, Phoenix	11/2006	Common carp	18
FL	Little Econlockhatchee River, Orlando	10/2006	Bowfin	17
IL	North Shore Channel, Chicago	09/2006	Largemouth bass	24
NM	East Fork Gila River (*Reference Site*)	11/2006	Sonora sucker	24
PA	Taylor Run, West Chester	08/2006	White sucker	24
TX	Trinity River, Dallas	10/2006	Smallmouth buffalo	18

2.5 Sample Analysis

Field packaging and labeling of fish composite samples at the Gila River reference site.

EPA contracted with an analytical laboratory at Baylor University in Waco, Texas to prepare and analyze fish tissue samples for the PPCP Fish Pilot Study. At the time that EPA initiated the study, research scientists at the university had developed the only analytical methods available in the country to screen fish tissue for a broad suite of pharmaceutical compounds and chemicals commonly used in personal care products, such as fragrances in soaps and ultraviolet filters in sunscreens. Staff at the laboratory prepared fillet and liver tissue samples from each fish composite sample to analyze both tissue types for pharmaceutical compounds and analyzed fillet tissue only for personal care product chemicals. They applied a method utilizing high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in the fillet and liver tissue analysis for pharmaceutical compounds. To analyze fillet samples for personal care products, they used gas chromatography with tandem mass spectrometry (GC-MS/MS). The laboratory also measured the lipid content in each of the fish tissue samples using gravimetry (weight-based method).

2.5.1 Fish Tissue Sample Preparation

EPA directed the laboratory to follow tissue preparation procedures in EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis, Third Edition* (USEPA 2000) and apply the batch method to prepare composite samples of fillet tissue for analysis. The primary steps in this process include:

- Removing the entire fillet (including the skin and belly flap) from both sides of each fish in the composite sample and using all the available tissue to prepare the fillet composite sample (i.e., the batch method),
- Grinding frozen cubes of fillet tissue to a fine powder using a high-speed blender and adding small amounts of dry ice during grinding to facilitate consistent blending of the tissue,
- Applying quartering, mixing, and re-grinding techniques described in the guidance document to produce a homogeneous composite mixture of fillet tissue, and
- Storing the homogenized fillet composite samples in a freezer at a temperature ≤ −20° C until the laboratory was ready to analyze them for PPCPs.

To prepare liver composite samples, the laboratory applied tissue dissection and homogenization techniques developed for prior studies conducted by Baylor University to characterize concentrations of PPCPs in fish tissue (Brooks et al. 2005). These techniques involved the following steps:

- Removing the liver from each fish in the composite (total of three or four livers, depending on the sampling location) and placing all of them in a clean glass container,
- Homogenizing the liver tissue using a motor-driven tissue homogenizer set to rotate at 30,000 revolutions per minute (rpm), and
- Storing the liver homogenate samples in the freezer at a temperature ≤ ⁻20° C until the laboratory was ready to analyze them for pharmaceutical compounds.

2.5.2 Analytical Methods

When EPA initiated the PPCP Fish Pilot Study in 2006, the agency did not have methods available to analyze tissue for PPCPs. However, researchers at Baylor University had developed and successfully applied two analytical methods to screen fish tissue for a wide range of PPCPs. Based on this experience, EPA arranged for the laboratory at Baylor University to analyze pilot study fish tissue samples since they were the only laboratory in the country at that time with the capability to screen tissue for three dozen PPCPs. The laboratory also measured the lipid content of each of the fish tissue samples. This section of the report provides a brief description of the methods for PPCP and lipid analysis, and Appendix A contains a summary of the extraction and analytical procedures associated with each method.

2.5.2.1 Analysis of Fillet and Liver Tissue for Pharmaceutical Compounds

The laboratory at Baylor University analyzed fillet tissue samples for 24 pharmaceutical compounds and liver tissue samples for 23 pharmaceuticals with a method that applies high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) using procedures described by Ramirez et al. (2007). This method specifies a fillet tissue weight of 1.0 gram and a liver tissue weight of 0.5 gram for analysis. It provides results for a range of prescription and over-the-counter drugs, including antibiotics, analgesics, antihistamines, and drugs to treat high blood pressure and high cholesterol, depression, seizures, and fungal infections. Table 5 contains a list of the target chemicals for this pharmaceutical method, along with the method detection limits (MDLs) for both the fillet and liver tissue. Note that the pharmaceutical method (Ramirez et al. 2007) could reliably detect and quantify the antifungal miconazole in fillet tissue, but not in liver tissue.

Table 5. Method Detection Limits for Pharmaceutical Chemicals Analyzed by HPLC-MS/MS in Fish Fillet and Liver Composite Samples.

Chemical	MDL[a] (ng/g)	
	Fillet	Liver
Acetaminophen	4.40	34.28
Atenolol	1.48	12.86
Caffeine	3.93	25.47
Carbamazepine	0.54	1.86
Cimetidine	1.04	5.18
Codeine	6.11	31.49
Diltiazem	0.12	0.26
1,7-Dimethylxanthine (caffeine metabolite)	1.10	5.84
Diphenhydramine	0.05	0.26
Erythromycin	6.42	43.03
Fluoxetine	6.74	12.41
Gemfibrozil	6.68	24.82
Ibuprofen	45.96	172.81
Lincomycin	5.53	56.14
Metoprolol	2.50	8.90
Miconazole	10.83	NA[b]
Norfluoxetine (Fluoxetine metabolite)	2.90	15.31
Propranolol	1.07	3.77
Sertraline	3.56	17.29
Sulfamethoxazole	2.29	13.95
Thiabendazole	2.63	7.84
Trimethoprim	2.15	8.00
Tylosin	5.02	34.67
Warfarin	0.86	2.70

[a] MDL is the method detection limit.

[b] Miconazole was not reliably measured in liver using existing analytical method.

2.5.2.2 Analysis of Fillet Tissue for Personal Care Product Chemicals

Laboratory staff extracted and analyzed only fillet tissue samples for 12 personal care product chemicals with a method that applies gas chromatography with tandem mass spectrometry (GC-MS/MS) using procedures described by Mottaleb et al. (2009). This method requires a fillet tissue volume of 1.0 gram for analysis. It provides results for a range of chemicals commonly used in personal care products, including a number of fragrances or musks, ultraviolet light filters, surfactants, an antimicrobial, and an insect repellent. Table 6

contains a list of the specific chemicals that can be detected with this personal care product (PCP) method, along with the detection limits for the fillet tissue.

Initially, the laboratory attempted to analyze liver tissue using the PCP method and encountered problems with interference due to the high lipid content of the liver tissue. In response, the laboratory experimented with several modifications to the clean-up procedures for the method to mitigate these problems. None of the PCP method modifications resolved the interference problems sufficiently to produce reliable liver results, so no PCP data are available for liver tissue.

Table 6. Method Detection Limits for Personal Care Products Analyzed by GC-MS/MS in Fish Fillet Tissue Composite Samples.

Chemical	Fillet MDL[a] (ng/g)
Benzophenone	16.4
Celestolide (ADBI)	17.7
Galaxolide	12.2
4-Methylbenzylidene-Camphor (4MBC)	120.5
Musk Ketone	321.2
Musk Xylene	397.1
p-Nonylphenol	9.7
Octocrylene	36
p-Octylphenol	8.2
m-Toluamide (DEET)	5.1
Tonalide	13.4
Triclosan	37.8

[a] MDL is the method detection limit.

2.5.2.3 Analysis of Fish Tissue for Lipids

To identify any correlations between PPCP and lipid concentrations, the laboratory measured the lipid content of each fillet and liver composite sample prepared from the six fish composite samples collected at every sampling location. The method for lipid analysis involves extracting lipids from two grams of tissue using a mixture of solvents, evaporating the solvents from the tissue mixture, determining the lipid content gravimetrically (i.e., measuring the lipid content based on weight) after drying the residue to a constant weight, and calculating the percent lipid by dividing the weight of the lipid residue by the initial weight of the tissue aliquot (approximately two grams). Appendix A provides a detailed description of the lipid method.

EPA requested that the laboratory also use lipid analysis as a quality control procedure to assess the homogeneity of the fillet and liver tissue samples. This procedure included triplicate lipid testing of all six fillet tissue samples and one of the six liver tissue samples

prepared from the six fish composite samples collected at every sampling location. For every fillet tissue sample, the laboratory analyzed three 2-gram tissue aliquots for lipids using the method summarized above. Since liver tissue was scarce compared to the fillet tissue, the laboratory tested three 2-gram tissue aliquots for lipid content from only one in every set of six liver tissue samples. If the relative standard deviation for the triplicate lipid measurements was less than 15%, then the tissue sample met the criterion for homogeneity.

2.6 Data Analysis

The analytical laboratory reported quantitative results for each pharmaceutical compound and personal care product chemical in fish fillets, and for each pharmaceutical compound in fish liver tissue. The MDL was the reporting limit for the PPCP data, so only values greater than or equal to the MDL were included when determining frequency of detection, mean detected concentration, or maximum detected concentration. Frequency of detection is defined as equal to the number of composites in which a chemical was detected at a concentration greater than or equal to the MDL compared to the total number of composites. Similarly, the mean and maximum detected concentrations presented in this report refer to the mean or maximum of the detected concentrations greater than or equal to the MDL.

The final results do not include data for three chemicals. Miconazole, an antifungal pharmaceutical, is not reported for fish liver tissue samples because the laboratory determined that it could not be reliably measured using the specified analytical method. Benzophenone and octocrylene, UV filters found in personal care products, were identified in blank control samples at concentrations comparable to those reported in the analytical samples. Therefore, the analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

2.7 Identifying and Quantifying Target Chemicals

The HPLC-MS/MS and GC-MS/MS methods used several characteristics to identify the target analytes. The first characteristic was retention time, or the time at which chemicals elute from the liquid or gas chromatograph. Retention time is a key indicator of the presence of a compound of interest in chromatographic analysis. Retention time profiles were determined by analysis of mixtures of high concentration standards during the development of chromatographic conditions. In addition to retention time, mass spectral data were used to confirm the identification of chemicals of interest and yield optimized quantitation of target chemicals of interest in the presence of co-eluting non-target interferences (i.e., chemicals not included in the study that produce peaks on the chromatogram that overlap with peaks of chemicals included in the study). While the retention time of a specific peak in the chromatogram may suggest the presence of a chemical of interest, it is the presence of specific precursor and quantitation ions in the tandem mass spectrometers that allows certainty in the identification.

After a chromatographic peak is identified as a chemical of interest, its concentration in the sample is calculated by the instrument data system. The tandem MS/MS detectors used in the HPLC-MS/MS and GC-MS/MS methods typically produce two or more ions that are characteristic of the chemical. The responses (i.e., peak areas) for these characteristic ions are used to calculate the concentrations in the sample. The pharmaceutical method used matrix-matched calibration standards (i.e., fish tissue matrix to the extent possible), and both instrumental methods used internal standard calibration and quantitation equations. Appendix A provides details on internal standard calibrations and general quantitation reporting.

3.0 Results

The PPCP Fish Pilot Study is the first research effort to screen fish tissue samples from several sites across the United States for a broad suite of PPCPs. Since the focus of the study was to investigate the occurrence of PPCPs in fish, EPA selected sampling locations for this national pilot study based on factors that could increase the likelihood of encountering PPCPs in the environment. Refer to Section 2.1.1 for a list of these factors. With the targeted study design, it is appropriate to apply routine calculations for statistical analysis of the fish tissue concentration data (e.g., mean concentrations and corresponding standard deviations).

Bowfin, Orlando, Florida

Sonora sucker, East Fork Gila River

Sections 3.1 and 3.2 describe the occurrence of PPCPs in fish tissue. Tables 7 and 8 in Section 3.1 list the pharmaceuticals and personal care products not detected in any of the fish tissue samples, respectively. Tables 9, 10, and 11 in Section 3.2 identify the method detection limit associated with each pharmaceutical compound detected in fillet and liver samples (Tables 9 and 10) and with each personal care product detected in fillet samples (Table 11). Additional information about method detection limits is provided below. These three tables also report the frequency of detection (ratio based on a total of six composite samples from each site) and the mean and maximum tissue concentrations for each of the five sampling locations (Chicago, Dallas, Orlando, Phoenix, and West Chester, PA). Ramirez et al. (2009) provides summary-level data and a discussion of the pilot study results. Consistent with the reporting approach in Ramirez et al. (2009), the mean fish tissue concentrations reported in the three tables were derived from detected concentrations only (i.e., only values from detected concentrations were used to calculate the mean concentrations without assigning any values to non-detects and factoring them into the mean concentration calculations). Table 12 presents quality control data for pharmaceutical compounds detected in liver composite samples. These data

can be applied in interpretation of the liver results at four of the sampling locations. In addition, three appendices provide site-specific PPCP data for the six fillet and liver composites analyzed from each sampling site: Appendix B contains the pharmaceutical data for fillet samples; Appendix C contains the pharmaceutical data for liver samples; and Appendix D contains the personal care products data for fillet samples.

