ARE YOU GONNA EAT THAT?
ARSENIC AND MERCURY LEVELS IN ALLEGHENY RIVER CATFISH AND IMPLICATIONS FOR HUMAN CONSUMPTION

by

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This study examined arsenic and mercury concentrations in channel catfish (*Ictalurus punctatus*) caught for human consumption in the Allegheny River. Arsenic is a known human carcinogen and mercury is known to cause neurological disorders, particularly in fetuses and children. Subsistence and semi-subsistence anglers and their families are at risk of exposure.

Catfish were caught at 4 distinct sites – Pittsburgh, Cheswick, Freeport and Ford City. They were measured for general characteristics such as weight, length, and sex, and tissue samples were taken and analyzed for heavy metal content. The study addressed main questions: Do levels of mercury and arsenic vary among the 4 sites and, if so, how? Do the levels of mercury and arsenic in these fish pose a threat to people who eat them regularly?

Analysis of variance was used to determine group differences by location. Contrasts were performed to test for specific differences: Pittsburgh from the other three sites, and Cheswick, Freeport and Ford City from each other. Multiple regression analyses were conducted to determine if any of the other factors, weight, length or sex, had an impact on metals levels in addition to location. Assessments of risk to human consumers of these fish were conducted using US EPA guidelines and formulae.

The Pittsburgh fish were found to have significantly different concentrations of both arsenic and mercury than the fish from the other sites. Mean levels of arsenic and mercury were

observed to be lower in the Pittsburgh fish. No significant differences in contaminant levels were found between the Cheswick, Freeport and Ford City fish. Subsequent analyses were conducted combining these three locations into the Allegheny River group. Regression analyses showed minimal impact of weight and no impact of any other factor when controlling for location.

Public Health Implications: Risk assessments found hazard quotients above 1 for all populations (children 3-8, children 9-15, women of childbearing age, other adults) based on 95% confidence intervals for mean concentrations of mercury. Arsenic levels also showed excess cancer risk for all populations. Current fish consumption advisories are inadequate to protect the health of regular consumers of these fish.

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PREFACE

Thanks to Dr. Dan Volz for providing the data used in this study and to the many researchers and community members who participated in the Pittsburgh Fish Consumption Study and the Allegheny River Stewardship Project. Thanks to Dr. John Wilson and Dr. Richard Day for their invaluable statistical advice

1.0   INTRODUCTION

Fish are generally considered one of the healthiest foods humans eat. H.O. Bang and his colleagues were among the first to formally study the connection between consumption of fish and human health (Bang, et al., 1980). They compared the diets and health profiles of Greenland Eskimos to the local Danish population. They noticed that although the Eskimo diet was high in fat, from seal and fish meat, the incidence of heart disease was much lower in the Eskimo population than in the Danish population. They hypothesized that this was at least partly due to the type of fatty acids contained in the seal and fish fats. This led to much further study and it is now widely accepted that eating fish reduces the risk of cardiovascular disease (He, et al., 2004a). The American Heart Association now recommends that Americans eat fish at least twice a week (Lichtenstein, et al., 2006).

High in protein and low in fat, fish also provide a number of important nutrients including iron, zinc, and calcium. Perhaps most significantly, fish flesh contains large amounts of alpha-linolenic acid (ALA).

ALA is an omega-3 fatty acid important for human growth and development. Human bodies cannot produce ALA, so it must come from food. In the body, ALA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA helps to regulate cell division and growth, blood clotting, and muscle activity. In addition, EPA is thought to protect against cardiovascular disease and some inflammatory diseases such as arthritis, lupus and

asthma. DHA, on the other hand, is critical to brain development and function and is a major component of the retina of the eye (NIH, 2005).

Fish consumption has been linked to a lower risk of stroke, though the specific mechanism is not fully understood (He, et al., 2004b). Studies have also found that eating fish has protective effects against depression, and mental decline with age (Morris, et al., 2005). DHA is likely an important factor in these connections.

Eating fish is especially recommended for pregnant and nursing women to improve the health of their children. The fatty acid DHA has been shown to improve eyesight and cognitive development in infants (Innis, 2008). J.T. Cohen and colleagues conducted a review study indicating that for each gram increase in maternal DHA consumption the child's IQ increased 0.8 – 1.8 points (Cohen, et al., 2005). DHA is also believed to decrease the chance of preterm birth.

However, fish are also a vector for human intake of environmental contaminants. Consumption of fish is the primary source of mercury exposure in humans (WHO, 1990), and 80% of human arsenic exposure comes from animal flesh, including fish (ATSDR, 1999). Fish absorb toxins from their environment, both through their skin and gills and through the food they eat, especially if they eat other fish (Burger, et al., 2002). Some of these toxins are readily excreted, but some take a very long time to leave the body. Mercury, for example, builds up in animal tissues and bioaccumulates. Other toxins, such as arsenic, are excreted over time.

1.1   ARSENIC

Arsenic is one of the most worrisome environmental contaminants. Arsenic occurs naturally and is present in air, water, soil and living things. It comes in elemental, organic and

inorganic forms. It is often called a metal, but is actually a metalloid. Inorganic arsenic is the most toxic, and has been identified as a human carcinogen (Eisler, 1988). The valence state of arsenic affects its toxicity. Elemental arsenic (As(0)) is insoluble in water and human tissue and is generally non-poisonous. Trivalent inorganic arsenic (As(III)) is easily absorbed by the body, and can cause damage to most organ systems. Pentavalent inorganic arsenic (As(V)) is just as toxic as As(III), but is not as easily absorbed, so exposure is not as common (ATSDR, 2000).