Section 3.3 presents the lipid results for the pilot study fish tissue samples. Table 13 in Section 3.3 provides a summary of the mean lipid content (% lipid) in the fillet and liver samples from each sampling location and the standard deviations that correspond to the mean lipid values. Appendix E contains a series of tables that provide the site-specific lipid data for the fillet and liver samples. All of the lipid data in Section 3.3 and Appendix E, along with the site-specific PPCP data in Appendices B, C, and D, are unique to this report. None of the site-specific data were published in Ramirez et al. (2009).

All of the data tables report the fish tissue results as wet-weight concentrations. These concentrations are expressed as the mass of the chemical per unit of fish tissue mass. The reporting unit for both the pharmaceutical method (HPLC-MS/MS) and the personal care product method (GC-MS/MS) is nanograms per gram (ng/g) or parts per billion (ppb).

The Code of Federal Regulations (CFR) provides a definition and description of the method detection limit (MDL) in 40 CFR, part 136, Appendix B. The MDL varies for different chemicals, matrices (e.g., water or tissue), and analytical methods. Tables 5 and 6 (Section 2.5.2) list method detection limits for each target pharmaceutical compound and personal care product chemical, respectively. The MDL is designed to provide a 99% level of confidence that when a chemical is reported as being present at the MDL level, it is really present. The opposite is not true, however. If a chemical is reported as not being present at the MDL level, there is a 50% possibility that the chemical is really present (i.e., the result is a false negative).

3.1 Chemicals Not Detected

3.1.1 Reference Site Results

One challenge that EPA faced in selecting sampling sites for the pilot study was identifying a reference site free from human influence with respect to the 36 chemicals of interest for the pilot study (Tables 2 and 3 in Section 2.1.2). Fishery biologists from the New Mexico Environment Department offered their technical expertise about southwest fisheries to assist EPA in selecting the reference site. They recommended a site on the East Fork of the Gila River that flows through the Gila Wilderness Area in southwest New Mexico. EPA followed their recommendation and it turned out to be an appropriate one. Results from the analysis of fillet and liver tissue from the six reference site fish composite samples showed that none of the 36 target PPCPs were detected in either type of fish tissue samples.

3.1.2 Pharmaceutical Compounds

Results for the 24 target pharmaceutical compounds in fillet tissue samples and 23 target pharmaceutical compounds in liver tissue samples revealed that the majority of these compounds did not occur in the fish tissue. Seventeen of the pharmaceutical compounds were not detected in any of the fillet or liver samples from fish composite samples collected at the five sites located on effluent-dominated rivers or streams. None of the six antibiotics (e.g., erythromycin) or the three analgesics (e.g., ibuprofen) occurred at detectable levels in the fish tissue samples. Three of the four pharmaceuticals used to treat high blood pressure (atenolol, metoprolol, and propranolol) were also not found in the fish tissue. Table 7 contains a complete list of the 17 pharmaceuticals not detected in any of the fish tissue samples.

Table 7. Pharmaceutical Chemicals Not Detected in Fillet or Liver Tissue.

Chemicals Not Detected	Use
Acetaminophen	Analgesic
Atenolol	Anti-hypertension
Caffeine	Stimulant
Cimetidine	Anti-acid reflux
Codeine	Analgesic
1,7-Dimethylxanthine	Antispasmodic
Erythromycin	Antibiotic
Ibuprofen	Analgesic
Lincomycin	Antibiotic
Metoprolol	Anti-hypertension
Miconazole	Antifungal
Propranolol	Anti-hypertension
Sulfamethoxazole	Antibiotic
Thiabendazole	Antibiotic
Trimethoprim	Antibiotic
Tylosin	Antibiotic
Warfarin	Anticoagulant

3.1.3 Personal Care Product Chemicals

Results for the 12 target chemicals commonly found in personal care products showed that 10 of these compounds did not occur in the fish tissue. The chemicals not detected in fillet samples included triclosan and *m*-toluamide, which are used widely in hand soaps and insect repellents, respectively. This group also included three chemicals that act as ultraviolet filters in sunscreens (benzophenone, octocrylene, and 4-methylbenzylidene camphor or 4-MBC). Table 8 provides a list of the 10 personal care product chemicals not detected in any of the

fillet samples. Liver tissue samples were not analyzed for any of the chemicals in personal care products as explained in Section 2.5.2.2.

Table 8. Personal Care Product Chemicals Not Detected in Fillet Tissue.

Chemicals Not Detected	Use
Benzophenone	UV filter
Celestolide (ADBI)	Fragrance/Musk
4-Methylbenzylidene Camphor (4-MBC)	UV filter
Musk Ketone	Fragrance/Musk
Musk Xylene	Fragrance/Musk
p-Nonylphenol	Surfactant
Octocrylene	UV filter
p-Octylphenol	Surfactant
m-Toluamide (DEET)	Insecticide
Triclosan	Antimicrobial

3.2 Detected PPCP Chemicals

This report summarizes the fillet and liver concentration data for PPCPs detected in the fish tissue composite samples using the same approach as Ramirez et al. (2009). The fillet concentration data in Tables 9 (pharmaceuticals) and 11 (personal care products) and the liver concentration data in Table 10 (pharmaceuticals) are based on six individual tissue composite sample results from each of the five sampling sites (i.e., 30 fillet composite samples and 30 liver composite samples). All values are expressed in nanograms per gram (ng/g) or parts per billion (ppb) mass of compound per mass of wet-weight fish tissue. In addition to the summary-level data, this report provides results for individual tissue samples at each site in Appendix B through Appendix D. Data from these appendices were used to generate the result summaries reported in Tables 9-11.

Tables 9 and 10 present the pharmaceutical results for the fillet and liver samples analyzed from each site. These data include frequency of occurrence, mean tissue concentration, and maximum tissue concentration for each detected pharmaceutical compound at every sampling location. The frequency of occurrence is a ratio identifying the number of fillet or liver samples with quantifiable detections for a specific chemical out of the six samples analyzed from each location. In Table 9, for example, the frequency reported under Chicago for diphenhydramine is 6/6, which indicates that this antihistamine was detected in all six fillet samples analyzed from Chicago. Another example is the frequency of 2/6 reported under Chicago for norfluoxetine (a metabolite or breakdown product of the commonly prescribed antidepressant fluoxetine), indicating that this chemical was detected in only one-third of the Chicago fillet samples. Note that frequencies greater than zero are highlighted in bold type. As described in Section 3.0, the mean tissue concentrations were calculated using only the values from detected concentrations. The two fillet concentrations measured above

the detection limit for norfluoxetine at Chicago were 3.19 ng/g and 3.21 ng/g (Table B-1, Appendix B). In Table 9, the mean concentration for these two detected values is 3.2 ng/g (or ppb). In this particular case, the maximum concentration reported to two significant figures is also 3.2 ng/g (or ppb). Tables B-1 (Chicago), B-2 (Dallas), B-3 (Orlando), B-4 (Phoenix), and B-5 (West Chester, PA) contain site-specific concentration data for the pharmaceutical compounds detected in individual fillet samples. These data were used to derive the summary results displayed in Table 9. Tables C-1 through C-5 in Appendix C provide the site-specific pharmaceutical data for individual liver samples that were used to derive the data presented in Table 10.

Table 11 provides summary-level concentration data for personal care product chemicals in fillet samples. There are only fillet tissue results for this group of chemicals, and they are reported in the same format described above for the pharmaceutical compounds. Tables D-1 through D-5 in Appendix D contain the site-specific data for personal care products detected in the individual fillet samples that were used to derive the data summarized in Table 11.

Table 9. Analytical Results for Pharmaceutical Compounds in Fillet Composite Samples.

Chemical	MDL (ng/g)	Chicago			Dallas			Orlando			Phoenix			West Chester		
		Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max
Carbamazepine	0.54	**6/6**	2.3	3.1	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*
Diltiazem	0.12	**5/6**	0.13	0.16	0/6	*	*	0/6	*	*	0/6	*	*	**3/6**	0.15	0.20
Diphenhydramine	0.05	**6/6**	1.4	1.7	0/6	*	*	0/6	*	*	**6/6**	1.2	1.4	**6/6**	1.7	2.5
Fluoxetine	6.7	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*
Gemfibrozil	6.7	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*
Norfluoxetine	2.9	**2/6**	3.2	3.2	0/6	*	*	0/6	*	*	**4/6**	4.0	4.8	**6/6**	3.9	5.0
Sertraline	3.6	0/6	*	*	0/6	*	*	0/6	*	*	**6/6**	5.0	6.5	**6/6**	11	19

Table 10. Analytical Results for Pharmaceutical Compounds in Liver Composite Samples.

Chemical	MDL (ng/g)	Chicago			Dallas			Orlando			Phoenix			West Chester		
		Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max
Carbamazepine	1.9	**6/6**	6.0	8.0	0/6	*	*	0/6	*	*	0/6	*	*	0/6	*	*
Diltiazem	0.26	**6/6**	0.71	0.90	0/6	*	*	0/6	*	*	**4/6**	0.32	0.44	**6/6**	0.69	0.76
Diphenhydramine	0.26	**6/6**	7.0	9.6	**5/6**	0.52	0.93	0/6	*	*	**6/6**	6.7	11	**6/6**	10	11
Fluoxetine	12	**3/6**	19	23	**2/6**	13	14	0/6	*	*	0/6	*	*	**6/6**	70	80
Gemfibrozil	25	0/6	*	*	0/6	*	*	0/6	*	*	**6/6**	70	90	**2/6**	27	27
Norfluoxetine	15	**6/6**	73	130	**4/6**	37	48	**5/6**	57	78	**5/6**	33	44	**6/6**	38	48
Sertraline	17	**6/6**	84	150	**4/6**	27	28	**1/6**	NA	21	**6/6**	71	110	**6/6**	380	550

Table 11. Analytical Results for Personal Care Product Chemicals in Fillet Composite Samples.

Chemical	MDL (ng/g)	Chicago			Dallas			Orlando			Phoenix			West Chester		
		Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max	Freq	Mean	Max
Galaxolide	12	**6/6**	1,300	1,800	**6/6**	840	1,800	**5/6**	110	290	**6/6**	1,800	2,100	**6/6**	1,800	2,000
Tonalide	13	**6/6**	150	230	**6/6**	72	150	**1/6**	NA	21	**6/6**	240	290	**6/6**	55	72

3.2.1 Pharmaceutical Results in Fillets

Results for pharmaceutical compounds show that five of the 24 target chemicals in this group were found in the fillet samples (Table 9). In order of decreasing detection frequency, they include:

- diphenhydramine (18 of 30 samples),
- norfluoxetine and sertraline (12 of 30 samples for each antidepressant),
- diltiazem (8 of 30 samples), and
- carbamazepine (6 of 30 samples).

There are notable differences in the frequency of detections among sites. For example, none of the pharmaceuticals were detected in fillet samples from Dallas and Orlando. The wastewater treatment plants (WWTPs) discharging to streams near the sampling locations in both cities employ advanced treatment technologies that may provide more effective removal of pharmaceuticals from the waste stream. Diphenhydramine occurred in every fillet sample from the remaining three sites (Chicago, Phoenix, and West Chester near Philadelphia). These data suggest widespread discharge of this active ingredient in over-the-counter cold medications into surface waters. In contrast, carbamazepine occurred in all the fillet samples at a single site (Chicago). On a site-specific basis, the largest number of pharmaceutical detections occurred in the fillet samples from West Chester (21 of 30 possible detections), followed by Chicago (19) and Phoenix (16).

All of the fillet concentrations for the detected pharmaceutical compounds were measured in the low parts per billion (ppb) range. None of the concentrations exceeded 20 ng/g (or ppb), and the majority of these concentrations were below 5 ng/g. The highest fillet concentration reported for any pharmaceutical compound was 19 ng/g. This was the level of sertraline (antidepressant) measured in a fillet sample from West Chester, PA. Sertraline was only detected in the fillet samples from West Chester and Phoenix (all six samples from each site). The mean concentration of sertraline in the West Chester samples (11 ng/g) was greater than twice the mean sertraline concentration in the Phoenix samples (5 ng/g).

3.2.2 Pharmaceutical Results in Livers

Seven of the 23 pharmaceutical compounds were detected in the liver samples. These compounds include fluoxetine (an antidepressant) and gemfibrozil (a drug used to treat high cholesterol), in addition to the five pharmaceuticals found in the fillet samples (i.e., carbamazepine, diltiazem, diphenhydramine, norfluoxetine, and sertraline). Pharmaceutical compounds were detected more frequently in the liver samples than in the fillet samples. Norfluoxetine and sertraline, two of the antidepressants, occurred in liver samples from all five sampling sites. Their detection frequencies were 26 and 23 per 30 samples, respectively. Diphenhydramine was detected in the liver samples with the same frequency as sertraline (23 detections in the 30 samples). Differences in detection frequencies for pharmaceuticals in the liver samples are apparent among the sampling sites. Consistent with the fillet results, carbamazepine occurred in only the liver samples from Chicago. The fewest number of

detections occurred at Orlando and Dallas, two sites where WWTPs apply advanced treatment technologies before discharging effluents into the streams. Site-specific data show that six of the seven pharmaceuticals found in liver samples were detected in the fish liver samples from Chicago and West Chester. Gemfibrozil was not detected in Chicago liver samples and carbamazapine was not detected in West Chester liver samples.

Compared to the fillet concentrations, the concentrations of pharmaceutical compounds measured in fish liver samples were greater. Differences among their mean concentrations ranged from a factor of nearly three to more than 20. All of the diltiazem concentrations in the liver samples were less than 1 ng/g (1 ppb). Sertraline had the highest concentrations reported for pharmaceuticals in liver samples, which included maximum concentrations of 100 ng/g at Phoenix, 150 ng/g at Chicago, and 550 ng/g at West Chester. The levels of sertraline in the liver samples from West Chester and Phoenix may be anomalous based on recovery data for quality control samples analyzed from these locations (i.e., matrix spike samples) that exceeded the defined acceptability limits.

Matrix spike samples for liver tissue from West Chester exceeded the acceptable limit for sertraline recovery by a factor of about three (acceptable limit for percent recovery of 150% and percent recovery of 473%), while the recovery of sertraline from Phoenix liver tissue was 172% compared to the acceptable limit of 150%. Table 12 provides the matrix spike recovery data for the four pharmaceutical compounds detected in liver tissue with recoveries that exceeded the acceptable limit (fluoxetine, gemfibrozil, norfluoxetine, and sertraline). Note that the recovery data for liver samples from Chicago and the reference site did fall within the acceptable limits, so the liver results for Chicago and reference site samples are not affected by the matrix spike recovery issue. However, the matrix spike recovery data may be a confounding factor for the liver results reported for these four pharmaceuticals at Dallas, Orlando, Phoenix, and West Chester.

Table 12. Matrix Spike Recovery Data for Four Pharmaceutical Compounds Detected in Liver Composite Samples that Exceeded Acceptable Limits[a].

Chemical	Matrix Spike Recovery Percentage					
	Chicago	Dallas	Orlando	Phoenix	West Chester	Reference (New Mexico)
Fluoxetine	144	335	349	271	362	105
Norfluoxetine	92	398	350	197	247	115
Sertraline	96	584	407	172	473	120
Gemfibrozil	172	166	527	246	218	106

[a] The acceptable range was from 60% to 150%.

3.2.3 Results for Personal Care Product Chemicals in Fillets

Concentration data for personal care product (PCP) chemicals in fish tissue are available only for fillet samples. In applying their PCP method to liver samples, Baylor University encountered problems with interferences due to the high lipid content of the liver tissue

(Section 2.5.2.2). PCP results show that two of the 12 target chemicals occurred in the fillet samples. These chemicals include galaxolide and tonalide, which are both fragrances added to common products like cosmetics and detergents. Both chemicals were detected in the fillet samples at all five sites. Galaxolide was detected more frequently in the fillet samples than any of the pharmaceutical compounds were detected in either the fillet or liver samples. It occurred in 29 of the 30 fillet samples. Tonalide was detected in 26 of the 30 fillet samples, a detection frequency that matched the occurrence of norfluoxetine in liver samples. Site-specific data reveal that galaxolide and tonalide occurred in every fillet sample from Chicago, Dallas, Phoenix, and West Chester. Orlando fillet samples had the lowest frequencies of detection for the PCP chemicals with five detections for galaxolide and one for tonalide.

Fillet concentrations of galaxolide occurred in the low parts per million range at a majority of the sites. The mean concentrations of galaxolide exceeded 1,000 ng/g or 1 part per million (ppm) in fillet samples from Chicago, Phoenix, and West Chester (1,300 ng/g for Chicago samples and 1,800 ng/g for Phoenix and West Chester samples). The maximum concentrations of galaxolide ranged from 1,800 ng/g to 2,100 ng/g (1.8 ppm to 2.1 ppm) in fillet samples from Chicago, Dallas, Phoenix, and West Chester. Orlando fillet samples contained the lowest concentrations of galaxolide with a mean concentration of 100 ng/g (0.1 ppm) and a maximum concentration of 290 ng/g (0.29 ppm). Tonalide concentrations in the fillet samples were about an order of magnitude lower than the galaxolide concentrations. The mean tonalide concentrations in the fillet samples from the five sites ranged from 55 ng/g to 240 ng/g, and the maximum tonalide concentration of 290 ng/g was measured in a fillet sample from Phoenix.

3.3 Lipid Results

Fish sampling efforts resulted in the collection of different species at each site since field teams found that the resident species differed from site to site. Table 13 lists the fish species collected at each sampling location, along with the mean percentage of lipids measured in the fillet and liver tissue samples for the individual species. The lipid content of both the fillet and liver samples varied widely among the six species analyzed for this study. The mean lipid measurements ranged from 1.0% to 4.9% in the fillet samples and from 2.2% to 11.6% in the liver samples. Sonora sucker fillet samples from the reference site in New Mexico had the highest mean percentage of lipids in the fillet tissue, while the common carp samples from Phoenix contained the highest mean percentage of lipids in liver tissue. Appendix E contains tables that provide the site-specific lipid data used to summarize the data in Table 13.

Lipid concentrations in fish are often closely correlated to the accumulation of chemicals in their tissue. Groups of non-polar organic chemicals, such as pesticides, polychlorinated biphenyls (PCBs), and dioxins and furans, tend to accumulate in lipid-rich tissues, particularly in the livers. For this study, Ramirez et al. (2009) examined the relationship between lipid content and accumulation of PPCPs in fish tissue by plotting percent lipid versus

chemical concentration for all six composite samples from each sampling site. The plots indicated that there was no relationship between these two variables for any of the pharmaceutical compounds detected in the fish tissue. In contrast, the two PCP chemicals detected in fillet samples, galaxolide and tonalide, showed significant positive correlations between lipid content and chemical concentrations in the fillet samples at Orlando and Dallas for galaxolide and at Chicago and Dallas for tonalide.

Table 13. Lipid Percentage in Fish Fillet and Liver Composite Samples.

Location	Species	% Lipid in Fillets		% Lipid in Liver	
		Mean	SD	Mean	SD
Chicago	Largemouth bass	2.3	0.6	2.2	0.4
Dallas	Smallmouth buffalo	2.2	1.1	8.1	2.7
Orlando	Bowfin	1.0	0.7	2.9	1.6
Phoenix	Common carp	3.9	0.8	11.6	2.1
West Chester	White sucker	1.9	0.4	4.7	0.9
Reference (NM)	Sonora sucker	4.9	1.6	4.9	2.5

4.0 Conclusions and Future Research

To advance the science related to detecting PPCPs in the environment, EPA teamed with research scientists at Tetra Tech, Inc. and Baylor University to conduct the first national pilot study for investigating the occurrence of PPCPs in fish tissue. Since the majority of human pharmaceuticals and personal care product chemicals enter surface waters from point-source release of WWTP effluents (Daughton and Ternes 1999), EPA obtained fish for the study from locations on effluent-dominated streams near WWTP discharges at five sites across the country. Chemists at Baylor University applied methods they developed to analyze fillet and liver samples for up to 24 pharmaceuticals (Ramirez et al. 2007) and fillet samples for 12 chemicals commonly used in personal care products (Mottaleb et al. 2009). Of the 24 pharmaceuticals and 12 PCPs analyzed for this study, 17 of the pharmaceuticals (71%) and 10 of the PCPs (83%) were not detected in the fish tissue samples even though EPA targeted sampling locations that potentially represented worst-case scenarios for studying occurrence of PPCPs. However, some of the PPCPs that occurred in the fish tissue samples were widely distributed among the study sites, including the following compounds: the antihistamine, diphenhydramine; the antidepressant, sertraline; and the musk fragrances, galaxolide and tonalide.

This final section of the report provides information that relates pilot study results to results from earlier studies of PPCPs in fish tissue, summarizes significant findings from the pilot study, and describes work that EPA is pursuing to expand investigations of the occurrence of contaminants of emerging concern (CECs) in fish tissue. Ramirez et al. (2009) contains a detailed discussion of the pilot study results, and this report summarizes highlights of that discussion in Section 4.1. Section 4.2 presents the pilot study conclusions and Section 4.3 describes further research that EPA is conducting on CECs in fish tissue.

4.1 Discussion of Pilot Study Results

An important topic of discussion in Ramirez et al. (2009) is the greater occurrence of PPCPs in effluent-dominated rivers and streams compared to other aquatic environments based on results from this study and from previous research. WWTPs releasing effluents into rivers and streams continually introduce PPCPs not removed by plant treatment processes into these aquatic systems. Fish living in the vicinity of these point-source discharges over their entire life cycle encounter exposures to constantly replenished concentrations of PPCPs that can be characterized as pseudopersistent exposures. Given this continuous exposure scenario, PPCPs can accumulate in fish tissue as demonstrated by the high concentrations of galaxolide measured in the fillet samples analyzed for the pilot study.

The core discussion in Ramirez et al. (2009) compares results of the pilot study to results from previous studies of PPCPs in fish tissue. This comparison emphasizes earlier work

conducted by Baylor University on fish tissue samples collected from Pecan Creek (an effluent-dominated stream in the Denton area of north central Texas) and analyzed for PPCPs (Brooks et al. 2005). Pharmaceutical results from these studies show detections for the same pharmaceutical compounds as the pilot study (i.e., carbamazepine, diltiazem, diphenhydramine, fluoxetine, norfluoxetine, and sertraline) except for gemfibrozil. In addition, the concentration ranges measured for the pharmaceuticals in the fish tissue samples are similar between the Pecan Creek and national pilot studies. Chemists at Baylor University also detected and quantified the same PCPs in fillet samples from Pecan Creek that occurred in the pilot study fillet samples, i.e., galaxolide and tonalide, although at slightly lower concentrations. Overall, there is good agreement between the Brooks et al. (2005) research and the pilot study results for the number and types of PPCPs detected in fish tissue and for the range of concentrations measured in the tissue. The discussion of pilot study results in Ramirez et al. (2009) includes data cited from Canadian, Danish, German, and Swiss studies of PPCPs in fish tissue. These data comparisons demonstrate similarities in the specific PPCPs detected and in the concentrations of those compounds.

Fillet concentrations of musk compounds detected in fish tissue from this study, specifically galaxolide, are up to an order of magnitude higher than whole-body concentrations detected in recent studies (Osemwengi and Gerstenberger 2004). Given the potential for both ecological and human health effects of galaxolide (Luckenbach and Epel 2005), its detection in fish tissue at the ppm-level may be of concern.

4.2 Pilot Study Conclusions

Data from EPA's National Pilot Study of PPCPs in Fish Tissue support conclusions from earlier studies and offer new insights, particularly with respect to the benefits of advanced wastewater treatment technologies on the occurrence of PPCPs in fish tissue. Conclusions for this study derive primarily from differences in PPCP detections and concentrations related to type of tissue, geographic location, and level of treatment technologies applied in WWTPs before discharge of effluents into rivers or streams. They include the following:

- At all five of the sampling locations, pharmaceutical compounds occurred in greater numbers and at higher detection frequencies and concentrations in liver samples than in fillet samples. No significant relationships were observed between lipid content and accumulation of pharmaceuticals in either fillet or liver tissue. Ramirez et al. (2009) noted that these differences are consistent with the liver being the primary site of metabolism of xenobiotics (compounds that are foreign to an organism, such as drugs and environmental pollutants) in fish.
- Differences in wastewater treatment technologies can substantially affect the removal efficiency of pharmaceutical compounds from wastewater discharges. By design, the pilot study included sampling locations near discharges from WWTPs that apply various levels and types of wastewater treatment processes. Fewer detections at lower frequencies and concentrations occurred at sites with more advanced wastewater treatment (Dallas, TX and Orlando, FL) than those with lower levels of wastewater

treatment (Chicago, IL and West Chester, PA). The advanced wastewater treatment at the Dallas WWTP involves ozonation, while the process line at the Orlando WWTP diverts nearly half of the daily load of treated wastewater through a constructed wetland to significantly reduce the amount of wastewater directly discharged into the adjacent river. In contrast, the wastewater entering the Chicago and West Chester WWTPs receives a less advanced level of treatment (secondary treatment) before being discharged from the plants.