People have used inorganic arsenic as a poison for centuries. Acute exposure results in severe abdominal pain, diarrhea, vomiting, shock, convulsions, heart failure and possibly death. Fortunately, acute exposure is extremely rare. Homicide, suicide and accidental ingestion of pesticides account for most acute exposures.

Chronic exposure is much more common, and insidious. Drinking water is the primary route of chronic human exposure to inorganic arsenic. Several sites around the world have well known problems with aquifers containing high amounts of arsenic. Communities in Bandgladesh (Bagla & Kaiser, 1996), Taiwan (Chiou, et al., 2001) and Chile (Smith, et al., 2000) have had to abandon their wells due to high concentrations of arsenic.

Early symptoms of chronic exposure include numbness or tingling in fingers or toes, skin lesions (called Blackfoot Disease) and anemia. Long term exposures lead to skin cancer, bladder cancer and lung cancer (Smith, et al., 2009), and are implicated in other cancers (ATSDR, 2000). Developing fetuses and children are at particular risk. Chronic arsenic exposure is associated with spontaneous abortion and stillbirth (Milton, et al., 2005). Recently, arsenic has also been linked to cardiovascular disease (States, et al., 2009).

Arsenic in living things occurs mostly in its organic form. The percentage of total arsenic that is inorganic ranges widely. The US EPA uses an estimate of 10% in its analyses. In

contaminated areas, however, fish and other seafood can contain amounts of inorganic arsenic up to 41% of the total arsenic load (Buchet, et al., 1996).

Arsenic is processed by the body relatively efficiently and does not build up over time. However, since it is carcinogenic, even a small exposure can increase one's risk of developing cancer.

1.2   MERCURY

Like arsenic, mercury is also a naturally occurring element. It is ubiquitous, present in air water, and soil. Mercury takes three basic forms – elemental (or metallic), inorganic compounds and organic compounds (Keating, et al., 1997). Most naturally occurring mercury is elemental or inorganic and serious health consequences from exposure to these forms of mercury are rare. Organic mercury is mercury that has bonded to carbon and formed a compound. When mercury bonds to carbon and methane, it forms methylmercury, $CH3Hg^+$, or MeHg. Methylmercury is a potent neurotoxin, and the most common form of organic mercury.

Methylmercury is created when elemental or inorganic mercury enters water or soil and is processed by microorganisms such as bacteria. In water, bacteria release the methylmercury and it attaches to tiny particles and makes its way into the food chain (ATSDR, 1999). Small fish eat algae and other small plants containing methylmercury, or simply absorb methylmercury through their skin. Methylmercury is not processed by the body and eliminated very efficiently, so it bioaccumulates, or builds up in the body over time. When bigger fish eat the small fish, they absorb all the methylmercury from the smaller fish. The higher an organism is on the food

chain, the higher mercury concentration its body is likely to contain. This process is known as biomagnification (see Figure 1).
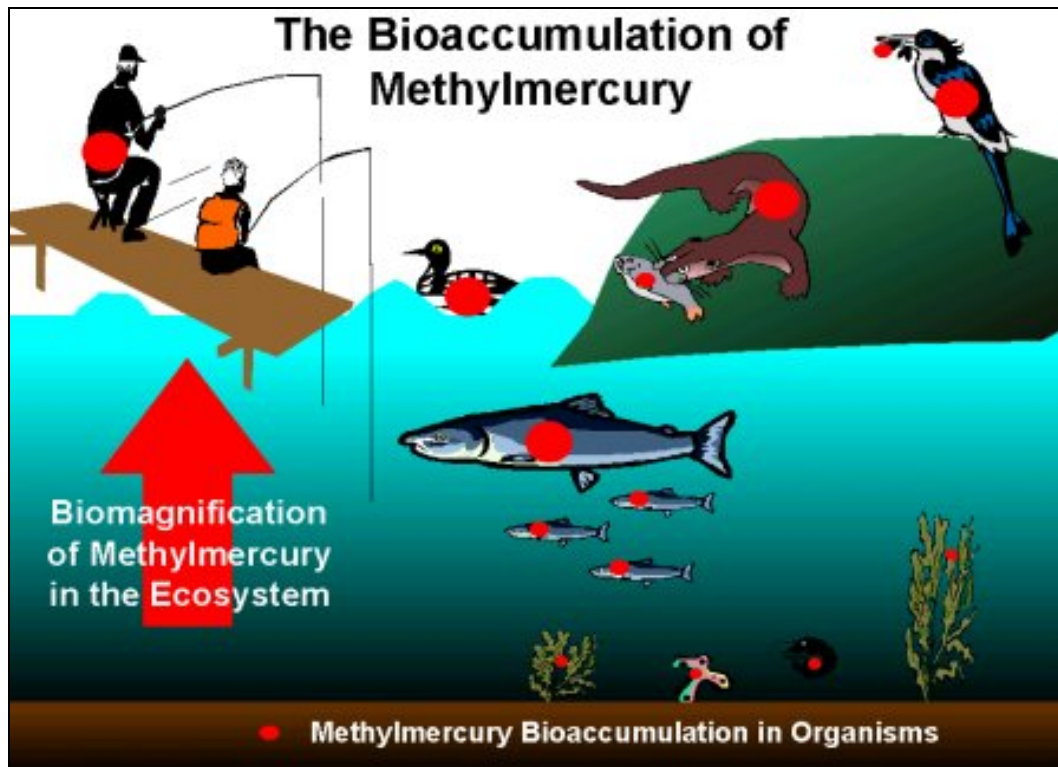
Figure 1. Bioaccumulation of Methylmercury in the Food Chain
(Environment Canada, 2004)

Several instances of large scale mercury poisoning have occurred in the last 50 years. Minamata Bay, Japan in the 1950's was one of the first and largest; its repercussions are still being felt (Harada, 1995). In that incident, mercury-containing wastewater was released into the bay on which the community relied heavily for food. Thousands of people died and thousands more were permanently crippled. Another famous incident occurred in Basra, Iraq, in 1971. Seed grain treated with methylmercury and intended for planting was stolen from storage and made directly into bread products. Warnings on the grain were printed in English and Spanish only, so the Arabic speaking population was unaware that they were eating poison. 6,500 cases

of mercury poisoning were recorded and hundreds of people died. Other large-scale incidents have occurred in Niigata Prefecture, Japan., and Ontario, Canada (D'Ltri & D'Ltri, 1978).