- Results from this study do not provide evidence that factors such as percentage of population age 65 and older or median income can serve as reliable indicators of areas where human pharmaceuticals are more likely to occur and accumulate in fish tissue. Based on EPA's evaluation of data from a limited number of sites, it appears that the wastewater treatment technologies applied in local WWTPs is a better predictor of pharmaceutical occurrence. However, demographics of local populations can influence geographic differences in detections of pharmaceutical compounds in fish tissue. For example, carbamazepine (an anti-seizure drug) was only detected in fillet and liver samples from Chicago.
- The widespread occurrence of norfluoxetine in fish tissue samples analyzed for this study provides further evidence of the importance of including metabolic products of target chemicals in future studies. Norfluoxetine is a metabolite of the antidepressant fluoxetine that has been identified in previous studies (Brooks et al. 2005). Koplin et al. (2002) documented the importance of obtaining data on metabolites for analysis of PPCPs in water, and results from EPA's PPCP Fish Pilot Study demonstrate the importance of targeting metabolites in fish tissue. It will be critical for future research efforts to identify other metabolites that are biologically active and persistent in fish. However, future analysis of these metabolic products may be limited by the availability of pure standards and the performance of the analytical method.

4.3 Future Research

Obtaining environmental data on CECs continues to be an area of interest for EPA. Since completing the PPCP Fish Pilot Study, EPA has initiated a national study of CECs in urban rivers and a regional assessment of CECs in the Great Lakes. The designs for both studies involve analysis of perfluorinated compounds (PFCs, including PFOA and PFOS) in fish fillet samples. Sections 4.3.1 and 4.3.2 provide summaries of the design and status for the urban river and Great Lakes studies, respectively.

4.3.1 National Urban River CEC Study

Results from recent studies (e.g., Barceló and Petrovic 2007, and Lau et al. 2007) prompted EPA to explore options to expand investigation of CECs in the environment to a national scale. In 2008, an opportunity to conduct a nationally representative study of CECs in surface water and fish tissue samples became available under EPA's National Rivers and Streams Assessment (NRSA). The NRSA is a probability-based study that involved collection of physical, chemical, and biological indicator data at approximately 1800 randomly

selected sites (across all stream orders) in the lower 48 states. Data from the NRSA will allow a statistically valid characterization of the condition of rivers and streams throughout the country.

The statistical design of the NRSA included a representative subset of 163 urban river sampling locations. EPA planned and is currently implementing a special study of CECs at these urban river sites called the National Urban River CEC Study. This is EPA's first broad assessment of CECs on a national level using a statistically based sampling design. The urban river study includes the following components:

- Sampling at 163 randomly selected urban river sites (5th order or larger) throughout the lower 48 states;
- Collecting one surface water grab sample and one fish composite sample (five similarly-sized adult fish of a single species that is commonly consumed by humans) from each site;
- Analyzing the surface water samples for 54 pharmaceutical compounds; and
- Analyzing fish fillet composite samples for 13 PFCs, and 6 musks (including galaxolide and tonalide).

Two EPA laboratories and one commercial laboratory are analyzing the urban river fish and water samples for CECs. EPA's National Exposure Research Laboratories in Cincinnati, OH and Las Vegas, NV analyzed surface water samples for pharmaceuticals and fish fillet samples for musks, respectively. AXYS Analytical in Sydney, British Columbia analyzed fillet samples for PFCs. The CEC assessments are in various stages of data review and analyses. EPA anticipates reporting CEC results in 2013.

4.3.2 Great Lakes Human Health Fish Tissue Study

EPA identified an opportunity to assess CECs in fish on a regional scale during planning for its 2010 National Coastal Condition Assessment (NCCA). EPA's field effort for the NCCA during 2010 consisted of collecting physical, chemical, and biological data from about 680 randomly selected marine sites along the coasts of the United States and from a representative set of 225 nearshore sites throughout the Great Lakes. EPA will use results from the NCCA to characterize the condition of the Nation's coastal and Great Lakes waters.

EPA's Office of Water, Great Lakes National Program Office, and Office of Research and Development combined resources and expertise to conduct the first statistically based assessment of Great Lakes fish contamination relevant to human health under the NCCA. The team initiated the Great Lakes Human Health Fish Tissue Study to collect and analyze fish samples for a number of contaminants, including mercury, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and PFCs. This assessment also includes analysis of fillet tissue for fatty acids to obtain species-specific data on compounds that may offer health benefits. Field crews collected fish samples from a representative subset of 157 Great Lakes sites (about 30 sites per lake) in the nearshore regions (up to 30 meters deep or

5 kilometers from shore). The Baltimore, MD Division of Microbac Laboratories prepared the fish samples for analysis (i.e., filleting and grinding the tissue). The following laboratories are analyzing Great Lakes fillet samples: Brooks Rand Labs in Seattle, WA for mercury; AXYS Analytical in Sydney, British Columbia for 209 PCB congeners; ALS Canada in Burlington, Ontario for 52 PBDEs; and TestAmerica Laboratories in West Sacramento, CA for 13 PFCs. Southwest Research Institute in San Antonio, TX is analyzing the tissue samples for 5 fatty acids. Results for the target chemicals will be available in 2013.

EPA will evaluate results from the National Urban River CEC Study and the Great Lakes Human Health Fish Tissue Study to determine future directions for assessment of CECs in fish tissue. EPA is archiving tissue from both studies to provide more cost-effective alternatives for assessing accumulation of new CECs in fish tissue. Forming creative partnerships with other public or private entities will also be critical for continuing agency efforts to monitor levels of CECs in fish.

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Appendix A

Analytical Procedures

Analysis of Pharmaceuticals by HPLC-MS/MS

Following homogenization, fillet and liver tissue samples were subsequently extracted and analyzed for 24 and 23 pharmaceutical compounds, respectively, by high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) using methods described in Ramirez et al. (2007). The method utilizes matrix-matched calibration standards (aliquots of control matrix from outside of the study area that are expected to be reasonably free of target compounds) spiked at a minimum of five concentrations, and extracted and analyzed along with study samples. By extracting standards, matrix effects and bias were minimized in the final analytical results.

Sample Extraction for Pharmaceuticals Analysis

Preparation of homogenates for analysis involved the following steps:

1) weighing out 1.0 gram (g) of fillet tissue and 0.5 g of liver tissue for each standard or sample composite individually to the nearest 0.01 g and placing each tissue aliquot into a 20-mL borosilicate glass screw-cap vial,
2) spiking with the appropriate surrogates (acetaminophen-d_4, diphenhydramine-d_3, carbamazepine-d_{10}, and ibuprofen-$^{13}C_3$) and standard mixtures (full target list spikes for calibration standards, control and matrix spike [MS/MSD] samples), as appropriate to complete the required batch quality control (method blank, low and high level control samples, and a pair of spiked MS/MSD samples from each site) in each analytical batch of 20 or fewer samples,
3) combining sample homogenates with 8 milliliters (mL) of 1:1 mixture of 0.1 M acetic acid buffer (pH 4) and methanol extraction solvent, and tightly replacing the cap,
4) sonicating the homogenate mixture in an ultrasonic bath for 15 minutes at 25° C,
5) shaking the mixture vigorously for 20 seconds to further ensure mixing and extraction,
6) quantitatively transferring each extract to a separate 50 mL polypropylene copolymer round-bottomed centrifuge tube with several rinses of the extraction solvent,
7) centrifuging the extracts at 16,000 rpm for 40 minutes at 4° C to achieve a full separation of residual solid and liquid phases,
8) transferring the supernatant into a clean 18 mL disposable borosilicate glass culture tube with rinses using disposable Pasteur pipettes,
9) evaporating the sample extracts to dryness under a stream of dry nitrogen at 45° C,
10) reconstituting the extracts in 1 mL of mobile phase 0.1% formic acid in reagent water,
11) adding internal standards (7-aminoflunitrazapam-d_7, fluoxetine-d_6, and meclofenamic acid),
12) sonicating for 1 min at 25° C,
13) filtering extracts using a Teflon membrane syringe filter into an amber HPLC injection vial, and
14) sealing the vial with a fluoropolymer lined cap.

Instrumental Analysis of Pharmaceuticals by HPLC-MS/MS

Pharmaceutical compounds were determined using analytical methods described in Ramirez et al. (2007). Calibration standard extracts (7 concentrations for fillet and 8 concentrations for liver tissue calibration) and sample extracts were analyzed on a Varian HPLC equipped with a binary pump and auto sampler connected to a 12.5 mm by 2.1 mm (inside diameter) C18 guard column, which preceded a 15 cm by 2.1 mm (inside diameter) C18 chromatographic column. Chromatographic separations were completed under a binary gradient consisting of 0.1% (v/v) formic acid in water and 100% methanol. Additional chromatographic parameters included:

- 10 microliter (µL) injection volume,
- 30°C column temperature, and
- 350 µL/min mobile phase flow rate.

Eluted analytes were monitored by MS/MS using a Varian triple-quadrupole mass analyzer equipped with an electrospray interface (ESI).

Target analytes were introduced individually into the mass spectrometer in both positive and negative ionization modes to determine the best ionization mode for analysis and to identify the most intense precursor ions for each target analyte. Once these variables were isolated, the energy at the collision cell was adjusted, while the third quadrupole was scanned to identify and optimize the intensity of a product ion for each compound. Precursor and product ions were identified for each target analyte. Additional instrumental parameters held constant for all analytes were as follows:

- nebulizing gas — nitrogen (N_2) at 60 pounds per square inch (psi),
- drying gas — N_2 at 19 psi,
- temperature —300 °C,
- needle voltage — 5000 V ESI+, 4500 V ESI-,
- declustering potential — 40 V, and
- collision gas — argon at 2.0 mTorr.

Sample results were calculated using the instrument software against a linear internal standard calibration curve for each tissue type. No additional multi-point calibration was required after the initial calibration curve analysis (i.e., analysis of a single calibration curve for fillets and a single calibration curve for livers). Analytical performance was verified through analysis of continuing calibration standards or daily standard calibration verification.

Analysis of Personal Care Products by GC-MS/MS

Following sample homogenization, fillet tissue samples were subsequently extracted and analyzed for 12 personal care product (PCP) chemicals by gas chromatography with tandem mass spectrometry (GC-MS/MS) using methods described by Mottaleb et al. (2009). Unlike the pharmaceutical method, matrix-matched calibration standards were not utilized in the PCP analytical method since matrix interferences were not as prevalent following the rigorous clean-ups used in the sample extraction procedures. Standards for the PCP analysis were prepared in solvent and subjected to derivatization to enhance measurement response, but were not subjected to the full preparation procedures used for study samples.

Sample Extraction for Personal Care Products Analysis

Preparation of homogenates for analysis involved the following steps:

1) weighing out 1.0 g of fillet tissue for each sample composite and 1.0 g of control matrix for each QC sample individually to the nearest 0.01 g and placing each tissue aliquot into a 20-mL borosilicate glass screw-cap vial,
2) spiking all samples with the appropriate surrogates (benzophenone-d_{10} and $^{13}C_6$ *p*-nonylphenol) and spiking QC samples (control and MS/MSD samples) with standard mixtures containing all target analytes, as appropriate, to complete the required batch quality control (method blank, low and high level control samples, and a pair of MS/MSD samples from each site) in each analytical batch of 20 samples or fewer,
3) adding 10 mL of acetone to each spiked homogenate aliquot,
4) sonicating samples for 15 min at 25° C,
5) shaking vigorously on removal for 20 seconds to further ensure mixing and extraction,
6) transferring samples into 50-mL polypropylene copolymer round-bottomed centrifuge tubes using 1 mL acetone as a rinse,
7) centrifuging at 16,000 rpm for 40 min at 4° C,
8) transferring the supernatant into 18-mL disposable glass test tubes,
9) evaporating the solvent to dryness under a stream of nitrogen at 30° C,
10) reconstituting the samples in 200 µL of 65:35 (v/v) hexane:acetone in preparation for silica gel clean-up,
11) loading sample extracts onto a preconditioned (8 mL of 65:35 hexane:acetone by volume) silica gel column (1 g), and eluting with 30 mL of hexane:acetone,
12) evaporating the resultant extract to near-dryness and reconstituting in 700 µL of methylene chloride to allow a solvent exchange in preparation for gel permeation chromatography (GPC) clean-up,

13) injecting one half (350 µL) of the extract into the GPC to separate the target analytes from co-extracted interferences by 5 mL/min elution through a cross-linked styrene divinylbenzene copolymer guard (30 mm×4.6 mm) and analytical (150 mm×19 mm) columns connected in series,
14) discharging co-extracted interferences to waste and collecting the fraction eluting between 11.4 and approximately 19.4 minutes,
15) concentrating the methylene chloride extract to near dryness and reconstituting it to approximately 200 µL in hexane:acetone,
16) adding 100 µL of MSTFA derivatizing agent, capping the GC vial, and heating the mixture in an oven at 60° C for 45 min,
17) concentrating the derivatized extract one final time to near dryness at room temperature under a stream of nitrogen, then reconstituting it in 180 µL of *n*-hexane, and
18) spiking the extracts prior to analysis with 20 µL of the internal standards (phenanthrene-d_{10} and mirex) as described by Mottaleb et al. (2009).

Instrumental Analysis of Personal Care Products by GC-MS/MS

Sample extracts were analyzed on a Varian GC system equipped with an autosampler and ion trap mass spectrometer. Analytes were separated using a 30 mm by 0.25 mm, 0.25 µm VF-5 MS capillary column. A temperature program, starting at 100° C and ramping up to 290° C, allowed separations in approximately 25 minutes with additional bake-out and equilibration resulting in approximately 50 minutes between injections. Helium was used as the carrier gas at a constant flow rate of 1 mL/min (linear velocity 37.2 cm/s). Injections of 1.0 µL were made using splitless mode and an injection port temperature of 275° C. The transfer line between the GC and the mass spectrometer was kept at 280° C.

Sample results were calculated using the available instrument software against a linear internal standard calibration curve. A total of two calibration curves were required to complete the analysis of samples from all 6 sites (i.e., once derivatized, standards exhibited a degraded response, and a second set of calibration curve and continuing calibration verification standards were required to complete the sample analyses). Initial daily calibration verification standards and frequent calibration verifications were distributed throughout the analytical sequences to minimize reanalysis required due to the cumulative effects of co-extractables (in excess of those removed during preparatory chromatography), which degraded chromatographic performance.

Analysis of Lipids

Three replicate tissue aliquots (approximately 2 g) from each fillet composite were analyzed for lipids using the procedure described in Mottaleb et al. (2009). This procedure was modified slightly for liver specimens. Due to limited sample mass, triplicate measurements were made for only one liver composite from each sampling site except Phoenix, for which a duplicate was analyzed (Appendix E). All other lipid determinations for liver samples were based on a single measurement.

Lipid analysis involved the following steps:

1) combining 2 g of tissue with 15 mL of a 1:1 mixture of dichloromethane:hexane in a borosilicate vial,
2) homogenizing each mixture for 3 minutes using a motor-driven tissue homogenizer,
3) placing the vials in an incubator for 24 hours at 35°C and periodically agitating by gentle end-over-end rotation,
4) adding 2 g of solid anhydrous sodium sulfate for each 1 g of sample (g Na_2SO_4 = 2 × g tissue) following extraction,
5) filtering the mixture through Grade 415 filter paper,
6) washing the solid residue with an additional 15 mL of 1:1 dichloromethane:hexane,
7) collecting the combined filtrate for each sample in a pre-weighed test tube,
8) evaporating the solvent with dry nitrogen for 8 hours at 45° C using an evaporator concentration workstation, and
9) drying each residue after evaporation to a constant weight in a vacuum oven at 40° C.

Lipid content was determined gravimetrically by weighing the three replicates from each sample. Percent lipid determinations were then calculated as shown in the following example, where t.t = test tube:

SAMPLE WEIGHT (g)	t.t WEIGHT (g)	t.t+LIPID WEIGHT (g)	LIPID W (g)	LIPID %
2.1630	9.6768	9.7060	0.0292	1.35

$$\begin{aligned} \text{Lipid weight} &= ((\text{t.t} + \text{lipid}) \text{ weight}) - \text{t.t weight} \\ &= (9.7060 - 9.6768)\text{g} \\ &= 0.0292\text{g} \end{aligned}$$

$$\begin{aligned} \text{Lipid \%} &= \frac{\text{Lipid weight}}{\text{Sample weight}} \times 100 \\ &= \frac{0.0292\text{g} \times 100}{2.1630\text{g}} \\ &= 1.35\% \end{aligned}$$

Quantifying Target Chemicals

The pharmaceutical method used matrix-matched (to the extent possible) calibration standards, and both instrumental methods used internal standard calibration and quantitation equations. Internal standard calibration required the determination of relative response factors (RRF) defined by the following equation:

$$RRF = \frac{R_x}{R_{is}} \times \frac{A_{is}}{A_x}$$

Where:

R_{is} = the area response of the quant ion m/z for the internal standard,

R_x = the area response of the quant ion m/z for the target chemical,

A_x = the amount (concentration in ng/g) of the target compound, and

A_{is} = the amount (concentration in ng/g) of the internal standard.

The quantitation ion (quant ion) is the primary ion used in the calculation of calibration curve coefficients (or linearity) and sample analysis. Generally speaking, quantitation ions are the most responsive, as they are compared to other spectral data to complete the qualitative confirmation both by the instrument data system software and by the analytical chemist conducting the measurements. At the onset of analysis, multiple quant ions may be selected in order to afford the analyst an opportunity to select the one least impacted by the sample matrix, if necessary.

Calibration data were evaluated through assessment of the relative standard deviation (RSD) of the RRFs in the calibration curve using the following equations:

$$RSD = \frac{s}{\bar{\chi}}$$

where s is the standard deviation, $\bar{\chi}$ is the mean RRF over the standard curve, and the standard deviation of the curve is calculated as follows:

$$s = \sqrt{\frac{\sum_{i=1}^{n} \left(\chi_i - \bar{\chi}\right)^2}{n-1}}$$

where χ_i is the measured value of the replicate, $\bar{\chi}$ is the mean of the measured values, and n is the number of replicates.

Both analytical methods included an optional relative standard deviation criterion of less than 30% to demonstrate linearity across the calibration range. If the calibration curve produced an evaluation of ≤ 30% RSD, the average RRF could be used to calculate sample results in subsequent analyses. Alternatively, a curve can be plotted and used to calculate sample results, provided the correlation coefficient (R^2) is greater than 0.995. The latter quantitation technique was used for sample analysis, as it is a more commonly used feature in the instrument's data handling capabilities and it gave the analyst the ability to assess data in real time.

Linear regression calibration curve output reports provided slope values for each target chemical, and sample results were calculated as in the following example for a control sample copied from one of the study reports. A requirement for all laboratory deliverables was to include an example calculation verifying the data system results for a control sample (the only samples where analytes are certain to be found).

General Quantitation Report

Data File: c:\varianws\data\tetra tech\2-21-2008\REF-CCV_F.SMS **Acquisition Date:** 2/21/2008 11:35
Comment: M3
SampleID: REF-CCV_F **Analyst:** AM

Calibration File: C:\VarianWS\data\Tetra Tech\2-05-2008\rerun cal levels (L1-L7)\L7_.SMS
Cal. Sample Dates: First: 2/5/2008 17:49 **Last:** 2/5/2008 22:41

Compound	R. T.	Scan#	Q Ion(s)	Area	Conc	Units	Match	RF
7)* Phenanthane-d10	10.40	1094	160.3	390415	40.00	ng/g	995	1.000
~~19)* Mirex~~	~~24.23~~	~~3776~~	~~237.0~~	~~2345~~	~~200.00~~	~~ng/g~~	~~999~~	~~1.000~~
1) m-Toluamide	7.14	481	145.0	13489	157.40	ng/g	999	0.009
3) Benzophenone	7.90	623	153.0	66833	299.92	ng/g	999	0.023
4) Celestolide	8.83	798	172.9	16490	219.69	ng/g	999	0.008
~~5) PCNB (surro)~~	~~9.72~~	~~967~~	~~262.9~~	~~8372~~	~~360.38~~	~~ng/g~~	~~999~~	~~0.002~~
8) Octylphenol	10.72	1153	178.9	21197	72.48	ng/g	100	0.030
9) Galaxolide	11.50	1303	213.1	49746	460.13	ng/g	975	0.011
10) Musk Xylene	11.65	1332	265.0	24349	4529.73	ng/g	991	0.001
11) Tonalide	11.69	1341	187.0	27683	509.39	ng/g	990	0.006
13) Nonylphenol	13.06	1615	178.9	37592	127.20	ng/g	100	0.030
~~14) 2,2-DinitroBP~~	~~16.28~~	~~2223~~	~~196.1~~	~~0~~	~~0.00~~	~~ng/g~~	~~912~~	~~0.000~~
15) 4MBC	16.41	2245	169.1	77836	2397.15	ng/g	945	0.003
16) Musk Ketone	16.47	2258	287.1	23502	8694.87	ng/g	925	0.000
17) Triclosan	17.30	2427	200.0	56898	212.40	ng/g	983	0.027
~~18) NPMOE~~	~~19.06~~	~~2780~~	~~292.1~~	~~0~~	~~0.00~~	~~ng/g~~	~~689~~	~~0.000~~
20) Octocrylene	25.02	3926	248.2	153167	320.26	ng/g	788	0.049

* Indicates Internal Standard.

Example calculation:

*Concentration of target chemical = (area of Target * IS concentration) / (area of the IS * slope of target)*

*m-toluamide concentration = (13489 * 40) / (390415 * 0.0088) = 539560 / 3435.65 = 157 ng/g*

The quantitation reports were consulted by the analyst to access data which were near or above established target detection limits, and that these data were used to further investigate spectral data for product ions and relative responses between the two to complete qualitative identification of results. While the "Match" column was a useful tool in identifying poor spectral matches, analyst judgment remained integral to analysis and interpretation of results. Initially, all sample data that provided spectral confirmation were tabulated with appropriate qualification indicating the results were estimates reported below the calibration range, or that the results were below the calculated method detection limit. Results were later more closely scrutinized and the decision was made to exclude only results below the method detection limit and report them as <MDL.

Appendix B

Site-specific Analytical Results Tables for Pharmaceuticals in Fillet Tissue

Table B-1. Analytical Results for Pharmaceuticals in Fish Fillet Samples from Chicago, Illinois.

Chemical	MDL ng/g (ppb)	Chicago 1	Chicago 2	Chicago 3	Chicago 4	Chicago 5	Chicago 6
Acetaminophen	4.40	*	*	*	*	*	*
Atenolol	1.48	*	*	*	*	*	*
Caffeine	3.93	*	*	*	*	*	*
Carbamazepine	0.54	**1.79**	**1.95**	**1.95**	**2.58**	**2.62**	**3.13**
Cimetidine	1.04	*	*	*	*	*	*
Codeine	6.11	*	*	*	*	*	*
Diltiazem	0.12	**0.14**	**0.12**	*	**0.12**	**0.12**	**0.16**
1,7-Dimethylxanthine	1.10	*	*	*	*	*	*
Diphenhydramine	0.05	**1.12**	**1.15**	**1.24**	**1.68**	**1.33**	**1.74**
Erythromycin	6.42	*	*	*	*	*	*
Fluoxetine	6.74	*	*	*	*	*	*
Gemfibrozil	6.68	*	*	*	*	*	*
Ibuprofen	45.96	*	*	*	*	*	*
Lincomycin	5.53	*	*	*	*	*	*
Metoprolol	2.50	*	*	*	*	*	*
Miconazole	10.83	*	*	*	*	*	*
Norfluoxetine	2.90	*	*	*	**3.19**	*	**3.21**
Propranolol	1.07	*	*	*	*	*	*
Sertraline	3.56	*	*	*	*	*	*
Sulfamethoxazole	2.29	*	*	*	*	*	*
Thiabendazole	2.63	*	*	*	*	*	*
Trimethoprim	2.15	*	*	*	*	*	*
Tylosin	5.02	*	*	*	*	*	*
Warfarin	0.86	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table B-2. Analytical Results for Pharmaceuticals in Fish Fillet Samples from Dallas, Texas.

Chemical	MDL ng/g (ppb)	Dallas 1	Dallas 2	Dallas 3	Dallas 4	Dallas 5	Dallas 6
Acetaminophen	4.40	*	*	*	*	*	*
Atenolol	1.48	*	*	*	*	*	*
Caffeine	3.93	*	*	*	*	*	*
Carbamazepine	0.54	*	*	*	*	*	*
Cimetidine	1.04	*	*	*	*	*	*
Codeine	6.11	*	*	*	*	*	*
Diltiazem	0.12	*	*	*	*	*	*
1,7-Dimethylxanthine	1.10	*	*	*	*	*	*
Diphenhydramine	0.05	*	*	*	*	*	*
Erythromycin	6.42	*	*	*	*	*	*
Fluoxetine	6.74	*	*	*	*	*	*
Gemfibrozil	6.68	*	*	*	*	*	*
Ibuprofen	45.96	*	*	*	*	*	*
Lincomycin	5.53	*	*	*	*	*	*
Metoprolol	2.50	*	*	*	*	*	*
Miconazole	10.83	*	*	*	*	*	*
Norfluoxetine	2.90	*	*	*	*	*	*
Propranolol	1.07	*	*	*	*	*	*
Sertraline	3.56	*	*	*	*	*	*
Sulfamethoxazole	2.29	*	*	*	*	*	*
Thiabendazole	2.63	*	*	*	*	*	*
Trimethoprim	2.15	*	*	*	*	*	*
Tylosin	5.02	*	*	*	*	*	*
Warfarin	0.86	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table B-3. Analytical Results for Pharmaceuticals in Fish Fillet Samples from Orlando, Florida.