Methylmercury is easily and efficiently (~95%) absorbed by the human gastro-intestinal tract. It then enters the bloodstream and circulates throughout the body. The brain and central nervous system (CNS) are most susceptible. In adults, symptoms include vision impairment, numbness, loss of fine and gross motor coordination, and renal failure. In severe and prolonged exposures such as in Minamata Bay, adults can die from methylmercury poisoning. However, healthy adults usually recover fully once the exposure to mercury is stopped (Baum, 1999).

Brains which are still developing are at much greater risk for long term and permanent damage due to methylmercury exposure. Methylmercury is passed from mother to fetus through the placenta, and fetal concentrations of mercury can be higher than the mother's (Kojima & Fujita, 1973). Methylmercury disrupts cell differentiation, leading to abnormal brain development. In some severe cases, a very undeveloped fetus will abort naturally. Fetuses affected later in development may exhibit symptoms of cerebral palsy and blindness. In less severe cases, children show developmental delays in motor skills and language acquisition, possibly accompanied by seizures (Keating, et al., 1997). Mercury continues to be a threat once the child is born, as it can also pass from mother to child through breast milk. Unlike in adults, the effects of methylmercury poisoning in infants and young children are not reversible.

Not all research into mercury exposure in fetuses and young children is so bleak. In response to growing concern about mercury exposure via fish consumption, studies have been done of communities who subsist primarily on mercury-containing fish, but who have not been subject to acute exposures. The most well-known and longest running of these studies is the Seychelles Child Development Study. Seychelles is a small island nation in the Indian Ocean,

whose population relies heavily on fish for food, eating an average of 12 meals of fish per week. Maternal hair measurements confirmed exposure to mercury, up to 40 ppm (Shamlaye, et al., 2004). The children of these mothers have been followed and their development assessed up to age 11 (so far). No developmental delays or mental or physical problems have been associated with mercury exposure (Davidson, et al., 2006). There seems to be a threshold dose below which no adverse effects are found.

1.3   THE STUDY AREA

The channel catfish in this study were caught at 4 sites in the Pittsburgh region. The city of Pittsburgh is known for and shaped by its 3 rivers – the Allegheny on the north, the Monongahela on the south and the Ohio on the west. These rivers were a major factor in Pittsburgh's development as an urban and industrial center. Another defining aspect of the Pittsburgh region is its rich coal deposits.   The rivers provided a convenient means of transportation for the mountains of coal and later steel that were produced in the Pittsburgh region.

The rivers also provided a convenient means of waste disposal. City Superintendent N.S. Sprague summed up the sewer philosophy succinctly: "Rivers are the natural and logical drains and are formed for the purpose of carrying the wastes to the sea."(Sprague, 1912). The use of the rivers as dumping sites for household and industrial waste has had long-lasting and devastating effects. Over the past couple of decades, due to cleanup efforts and the decline of the local steel industry, the rivers are much cleaner than they once were (Tarr, 2004). Coke-burning steel mills have been replaced as a major threat to river health by coal-fired power plants.

Despite their nickname "the Three Rivers", in the vicinity of Pittsburgh, the Allegheny, Monongahela and Ohio Rivers actually behave like a single, contained body of water. In order to make the rivers more navigable by the ships carrying the raw materials and the fruits of Pittsburgh's industry, series of locks and dams have been constructed all along the 3 rivers. On the Allegheny, there is the Highland Park Dam, on the Monongahela, the Braddock Dam, and on the Ohio, the Emsworth Locks and Dam (see Figure 2).

As a result, the three rivers around the city of Pittsburgh between the dams have been collectively called the "Pittsburgh Pool". Previous research has found no statistically significant differences in channel catfish caught in the Monongahela, Allegheny or Ohio rivers within the Pittsburgh Pool, in terms of size or contaminant levels (Liu, 2007).

However, 35 miles up the Allegheny in Kittaning, PA, catfish are very different. In 2005, the Pittsburgh Fish Consumption Study found that fish caught near the Kittaning Dam were significantly smaller than Pittsburgh Pool fish and had much higher levels of toxins. 23% of the Kittaning fish were found to have mercury levels higher than the EPA criterion for fish consumption. Making this a serious cause for concern is the fact that the Kittaning area was perceived by the local anglers as a much cleaner and safer place to fish (Liu, 2007).

1.4   THE FISH

In the current study, channel catfish are the vector of concern for arsenic and mercury. The channel catfish, *Ictalurus punctatus*, is one of the most commonly caught fish for recreation and consumption, both in the Pittsburgh region and nationwide. This piscivorous fish can be found in many types of freshwater aquatic environments, from small ponds to large rivers.

Though it will eat algae, plants, snails and insects, its primary source of food is other, smaller fish, dead or alive. 14 years is the average lifespan of a channel catfish, though fish as old as 40 years have been recorded. Average weight of catfish caught by anglers is 2-3 pounds (0.9 - 1.4 kg) (Wellborn, 1988).