Chemical	MDL ng/g (ppb)	Orlando 1	Orlando 2	Orlando 3	Orlando 4	Orlando 5	Orlando 6
Acetaminophen	4.40	*	*	*	*	*	*
Atenolol	1.48	*	*	*	*	*	*
Caffeine	3.93	*	*	*	*	*	*
Carbamazepine	0.54	*	*	*	*	*	*
Cimetidine	1.04	*	*	*	*	*	*
Codeine	6.11	*	*	*	*	*	*
Diltiazem	0.12	*	*	*	*	*	*
1,7-Dimethylxanthine	1.10	*	*	*	*	*	*
Diphenhydramine	0.05	*	*	*	*	*	*
Erythromycin	6.42	*	*	*	*	*	*
Fluoxetine	6.74	*	*	*	*	*	*
Gemfibrozil	6.68	*	*	*	*	*	*
Ibuprofen	45.96	*	*	*	*	*	*
Lincomycin	5.53	*	*	*	*	*	*
Metoprolol	2.50	*	*	*	*	*	*
Miconazole	10.83	*	*	*	*	*	*
Norfluoxetine	2.90	*	*	*	*	*	*
Propranolol	1.07	*	*	*	*	*	*
Sertraline	3.56	*	*	*	*	*	*
Sulfamethoxazole	2.29	*	*	*	*	*	*
Thiabendazole	2.63	*	*	*	*	*	*
Trimethoprim	2.15	*	*	*	*	*	*
Tylosin	5.02	*	*	*	*	*	*
Warfarin	0.86	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table B-4. Analytical Results for Pharmaceuticals in Fish Fillet Samples from Phoenix, Arizona.

Chemical	MDL ng/g (ppb)	Phoenix 1	Phoenix 2	Phoenix 3	Phoenix 4	Phoenix 5	Phoenix 6
Acetaminophen	4.40	*	*	*	*	*	*
Atenolol	1.48	*	*	*	*	*	*
Caffeine	3.93	*	*	*	*	*	*
Carbamazepine	0.54	*	*	*	*	*	*
Cimetidine	1.04	*	*	*	*	*	*
Codeine	6.11	*	*	*	*	*	*
Diltiazem	0.12	*	*	*	*	*	*
1,7-Dimethylxanthine	1.10	*	*	*	*	*	*
Diphenhydramine	0.05	**1.04**	**1.20**	**1.41**	**1.21**	**1.12**	**1.24**
Erythromycin	6.42	*	*	*	*	*	*
Fluoxetine	6.74	*	*	*	*	*	*
Gemfibrozil	6.68	*	*	*	*	*	*
Ibuprofen	45.96	*	*	*	*	*	*
Lincomycin	5.53	*	*	*	*	*	*
Metoprolol	2.50	*	*	*	*	*	*
Miconazole	10.83	*	*	*	*	*	*
Norfluoxetine	2.90	*	**3.42**	**4.83**	**4.19**	*	**3.53**
Propranolol	1.07	*	*	*	*	*	*
Sertraline	3.56	**4.05**	**4.55**	**4.97**	**5.36**	**4.70**	**6.47**
Sulfamethoxazole	2.29	*	*	*	*	*	*
Thiabendazole	2.63	*	*	*	*	*	*
Trimethoprim	2.15	*	*	*	*	*	*
Tylosin	5.02	*	*	*	*	*	*
Warfarin	0.86	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table B-5. Analytical Results for Pharmaceuticals in Fish Fillet Samples from West Chester, Pennsylvania.

Chemical	MDL ng/g (ppb)	W. Chester 1	W. Chester 2	W. Chester 3	W. Chester 4	W. Chester 5	W. Chester 6
Acetaminophen	4.40	*	*	*	*	*	*
Atenolol	1.48	*	*	*	*	*	*
Caffeine	3.93	*	*	*	*	*	*
Carbamazepine	0.54	*	*	*	*	*	*
Cimetidine	1.04	*	*	*	*	*	*
Codeine	6.11	*	*	*	*	*	*
Diltiazem	0.12	*	*	*	**0.14**	**0.12**	**0.20**
1,7-Dimethylxanthine	1.10	*	*	*	*	*	*
Diphenhydramine	0.05	**1.23**	**1.49**	**1.82**	**1.74**	**1.67**	**2.48**
Erythromycin	6.42	*	*	*	*	*	*
Fluoxetine	6.74	*	*	*	*	*	*
Gemfibrozil	6.68	*	*	*	*	*	*
Ibuprofen	45.96	*	*	*	*	*	*
Lincomycin	5.53	*	*	*	*	*	*
Metoprolol	2.50	*	*	*	*	*	*
Miconazole	10.83	*	*	*	*	*	*
Norfluoxetine	2.90	**3.15**	**2.95**	**4.19**	**4.39**	**3.74**	**4.99**
Propranolol	1.07	*	*	*	*	*	*
Sertraline	3.56	**7.01**	**6.51**	**13.85**	**12.64**	**9.98**	**18.63**
Sulfamethoxazole	2.29	*	*	*	*	*	*
Thiabendazole	2.63	*	*	*	*	*	*
Trimethoprim	2.15	*	*	*	*	*	*
Tylosin	5.02	*	*	*	*	*	*
Warfarin	0.86	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table B-6. Analytical Results for Pharmaceuticals in Fish Fillet Samples from Gila Wilderness Area, New Mexico.

Chemical	MDL ng/g (ppb)	Reference 1	Reference 2	Reference 3	Reference 4	Reference 5	Reference 6
Acetaminophen	4.40	*	*	*	*	*	*
Atenolol	1.48	*	*	*	*	*	*
Caffeine	3.93	*	*	*	*	*	*
Carbamazepine	0.54	*	*	*	*	*	*
Cimetidine	1.04	*	*	*	*	*	*
Codeine	6.11	*	*	*	*	*	*
Diltiazem	0.12	*	*	*	*	*	*
1,7-Dimethylxanthine	1.10	*	*	*	*	*	*
Diphenhydramine	0.05	*	*	*	*	*	*
Erythromycin	6.42	*	*	*	*	*	*
Fluoxetine	6.74	*	*	*	*	*	*
Gemfibrozil	6.68	*	*	*	*	*	*
Ibuprofen	45.96	*	*	*	*	*	*
Lincomycin	5.53	*	*	*	*	*	*
Metoprolol	2.50	*	*	*	*	*	*
Miconazole	10.83	*	*	*	*	*	*
Norfluoxetine	2.90	*	*	*	*	*	*
Propranolol	1.07	*	*	*	*	*	*
Sertraline	3.56	*	*	*	*	*	*
Sulfamethoxazole	2.29	*	*	*	*	*	*
Thiabendazole	2.63	*	*	*	*	*	*
Trimethoprim	2.15	*	*	*	*	*	*
Tylosin	5.02	*	*	*	*	*	*
Warfarin	0.86	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Appendix C

Site-specific Analytical Results Tables for Pharmaceuticals in Liver Tissue

Table C-1. Analytical Results for Pharmaceuticals in Fish Liver Tissue Samples from Chicago, Illinois.

Chemical	MDL ng/g (ppb)	Chicago 1	Chicago 2	Chicago 3	Chicago 4	Chicago 5	Chicago 6
Acetaminophen	34.28	*	*	*	*	*	*
Atenolol	12.86	*	*	*	*	*	*
Caffeine	25.47	*	*	*	*	*	*
Carbamazepine	1.86	**4.27**	**5.15**	**6.64**	**6.77**	**5.32**	**7.52**
Cimetidine	5.18	*	*	*	*	*	*
Codeine	31.49	*	*	*	*	*	*
Diltiazem	0.26	**0.50**	**0.54**	**0.78**	**0.88**	**0.64**	**0.90**
1,7-Dimethylxanthine	5.84	*	*	*	*	*	*
Diphenhydramine	0.26	**5.20**	**4.54**	**7.46**	**9.13**	**5.91**	**9.59**
Erythromycin	43.03	*	*	*	*	*	*
Fluoxetine	12.41	*	*	**18.42**	**14.44**	*	**22.76**
Gemfibrozil	24.82	*	*	*	*	*	*
Ibuprofen	172.81	*	*	*	*	*	*
Lincomycin	56.14	*	*	*	*	*	*
Metoprolol	8.90	*	*	*	*	*	*
Norfluoxetine	15.31	**41.06**	**20.96**	**127.71**	**81.33**	**38.26**	**129.65**
Propranolol	3.77	*	*	*	*	*	*
Sertraline	17.29	**41.19**	**42.08**	**96.40**	**148.70**	**34.15**	**140.93**
Sulfamethoxazole	13.95	*	*	*	*	*	*
Thiabendazole	7.84	*	*	*	*	*	*
Trimethoprim	8.00	*	*	*	*	*	*
Tylosin	34.67	*	*	*	*	*	*
Warfarin	2.70	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table C-2. Analytical Results for Pharmaceuticals in Fish Liver Tissue Samples from Dallas, Texas.

Chemical	MDL ng/g (ppb)	Dallas 1	Dallas 2	Dallas 3	Dallas 4	Dallas 5	Dallas 6
Acetaminophen	34.28	*	*	*	*	*	*
Atenolol	12.86	*	*	*	*	*	*
Caffeine	25.47	*	*	*	*	*	*
Carbamazepine	1.86	*	*	*	*	*	*
Cimetidine	5.18	*	*	*	*	*	*
Codeine	31.49	*	*	*	*	*	*
Diltiazem	0.26	*	*	*	*	*	*
1,7-Dimethylxanthine	5.84	*	*	*	*	*	*
Diphenhydramine	0.26	**0.93**	*	**0.26**	**0.80**	**0.31**	**0.28**
Erythromycin	43.03	*	*	*	*	*	*
Fluoxetine	12.41	**12.44**	*	*	**13.75**	*	*
Gemfibrozil	24.82	*	*	*	*	*	*
Ibuprofen	172.81	*	*	*	*	*	*
Lincomycin	56.14	*	*	*	*	*	*
Metoprolol	8.90	*	*	*	*	*	*
Norfluoxetine	15.31	**46.26**	*	**26.90**	**48.17**	**27.44**	*
Propranolol	3.77	*	*	*	*	*	*
Sertraline	17.29	**28.21**	*	**27.54**	**23.19**	**28.07**	*
Sulfamethoxazole	13.95	*	*	*	*	*	*
Thiabendazole	7.84	*	*	*	*	*	*
Trimethoprim	8.00	*	*	*	*	*	*
Tylosin	34.67	*	*	*	*	*	*
Warfarin	2.70	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table C-3. Analytical Results for Pharmaceuticals in Fish Liver Tissue Samples from Orlando, Florida.

Chemical	MDL ng/g (ppb)	Orlando 1	Orlando 2	Orlando 3	Orlando 4	Orlando 5	Orlando 6
Acetaminophen	34.28	*	*	*	*	*	*
Atenolol	12.86	*	*	*	*	*	*
Caffeine	25.47	*	*	*	*	*	*
Carbamazepine	1.86	*	*	*	*	*	*
Cimetidine	5.18	*	*	*	*	*	*
Codeine	31.49	*	*	*	*	*	*
Diltiazem	0.26	*	*	*	*	*	*
1,7-Dimethylxanthine	5.84	*	*	*	*	*	*
Diphenhydramine	0.26	*	*	*	*	*	*
Erythromycin	43.03	*	*	*	*	*	*
Fluoxetine	12.41	*	*	*	*	*	*
Gemfibrozil	24.82	*	*	*	*	*	*
Ibuprofen	172.81	*	*	*	*	*	*
Lincomycin	56.14	*	*	*	*	*	*
Metoprolol	8.90	*	*	*	*	*	*
Norfluoxetine	15.31	**48.27**	**44.09**	**48.99**	**78.39**	**62.95**	*
Propranolol	3.77	*	*	*	*	*	*
Sertraline	17.29	*	*	*	**21.18**	*	*
Sulfamethoxazole	13.95	*	*	*	*	*	*
Thiabendazole	7.84	*	*	*	*	*	*
Trimethoprim	8.00	*	*	*	*	*	*
Tylosin	34.67	*	*	*	*	*	*
Warfarin	2.70	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table C-4. Analytical Results for Pharmaceuticals in Fish Liver Tissue Samples from Phoenix, Arizona.

Chemical	MDL ng/g (ppb)	Phoenix 1	Phoenix 2	Phoenix 3	Phoenix 4	Phoenix 5	Phoenix 6
Acetaminophen	34.28	*	*	*	*	*	*
Atenolol	12.86	*	*	*	*	*	*
Caffeine	25.47	*	*	*	*	*	*
Carbamazepine	1.86	*	*	*	*	*	*
Cimetidine	5.18	*	*	*	*	*	*
Codeine	31.49	*	*	*	*	*	*
Diltiazem	0.26	*	*	**0.29**	**0.28**	**0.28**	**0.44**
1,7-Dimethylxanthine	5.84	*	*	*	*	*	*
Diphenhydramine	0.26	**6.32**	**4.38**	**5.79**	**5.48**	**7.28**	**11.09**
Erythromycin	43.03	*	*	*	*	*	*
Fluoxetine	12.41	*	*	*	*	*	*
Gemfibrozil	24.82	**74.43**	**77.60**	**60.10**	**49.14**	**66.95**	**90.39**
Ibuprofen	172.81	*	*	*	*	*	*
Lincomycin	56.14	*	*	*	*	*	*
Metoprolol	8.90	*	*	*	*	*	*
Norfluoxetine	15.31	*	**25.02**	**41.25**	**27.96**	**28.70**	**43.65**
Propranolol	3.77	*	*	*	*	*	*
Sertraline	17.29	**57.28**	**64.80**	**67.79**	**62.19**	**68.86**	**105.24**
Sulfamethoxazole	13.95	*	*	*	*	*	*
Thiabendazole	7.84	*	*	*	*	*	*
Trimethoprim	8.00	*	*	*	*	*	*
Tylosin	34.67	*	*	*	*	*	*
Warfarin	2.70	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table C-5. Analytical Results for Pharmaceuticals in Fish Liver Tissue Samples from West Chester, Pennsylvania.

Chemical	MDL ng/g (ppb)	W. Chester 1	W. Chester 2	W. Chester 3	W. Chester 4	W. Chester 5	W. Chester 6
Acetaminophen	34.28	*	*	*	*	*	*
Atenolol	12.86	*	*	*	*	*	*
Caffeine	25.47	*	*	*	*	*	*
Carbamazepine	1.86	*	*	*	*	*	*
Cimetidine	5.18	*	*	*	*	*	*
Codeine	31.49	*	*	*	*	*	*
Diltiazem	0.26	**0.59**	**0.60**	**0.71**	**0.75**	**0.72**	**0.76**
1,7-Dimethylxanthine	5.84	*	*	*	*	*	*
Diphenhydramine	0.26	**7.86**	**9.40**	**10.67**	**11.44**	**10.73**	**11.17**
Erythromycin	43.03	*	*	*	*	*	*
Fluoxetine	12.41	**63.24**	**66.13**	**66.28**	**65.11**	**79.70**	**78.26**
Gemfibrozil	24.82	*	*	**27.34**	*	**26.88**	*
Ibuprofen	172.81	*	*	*	*	*	*
Lincomycin	56.14	*	*	*	*	*	*
Metoprolol	8.90	*	*	*	*	*	*
Norfluoxetine	15.31	**37.56**	**47.73**	**33.29**	**38.02**	**34.40**	**37.79**
Propranolol	3.77	*	*	*	*	*	*
Sertraline	17.29	**358.62**	**431.96**	**432.17**	**545.34**	**326.03**	**189.66**
Sulfamethoxazole	13.95	*	*	*	*	*	*
Thiabendazole	7.84	*	*	*	*	*	*
Trimethoprim	8.00	*	*	*	*	*	*
Tylosin	34.67	*	*	*	*	*	*
Warfarin	2.70	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Table C-6. Analytical Results for Pharmaceuticals in Fish Liver Tissue Samples from the Gila Wilderness Area, New Mexico.

Chemical	MDL ng/g (ppb)	Reference 1	Reference 2	Reference 3	Reference 4	Reference 5	Reference 6
Acetaminophen	34.28	*	*	*	*	*	*
Atenolol	12.86	*	*	*	*	*	*
Caffeine	25.47	*	*	*	*	*	*
Carbamazepine	1.86	*	*	*	*	*	*
Cimetidine	5.18	*	*	*	*	*	*
Codeine	31.49	*	*	*	*	*	*
Diltiazem	0.26	*	*	*	*	*	*
1,7-Dimethylxanthine	5.84	*	*	*	*	*	*
Diphenhydramine	0.26	*	*	*	*	*	*
Erythromycin	43.03	*	*	*	*	*	*
Fluoxetine	12.41	*	*	*	*	*	*
Gemfibrozil	24.82	*	*	*	*	*	*
Ibuprofen	172.81	*	*	*	*	*	*
Lincomycin	56.14	*	*	*	*	*	*
Metoprolol	8.90	*	*	*	*	*	*
Norfluoxetine	15.31	*	*	*	*	*	*
Propranolol	3.77	*	*	*	*	*	*
Sertraline	17.29	*	*	*	*	*	*
Sulfamethoxazole	13.95	*	*	*	*	*	*
Thiabendazole	7.84	*	*	*	*	*	*
Trimethoprim	8.00	*	*	*	*	*	*
Tylosin	34.67	*	*	*	*	*	*
Warfarin	2.70	*	*	*	*	*	*

* Less than the Method Detection Limit (<MDL)

Appendix D

Site-specific Analytical Results Tables for Personal Care Product Chemicals

Table D-1. Analytical Results for Personal Care Products in Fish Fillet Samples from Chicago, Illinois.

CHEMICAL[†]	MDL ng/g (ppb)	CHICAGO 1	CHICAGO 2	CHICAGO 3	CHICAGO 4	CHICAGO 5	CHICAGO 6
Celestolide	17.7	*	*	*	*	*	*
Galaxolide	12.2	**657.50**	**624.60**	**1,211.95**	**1,582.40**	**1,806.64**	**1,760.75**
4-Methylbenzylidene Camphor (4-MBC)	120.5	*	*	*	*	*	*
Musk Ketone	321.2	*	*	*	*	*	*
Musk Xylene	397.1	*	*	*	*	*	*
p-Nonylphenol	9.7	*	*	*	*	*	*
p-Octylphenol	8.2	*	*	*	*	*	*
m-Toluamide	5.1	*	*	*	*	*	*
Tonalide	13.4	**80.99**	**79.09**	**138.02**	**176.57**	**230.21**	**222.67**
Triclosan	37.8	*	*	*	*	*	*

[†] Benzophenone and octocrylene were identified in blank control samples at concentrations comparable to those reported in the analytical samples. The analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

* Less than the Method Detection Limit (<MDL)

Table D-2. Analytical Results for Personal Care Products in Fish Fillet Samples from Dallas, Texas.

CHEMICAL†	MDL ng/g (ppb)	DALLAS 1	DALLAS 2	DALLAS 3	DALLAS 4	DALLAS 5	DALLAS 6
Celestolide	17.7	*	*	*	*	*	*
Galaxolide	12.2	**516.10**	**606.42**	**202.91**	**1,146.77**	**1,842.27**	**731.02**
4-Methylbenzylidene Camphor (4-MBC)	120.5	*	*	*	*	*	*
Musk Ketone	321.2	*	*	*	*	*	*
Musk Xylene	397.1	*	*	*	*	*	*
p-Nonylphenol	9.7	*	*	*	*	*	*
p-Octylphenol	8.2	*	*	*	*	*	*
m-Toluamide	5.1	*	*	*	*	*	*
Tonalide	13.4	**45.12**	**54.86**	**19.99**	**102.74**	**149.68**	**57.67**
Triclosan	37.8	*	*	*	*	*	*

† Benzophenone and octocrylene were identified in blank control samples at concentrations comparable to those reported in the analytical samples. The analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

* Less than the Method Detection Limit (<MDL)

Table D-3. Analytical Results for Personal Care Products in Fish Fillet Samples from Orlando, Florida.

CHEMICAL†	MDL ng/g (ppb)	ORLANDO 1	ORLANDO 2	ORLANDO 3	ORLANDO 4	ORLANDO 5	ORLANDO 6
Celestolide	17.7	*	*	*	*	*	*
Galaxolide	12.2	**288.53**	**115.17**	**62.44**	**39.66**	**33.42**	*
4-Methylbenzylidene Camphor (4-MBC)	120.5	*	*	*	*	*	*
Musk Ketone	321.2	*	*	*	*	*	*
Musk Xylene	397.1	*	*	*	*	*	*
p-Nonylphenol	9.7	*	*	*	*	*	*
p-Octylphenol	8.2	*	*	*	*	*	*
m-Toluamide	5.1	*	*	*	*	*	*
Tonalide	13.4	**21.44**	*	*	*	*	*
Triclosan	37.8	*	*	*	*	*	*

† Benzophenone and octocrylene were identified in blank control samples at concentrations comparable to those reported in the analytical samples. The analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

* Less than the Method Detection Limit (<MDL)

Table D-4. Analytical Results for Personal Care Products in Fish Fillet Samples from Phoenix, Arizona.

CHEMICAL†	MDL ng/g (ppb)	PHOENIX 1	PHOENIX 2	PHOENIX 3	PHOENIX 4	PHOENIX 5	PHOENIX 6
Celestolide	17.7	*	*	*	*	*	*
Galaxolide	12.2	**2,038.93**	**1,960.68**	**1,961.63**	**2,099.75**	**1,414.39**	**1,049.12**
4-Methylbenzylidene Camphor (4-MBC)	120.5	*	*	*	*	*	*
Musk Ketone	321.2	*	*	*	*	*	*
Musk Xylene	397.1	*	*	*	*	*	*
p-Nonylphenol	9.7	*	*	*	*	*	*
p-Octylphenol	8.2	*	*	*	*	*	*
m-Toluamide	5.1	*	*	*	*	*	*
Tonalide	13.4	**259.54**	**283.56**	**290.57**	**272.81**	**191.69**	**162.12**
Triclosan	37.8	*	*	*	*	*	*

† Benzophenone and octocrylene were identified in blank control samples at concentrations comparable to those reported in the analytical samples. The analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

* Less than the Method Detection Limit (<MDL)

Table D-5. Analytical Results for Personal Care Products in Fish Fillet Samples from West Chester, Pennsylvania.

CHEMICAL[†]	MDL ng/g (ppb)	W. CHESTER 1	W. CHESTER 2	W. CHESTER 3	W. CHESTER 4	W. CHESTER 5	W. CHESTER 6
Celestolide	17.7	*	*	*	*	*	*
Galaxolide	12.2	**2,017.65**	**1,812.86**	**2,006.32**	**1,864.33**	**1,960.51**	**1,236.54**
4-Methylbenzylidene Camphor (4-MBC)	120.5	*	*	*	*	*	*
Musk Ketone	321.2	*	*	*	*	*	*
Musk Xylene	397.1	*	*	*	*	*	*
p-Nonylphenol	9.7	*	*	*	*	*	*
p-Octylphenol	8.2	*	*	*	*	*	*
m-Toluamide	5.1	*	*	*	*	*	*
Tonalide	13.4	**72.26**	**53.16**	**66.93**	**62.48**	**40.82**	**35.57**
Triclosan	37.8	*	*	*	*	*	*

[†] Benzophenone and octocrylene were identified in blank control samples at concentrations comparable to those reported in the analytical samples. The analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

* Less than the Method Detection Limit (<MDL)

Table D-6. Analytical Results for Personal Care Products in Fish Fillet Samples from the Gila Wilderness Area, New Mexico.

CHEMICAL†	MDL ng/g (ppb)	REFERENCE 1	REFERENCE 2	REFERENCE 3	REFERENCE 4	REFERENCE 5	REFERENCE 6
Celestolide	17.7	*	*	*	*	*	*
Galaxolide	12.2	*	*	*	*	*	*
4-Methylbenzylidene Camphor (4-MBC)	120.5	*	*	*	*	*	*
Musk Ketone	321.2	*	*	*	*	*	*
Musk Xylene	397.1	*	*	*	*	*	*
p-Nonylphenol	9.7	*	*	*	*	*	*
p-Octylphenol	8.2	*	*	*	*	*	*
m-Toluamide	5.1	*	*	*	*	*	*
Tonalide	13.4	*	*	*	*	*	*
Triclosan	37.8	*	*	*	*	*	*

† Benzophenone and octocrylene were identified in blank control samples at concentrations comparable to those reported in the analytical samples. The analytical results for these two chemicals were considered inconclusive by the laboratory and are excluded from the final results.

* Less than the Method Detection Limit (<MDL)

Appendix E

Lipid Content in Fish Fillet and Liver Tissue Samples

Table E-1. Analytical Results for Lipid Content in Fish Fillet Samples from Chicago, Illinois.

	Chicago 1	Chicago 2	Chicago 3	Chicago 4	Chicago 5	Chicago 6
Replicate 1						
Lipid weight (g)	0.0292	0.0440	0.0419	0.0533	0.0647	0.0532
Sample weight (g)	2.1630	2.0040	2.0369	2.0717	2.0213	2.0439
Lipid %	1.3500	2.1956	2.0570	2.5728	3.2009	2.6029
Replicate 2						
Lipid weight (g)	0.0273	0.0451	0.0429	0.0513	0.0674	0.0594
Sample weight (g)	2.0724	2.0842	2.0211	2.0075	2.0327	2.2188
Lipid %	1.3173	2.1639	2.1226	2.5554	3.3158	2.6771
Replicate 3						
Lipid weight (g)	0.0304	0.0353	0.0425	0.0510	0.0693	0.0539
Sample weight (g)	2.1974	1.5580	2.1166	2.0566	2.1468	2.0682
Lipid %	1.3835	2.2657	2.0079	2.4798	3.2281	2.6061
Summary						
Average Lipid %	1.3502	2.2084	2.0625	2.5360	3.2483	2.6287
Standard Deviation	0.0331	0.0521	0.0575	0.0494	0.0600	0.0420

Table E-2. Analytical Results for Lipid Content in Fish Fillet Samples from Dallas, Texas.