Figure 2. Channel Catfish
(Rivers, 2003)

Their position near the top of the food chain makes the channel catfish susceptible to environmental contaminants contained in all of its food sources. Those contaminants can include polychlorinated biphenyls (PCBs), estrogens and heavy metals such as mercury, and metalloids such as arsenic.

The catfish data used in this study come from two field studies concerned with the health of Pittsburgh's rivers and the organisms, including people, that use them: the Pittsburgh Fish Consumption Study and the Allegheny River Stewardship Project. The Pittsburgh Fish Consumption Study was conducted in 2005-2006 by researchers at the University of Pittsburgh in collaboration with local interests. As a Community Based Participatory Research (CBPR) project, the Pittsburgh Fish Consumption Study involved community members, in this case, recreational, subsistence and semi-subsistence anglers. Researchers and local anglers determined where to catch the fish, based on sites of scientific interest and sites popular with fishermen.

Previous studies have shown that scientists studying wild caught fish catch significantly different fish than local anglers (Burger, et al., 2006). In order to avoid any biases in terms of

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the size of fish caught, researchers in the Pittsburgh Fish Consumption Study relied on the anglers to catch the fish.

The Allegheny River Stewardship Project is a collaborative effort between University of Pittsburgh researchers and Alle-Kiski Valley residents to determine the sources and types of river pollutants by monitoring the levels of toxins in fish living in the river. Also a CBPR project, the Allegheny River Stewardship Project recruited local anglers to assist researchers in catching fish at designated locations during 5 "fishing days" in the spring/summer of 2008.

1.5   PUBLIC HEALTH IMPLICATIONS

The public health implications of game fish contaminated with high levels of arsenic and/or mercury are clear. Populations eating such fish may be at risk of numerous health problems, both short-term and long-term. Many states and municipalities seek to address these concerns by issuing fish advisories, telling residents how much of fish is "safe" to eat. The Pennsylvania Fish and Boat Commission has issued a state-wide recommendation to eat no more than one meal per week of wild caught fish, with additional advisories about specific waterways. On most of the Allegheny River, there is an advisory about walleye, but nothing about channel catfish. Only the area around the Point in Pittsburgh has a warning about channel catfish. The concern is PCBs and the recommendation is to eat no more than one meal per month.

The current study seeks to determine if further advisories are warranted on the Allegheny River and to quantify the risks to those who eat fish from it.

1.6   RESEARCH QUESTIONS

1) Do levels of mercury and arsenic in catfish vary among the 4 sites (Pittsburgh, Cheswick,

   Ford City and Freeport)?

   a) Do the Pittsburgh fish differ from the Upper Allegheny (Cheswick, Ford City, Freeport)

      fish?

   b) Do the Upper Allegheny fish differ by site?

   c) How are any differences affected by other factors (weight, length, sex, etc.)?
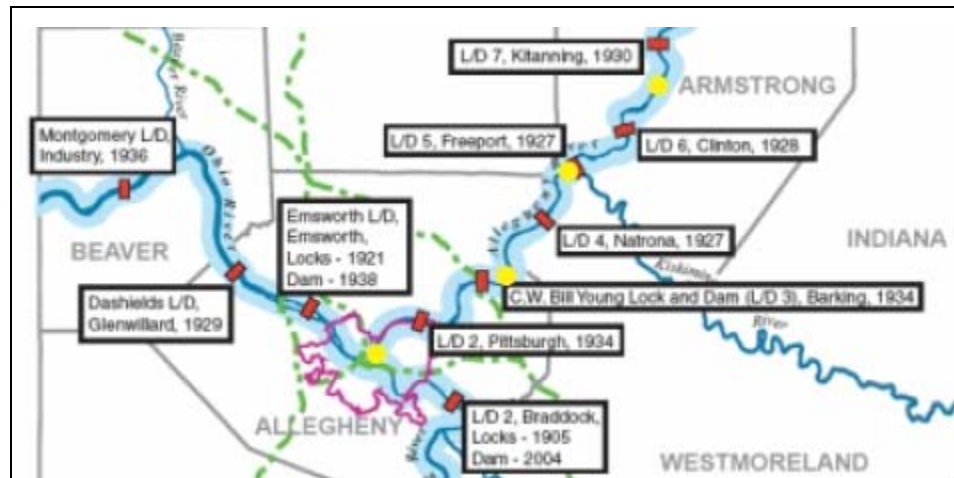
Figure 3. Map of Locks and Dams in the Pittsburgh Area, with fishing sites
(Port of Pittsburgh Commission, 2005)

As the map above shows, the four fishing sites (yellow dots) are separated by a series of

locks and dams, restricting fish movement between the sites. A previous study (Liu, 2007) has

shown that Pittsburgh Pool fish differ significantly from fish caught in the Kittanning area,

further up the Allegheny River than the 3 Allegheny River sites in the current study. Refining

the boundaries between those differences may help locate sources of contamination.

For contaminants like mercury that accumulate in the body and are not quickly or

completely excreted, it is hypothesized that older fish will have higher levels. In fish, size is a

good correlate of age, so the relationship between length, weight and metal levels will be examined.

2) Do the levels of mercury and arsenic in these fish pose a threat to people who eat them regularly?

a) Can we develop a risk assessment model?

b) Can we determine "safe" amounts of fish to eat?

As part of the Pittsburgh Fish Consumption Study, local anglers were surveyed about their consumption of fish from the rivers. Some groups rely on fishing for a significant portion of their food, eating an average of 4 meals of fish per week. Clearly, this is in excess of the recommended 1 meal per week. It is important to determine the true risk to these populations. In addition, the current study is designed to assess the adequacy of current fish consumption advisories.

2.0   METHODS

2.1   FISH SAMPLING METHODS

Catfish were all caught by rod and reel by local anglers and researchers, from shore and from boats. Sample size was determined by availability of fish, with a goal of at least 10 fish per site. Locations were chosen as representative of popular fishing sites.

Length, weight and sex were recorded at the time of catch. Fish were dissected and frozen in the field when possible. Frozen tissue was sent to an authorized lab for metals analysis.

Tissues were digested by a nitric acid/hydrogen peroxide method – typically 2 mL 12 M ('metal-free') HNO_3 + 1 mL 30% (w/w) H_2O_2 added to ~1 g tissue, dissolved in 2% HNO_3 after the instrument-controlled microwave-based digestion cycle. Microwave-based approaches prevent background contaminants from entering the samples.

Samples were analyzed using collision cell Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) for a suite of 29 metals including Arsenic (As), Cadmium (Cd), Chromium (Cr), Manganese (Mn), Lead (Pb), Selenium (Se), and Zinc (Zn). Mercury (Hg) was measured by isotope dilution cold vapor ICP-MS.

2.2 STATISTICAL METHODS

Data were analyzed using SPSS 15.0 and SAS 9.1. The assumption of the normal distribution and equal variances was checked for metal levels. Arsenic and mercury were not normally distributed (Shapiro-Wilk $W = 0.812$, $p < 0.0001$ for As; $W = 0.542$, $p < 0.0001$ for Hg) so a log transform was used. The appropriateness of the log transform was verified using the Box-Cox procedure (Box & Cox, 1964).

An initial analysis of variance by location was performed to answer the first research question. Contrasts were included to test for individual differences. The Pittsburgh site was contrasted against the Allegheny River sites and the Allegheny River sites were contrasted against each other. Residuals from each analysis were checked for normality.

In the case of non-normal residuals, permutation tests were conducted, following methods described by E. L. Lehmann (Lehmann & Romano, 2005). SAS procedure `proc multtest` with the `perms` option was used to create 10000 new samples with the location variable randomized. An ANOVA was run on each sample and the F statistic from each ANOVA was captured. The distribution of these F statistics represents a reasonable approximation of the true distribution of F statistics in this data under the null hypothesis of no difference between locations. The F statistic from the ANOVA on the original data was then compared to the distribution of F values from the permutations.

To determine whether any differences found by analysis of variance were affected by any other factors multiple regression analysis was also conducted. Variables of interest were: total weight (grams), standard length (length from snout to base of tail, not including fins, in cm), and sex (male/female/indeterminate). Centered forms of weight and standard length were created to

reduce collinearity in case quadratic terms were needed. Because some variables were missing for some fish, stepwise regression was done manually. Each factor was entered into the model, checked for significance and kept or rejected based on the change in error sum of squares.

Risk assessment methods followed US EPA recommendations and used US EPA developed formulae. Dry weight measurements of arsenic and mercury were converted to wet weight and compared to EPA criteria for "safe" levels in fish tissue. Average daily doses and hazard quotients were calculated for non-carcinogenic outcomes of arsenic and mercury. Excess cancer risk was calculated for carcinogenic outcomes related to inorganic arsenic. Recommended consumption levels were then back-calculated based on EPA reference doses and target cancer risk.

3.0   RESULTS

Table 1 shows the breakdown of location and sex, and mean values for weight, standard length, raw arsenic and raw mercury.

Table 1 -- Basic Fish Data

Location	N	Female	Male	Indeterminate	Mean Weight (grams)	Mean Std. Length (cm)	Mean Arsenic (mg/kg)	Mean Mercury (mg/kg)
Pittsburgh	39	13	20	6	1087	38.5	0.0314	0.1487
Cheswick	8	4	4	0	869	36.4	0.0700	0.5750
Freeport	17	5	9	3	690	34.5	0.0606	0.7176
Ford City	29	11	17	1	497	32.7	0.0957	0.5676
TOTAL	93	33	50	10	838	35.97	0.0566	0.4495

Potential differences by location are apparent even in the simple format of Table 1, with Pittsburgh having lower levels of metals than the other locations.   Mean values can be misleading, since arsenic and mercury are not normally distributed. The following boxplots show the distributions of weight, standard length, Log(As) and Log(Hg) by location.

Weight by location				
Min	310	350	300	48.8
Mean	1086.816	868.75	690.3529	497.4571
Max	2230	2000	2200	1210
N	38	8	17	21
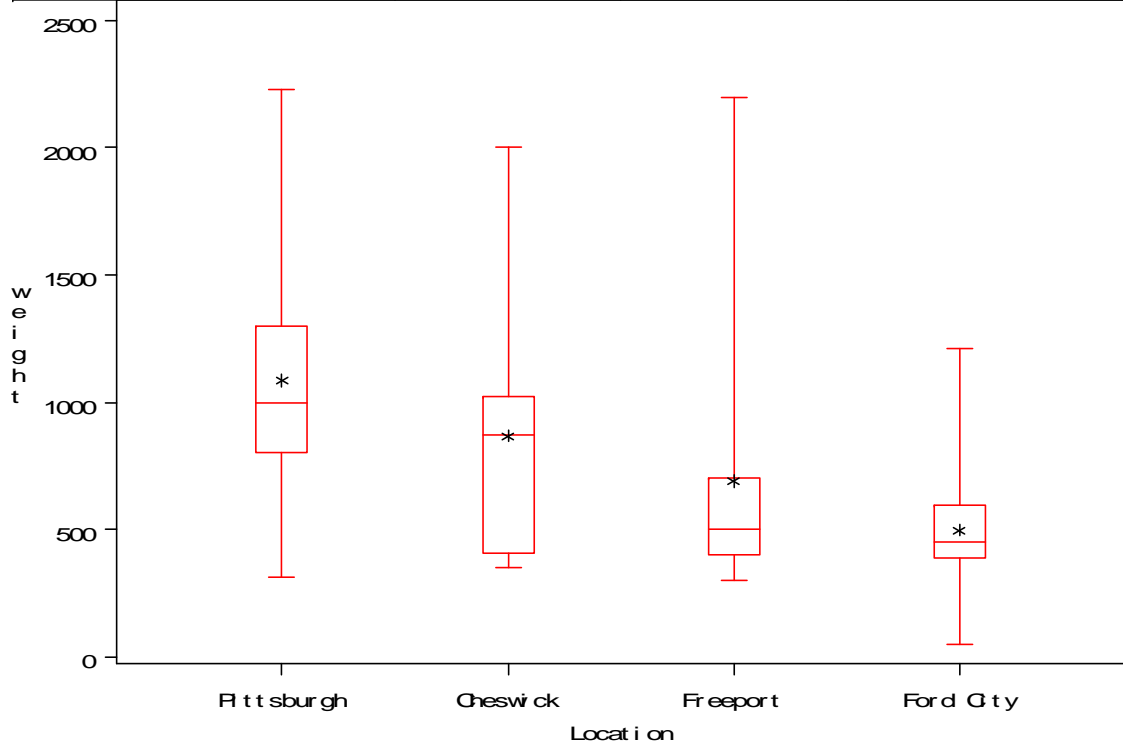
Figure 4. Boxplot of Weight by Location