	Dallas 1	Dallas 2	Dallas 3	Dallas 4	Dallas 5	Dallas 6
Replicate 1						
Lipid weight (g)	0.0357	0.0429	0.0181	0.0691	0.0875	0.0325
Sample weight (g)	2.1415	2.0787	2.0795	2.2466	2.1908	2.0099
Lipid %	1.6671	2.0638	0.8704	3.0758	3.9940	1.6170
Replicate 2						
Lipid weight (g)	0.0346	0.0418	0.0176	0.0593	0.0867	0.0387
Sample weight (g)	2.1395	2.0062	2.0221	2.0066	2.0905	2.1314
Lipid %	1.6172	2.0835	0.8704	2.9552	4.1473	1.8157
Replicate 3						
Lipid weight (g)	0.0334	0.0458	0.0162	0.0598	0.0790	0.0344
Sample weight (g)	2.0600	2.2592	2.0075	2.0463	2.0136	2.0865
Lipid %	1.6214	2.0273	0.8070	2.9223	3.9233	1.6487
Summary						
Average Lipid %	1.6352	2.0582	0.8493	2.9845	4.0215	1.6938
Standard Deviation	0.0277	0.0286	0.0366	0.0808	0.1145	0.1068

Table E-3. Analytical Results for Lipid Content in Fish Fillet Samples from Orlando, Florida.

	Orlando 1	Orlando 2	Orlando 3	Orlando 4	Orlando 5	Orlando 6
Replicate 1						
Lipid weight (g)	0.0488	0.0337	0.0182	0.0100	0.0100	0.0122
Sample weight (g)	2.0660	2.2852	2.1320	2.1662	2.1196	2.0219
Lipid %	2.3621	1.4747	0.8537	0.4616	0.4718	0.6034
Replicate 2						
Lipid weight (g)	0.0459	0.0334	0.0178	0.0097	0.0104	0.0111
Sample weight (g)	2.0712	2.1211	2.1038	2.0359	2.0596	2.1061
Lipid %	2.2161	1.5747	0.8461	0.4764	0.5050	0.5270
Replicate 3						
Lipid weight (g)	0.0455	0.0325	0.0180	0.0097	0.0098	0.0127
Sample weight (g)	2.0680	2.1734	2.1157	2.0998	2.0300	2.0854
Lipid %	2.2002	1.4954	0.8508	0.4619	0.4828	0.6090
Summary						
Average Lipid %	2.2595	1.5149	0.8502	0.4667	0.4865	0.5798
Standard Deviation	0.0892	0.0528	0.0038	0.0085	0.0169	0.0458

Table E-4. Analytical Results for Lipid Content in Fish Fillet Samples from Phoenix, Arizona.

	Phoenix 1	Phoenix 2	Phoenix 3	Phoenix 4	Phoenix 5	Phoenix 6
Replicate 1						
Lipid weight (g)	0.0721	0.0847	0.0969	0.1095	0.0841	0.0542
Sample weight (g)	2.1314	2.0962	2.1160	2.3087	2.0537	2.0260
Lipid %	3.3828	4.0406	4.5794	4.7429	4.0950	2.6752
Replicate 2						
Lipid weight (g)	0.0675	0.0799	0.0919	0.1037	0.0926	0.0591
Sample weight (g)	2.1020	2.0458	2.0174	2.1917	2.2806	2.2439
Lipid %	3.2112	3.9056	4.5554	4.7315	4.0603	2.6338
Replicate 3						
Lipid weight (g)	0.0677	0.0870	0.0951	0.0908	0.0859	0.0540
Sample weight (g)	2.0898	2.2060	2.0705	2.0013	2.0072	1.9991
Lipid %	3.2395	3.9438	4.5931	4.5371	4.2796	2.7012
Summary						
Average Lipid %	3.2778	3.9633	4.5760	4.6705	4.1450	2.6701
Standard Deviation	0.0920	0.0696	0.0191	0.1157	0.1179	0.0340

Table E-5. Analytical Results for Lipid Content in Fish Fillet Samples from West Chester, Pennsylvania.

	W. Chester 1	W. Chester 2	W. Chester 3	W. Chester 4	W. Chester 5	W. Chester 6
Replicate 1						
Lipid weight (g)	0.0312	0.0490	0.0442	0.0414	0.0510	0.0274
Sample weight (g)	2.1707	2.2533	2.1216	2.2320	2.2832	2.1425
Lipid %	1.4373	2.1746	2.0833	1.8548	2.2337	1.2789
Replicate 2						
Lipid weight (g)	0.0329	0.0433	0.0446	0.0378	0.0467	0.0256
Sample weight (g)	2.1878	2.0429	2.0096	2.0244	2.1188	2.0200
Lipid %	1.5038	2.1195	2.2193	1.8672	2.2041	1.2673
Replicate 3						
Lipid weight (g)	0.0326	0.0438	0.0447	0.0394	0.0475	0.0269
Sample weight (g)	2.0320	2.0120	2.0712	2.1265	2.0214	2.1129
Lipid %	1.6043	2.1769	2.1582	1.8528	2.3499	1.2731
Summary						
Average Lipid %	1.5151	2.1570	2.1536	1.8583	2.2625	1.2731
Standard Deviation	0.0841	0.0325	0.0681	0.0078	0.0770	0.0058

Table E-6. Analytical Results for Lipid Content in Fish Fillet Samples from the Gila Wilderness Area, New Mexico.

	Reference 1	Reference 2	Reference 3	Reference 4	Reference 5	Reference 6
Replicate 1						
Lipid weight (g)	0.0749	0.0918	0.1794	0.0852	0.1027	0.1055
Sample weight (g)	2.0917	2.1683	2.1260	2.0070	2.2311	2.0474
Lipid %	3.5808	4.2337	8.4384	4.2451	4.6031	5.1529
Replicate 2						
Lipid weight (g)	0.0699	0.0841	0.1748	0.0915	0.0968	0.0995
Sample weight (g)	2.0614	2.1780	2.2158	2.0980	2.0299	2.0297
Lipid %	3.3909	3.8613	7.8888	4.3613	4.7687	4.9022
Replicate 3						
Lipid weight (g)	0.0733	0.0838	0.1609	0.0897	0.0919	0.1069
Sample weight (g)	2.1426	2.0323	2.0893	2.0100	2.0239	2.1366
Lipid %	3.4211	4.1234	7.7011	4.4627	4.5407	5.0033
Summary						
Average Lipid %	3.4643	4.0728	8.0094	4.3564	4.6375	5.0195
Standard Deviation	0.1021	0.1913	0.3831	0.1089	0.1178	0.1261

Table E-7. Analytical Results for Lipid Content in Fish Liver Samples from Chicago, Illinois.

	Chicago 1	Chicago 2	Chicago 3	Chicago 4	Chicago 5	Chicago 6
Replicate 1						
Lipid weight (g)	0.0465	0.0327	0.0421	0.0474	0.0588	0.0236
Sample weight (g)	2.0089	1.9991	2.0839	2.0627	2.1197	1.0467
Lipid %	2.3147	1.6357	2.0203	2.2980	2.7740	2.2547
Replicate 2						
Lipid weight (g)						0.0214
Sample weight (g)						1.0236
Lipid %						2.0907
Replicate 3						
Lipid weight (g)						0.0244
Sample weight (g)						1.0198
Lipid %						2.3926
Summary						
Average Lipid %	2.3147	1.6357	2.0203	2.2980	2.7740	2.2460
Standard Deviation						0.1512

Table E-8. Analytical Results for Lipid Content in Fish Liver Samples from Dallas, Texas.

	DALLAS 1	DALLAS 2	DALLAS 3	DALLAS 4	DALLAS 5	DALLAS 6
REPLICATE 1						
Lipid weight (g)	0.1181	0.1809	0.1963	0.2067	0.2071	0.0759
Sample weight (g)	2.0128	2.0269	2.0876	2.0189	2.0648	2.0893
Lipid %	5.8674	8.9250	9.4031	10.2382	10.0300	3.6328
REPLICATE 2						
Lipid weight (g)					0.2175	
Sample weight (g)					2.0940	
Lipid %					10.3868	
REPLICATE 3						
Lipid weight (g)					0.2149	
Sample weight (g)					2.0677	
Lipid %					10.3932	
SUMMARY						
Average Lipid %	5.8674	8.9250	9.4031	10.2382	10.2700	3.6328
Standard Deviation					0.2079	

Table E-9. Analytical Results for Lipid Content in Fish Liver Samples from Orlando, Florida.

	Orlando 1	Orlando 2	Orlando 3	Orlando 4	Orlando 5	Orlando 6
Replicate 1						
Lipid weight (g)	0.0750	0.1157	0.0612	0.0227	0.0334	0.0497
Sample weight (g)	2.0795	2.0509	2.0524	2.0153	2.0127	2.0190
Lipid %	3.6066	5.6414	2.9819	1.1264	1.6595	2.4616
Replicate 2						
Lipid weight (g)	0.0755					
Sample weight (g)	2.0125					
Lipid %	3.7516					
Replicate 3						
Lipid weight (g)	0.0720					
Sample weight (g)	2.0244					
Lipid %	3.5566					
Summary						
Average Lipid %	3.6383	5.6414	2.9819	1.1264	1.6595	2.4616
Standard Deviation	0.1012					

Table E-10. Analytical Results for Lipid Content in Fish Liver Samples from Phoenix, Arizona.

	PHOENIX 1	PHOENIX 2	PHOENIX 3	PHOENIX 4	PHOENIX 5	PHOENIX 6
REPLICATE 1						
Lipid weight (g)	0.2146	0.3042	0.2140	0.2703	0.1965	0.2069
Sample weight (g)	2.0390	2.0138	2.0276	2.0882	2.0918	2.0071
Lipid %	10.5248	15.1058	10.5543	12.9442	9.3938	10.3084
REPLICATE 2						
Lipid weight (g)						0.2225
Sample weight (g)						2.0336
Lipid %						10.9412
REPLICATE 3						
Lipid weight (g)						0.2189
Sample weight (g)						2.0493
Lipid %						10.6817
REPLICATE 4						
Lipid weight (g)						0.2225
Sample weight (g)						2.0211
Lipid %						11.0089
REPLICATE 5						
Lipid weight (g)						0.2262
Sample weight (g)						2.0109
Lipid %						11.2487
REPLICATE 6						
Lipid weight (g)						0.2114
Sample weight (g)						2.0006
Lipid %						10.5668
SUMMARY						
Average Lipid %	10.5248	15.1058	10.5543	12.9442	9.3938	10.7926
Standard Deviation						0.3390

Table E-11. Analytical Results for Lipid Content in Fish Liver Samples from West Chester, Pennsylvania.

	W. Chester 1	W. Chester 2	W. Chester 3	W. Chester 4	W. Chester 5	W. Chester 6
Replicate 1						
Lipid weight (g)	0.0946	0.1003	0.0665	0.0372	0.0573	0.0753
Sample weight (g)	2.0732	1.9288	1.1284	1.0104	1.0637	2.0077
Lipid %	4.5630	5.2001	5.8933	3.6817	5.3869	3.7506
Replicate 2						
Lipid weight (g)			0.0531			
Sample weight (g)			1.0054			
Lipid %			5.2815			
Replicate 3						
Lipid weight (g)			0.0652			
Sample weight (g)			1.0114			
Lipid %			6.4465			
Summary						
Average Lipid %	4.5630	5.2001	5.8738	3.6817	5.3869	3.7506
Standard Deviation			0.5828			

Table E-12. Analytical Results for Lipid Content in Fish Liver Samples from the Gila Wilderness Area, New Mexico.

	Reference 1	Reference 2	Reference 3	Reference 4	Reference 5	Reference 6
Replicate 1						
Lipid weight (g)	0.0838	0.0928	0.0745	0.0613	0.2048	0.0910
Sample weight (g)	2.0757	2.0269	2.0059	2.0621	2.0853	2.1054
Lipid %	4.0372	4.5784	3.7140	2.9727	9.8211	4.3222
Replicate 2						
Lipid weight (g)			0.0654			
Sample weight (g)			2.0350			
Lipid %			3.2138			
Replicate 3						
Lipid weight (g)			0.0692			
Sample weight (g)			2.0138			
Lipid %			3.4363			
Summary						
Average Lipid %	4.0372	4.5784	3.4547	2.9727	9.8211	4.3222
Standard Deviation			0.2506			