As the plot shows, weight is highly variable, with Pittsburgh, Cheswick and Freeport having similar ranges.

Std. Length by location				
Min	25	28.5	28.6	25.5
Mean	38.25897	36.45	34.55294	32.7
Max	48.5	48.5	49.4	42.5
N	39	8	17	21
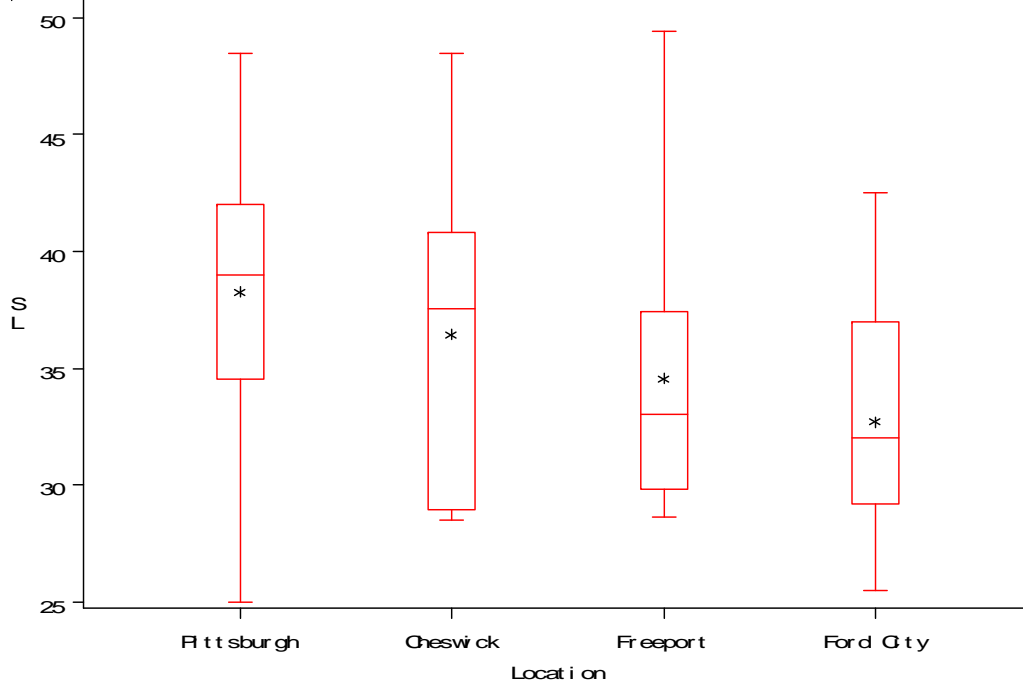
Figure 5. Boxplot of Standard Length by Location

Standard length is also quite variable, though all sites appear to have similar ranges.

As_Log by location				
Min	−5.22	−3.21	−3.91	−3.91
Mean	−3.61923	−2.68375	−2.88412	−2.65379
Max	−1.54	−2.3	−2.2	−1.2
N	39	8	17	29
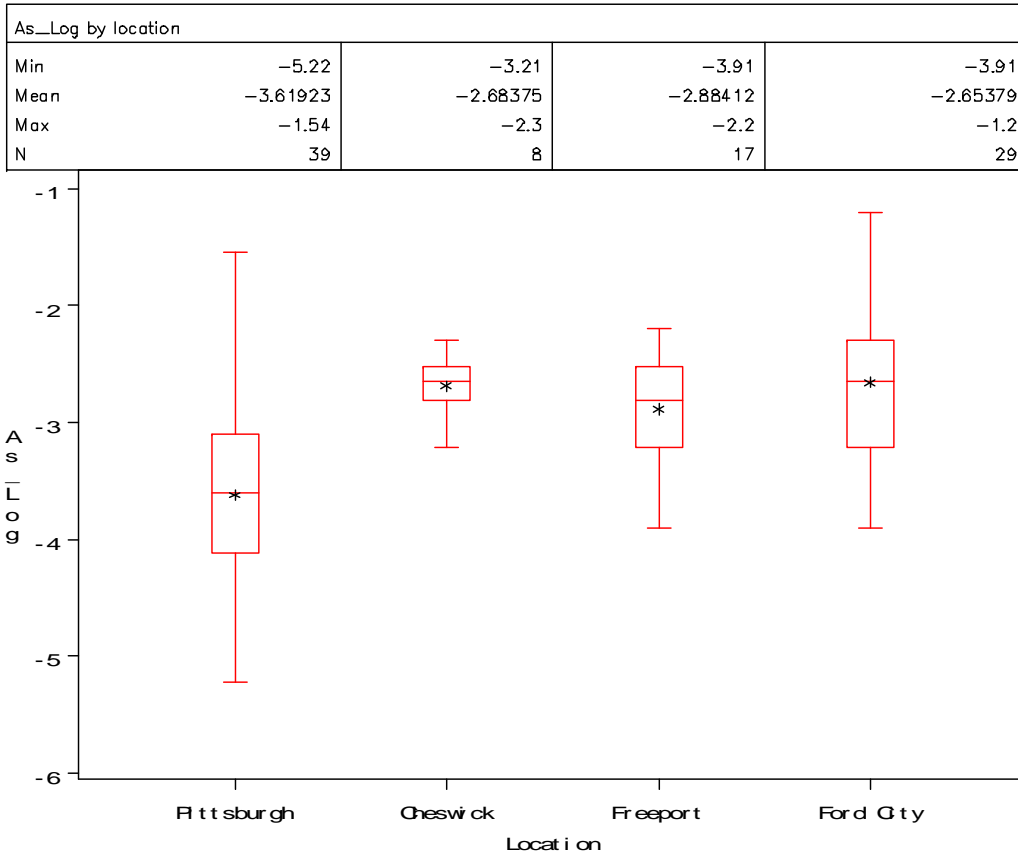
Figure 6. Boxplot of Log(arsenic) by Location

Log(As) appears to differ by location, though the range of values in Pittsburgh is wider than any of the others.

Hg_Log by location				
Min	−4.52	−1.71	−1.51	−2.99
Mean	−2.09692	−0.67625	−0.75	−0.75793
Max	−0.74	−0.08	1.53	1.23
N	39	8	17	29

Figure 7. Boxplot of Log(mercury) by Location

Log(Hg) also seems to differ by location, though the ranges for all sites are fairly small.

3.1   ANALYSES OF VARIANCE

3.1.1   Arsenic

The analysis of variance for Log(As) showed group differences, indicating that at least one location has a significantly different mean than the others. The residuals for the overall

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ANOVA were normally distributed (Shapiro-Wilk W = 0.981, p = 0.205), supporting the assumption that the sample of Log(As) measurements comes from a normally distributed population.

SAS `proc glm` output:

```
Dependent Variable: As_Log

                            Sum of
Source              DF      Squares     Mean Square    F Value    Pr > F
Model                3    18.40091008    6.13363669      14.26    <.0001
Error               89    38.28238509    0.43013916
Corrected Total     92    56.68329518
```

The contrast of Pittsburgh against the other locations showed that Pittsburgh is significantly different from Cheswick, Freeport and Ford City.

```
Contrast          DF    Contrast SS    Mean Square    F Value    Pr > F
Pittsburgh         1    15.41108274    15.41108274      35.83    <.0001
```

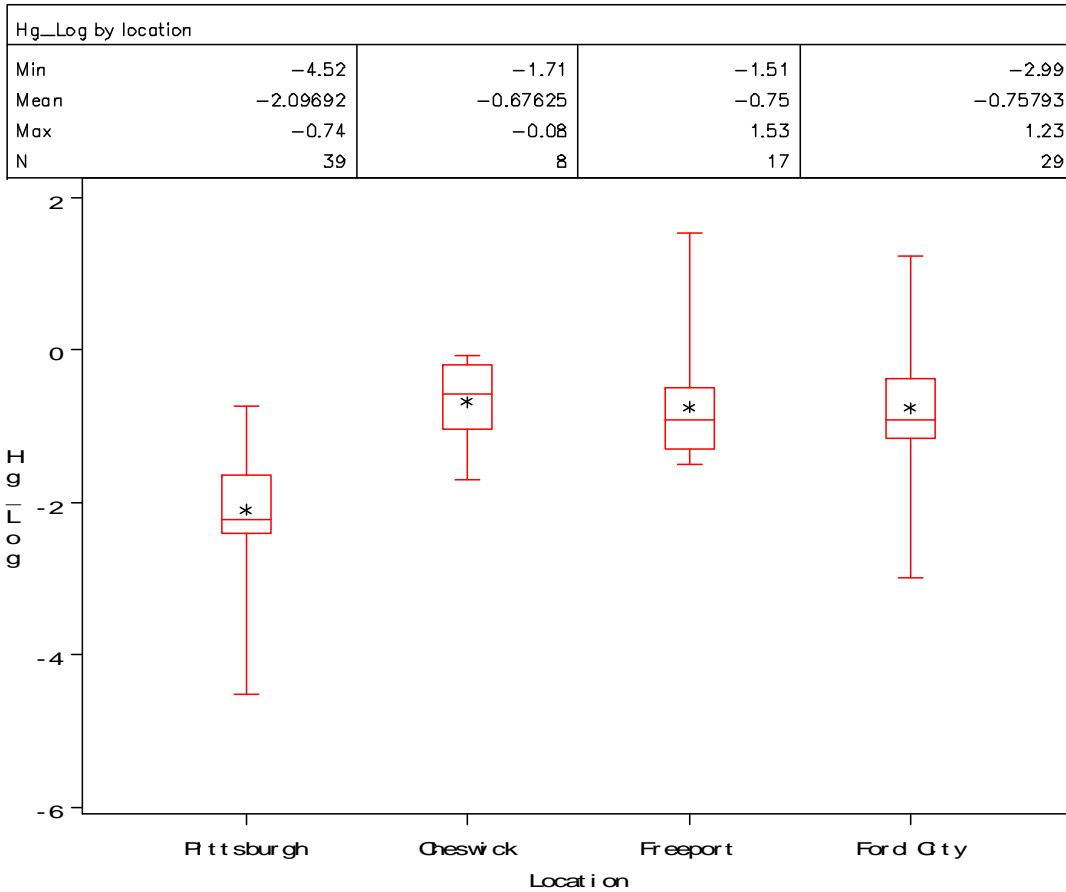The contrast of Cheswick, Freeport and Ford City (noted Allegheny River (A.R.) Group) against each other found no significant differences in arsenic level.

```
Contrast          DF    Contrast SS    Mean Square    F Value    Pr > F
A.R. Group         2     0.59494432     0.29747216       0.69    0.5035
```

3.1.2 Mercury

The analysis of variance for Log(Hg) showed group differences, indicating that at least one location has a significantly different mean than the others. However, the residuals were not normally distributed (Shapiro-Wilk W = 0.96, p = 0.0061). This calls into question the assumption that the sample of Log(Hg) measurements comes from a normally distributed population, which makes the ANOVA results unreliable without further confirmation.

```
Dependent Variable: Hg_Log

                                Sum of
Source                  DF      Squares      Mean Square    F Value    Pr > F
Model                    3    41.59933020    13.86644340      24.17    <.0001
Error                   89    51.06958913     0.57381561
Corrected Total         92    92.66891933
```

To confirm the ANOVA results, permutation tests were conducted. Based on the null hypothesis that the mean of Log(Hg) is the same across locations, permutations of the sample were constructed with location randomly assigned to each Log(Hg) value. SAS procedure `proc multtest` with the `perms` option was used to create 10000 new samples (N=93, same as the original sample). An ANOVA was run on each sample and the F statistic from each ANOVA was captured. The distribution of these F statistics represents a reasonable approximation of the true distribution of F statistics for Log(Hg) in this data under the null hypothesis (see figure 7). The F value from the initial ANOVA (24.17) is far outside the null hypothesis range of F values (max F = 6.91). Thus the finding of group differences is confirmed.
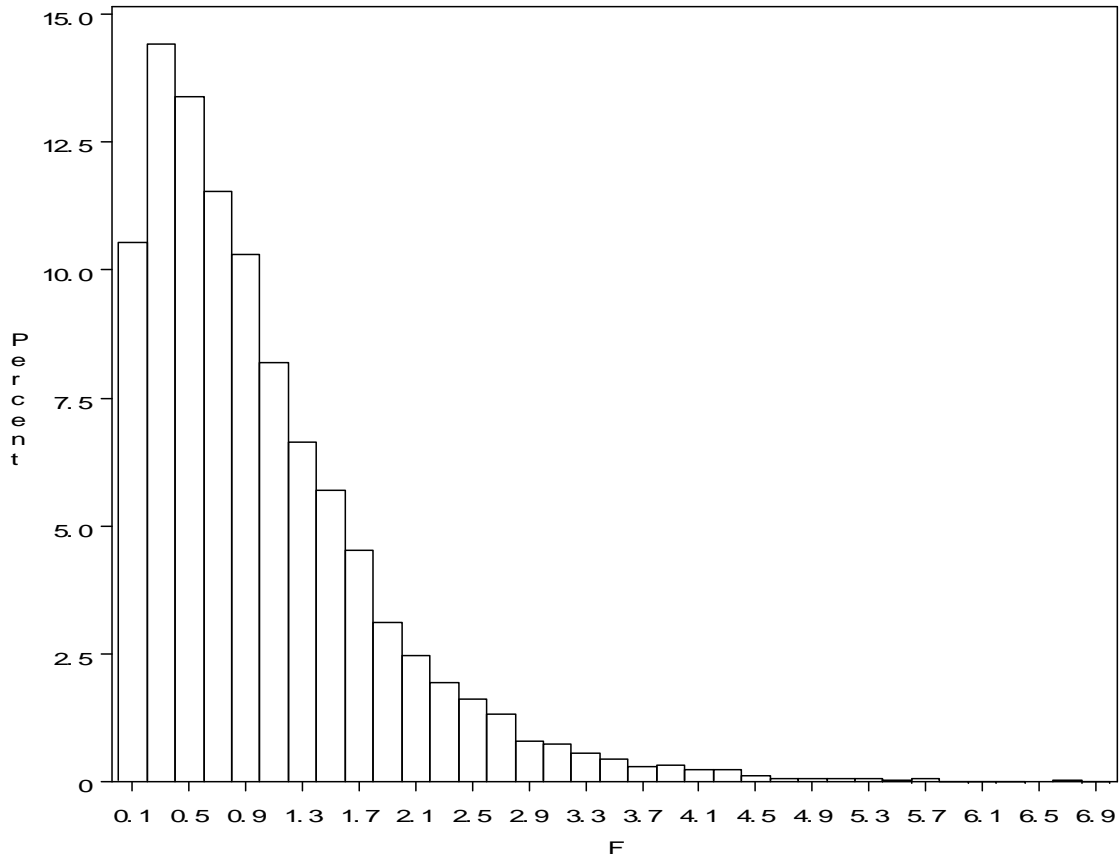
Figure 8. Distribution of F Values from Permutations for Overall ANOVA

The permutation procedure was repeated to find null hypothesis F distributions for the Pittsburgh contrast and the Allegheny River contrast. The F value found in the Pittsburgh contrast (65.83) was again far outside the range of F values under the null hypothesis (max F = 15.64). The mercury level in Pittsburgh is significantly different from the other groups.

The F value found by the Allegheny River contrast was within the range of F values under the null hypothesis (F = 0.03, range = 0.00-12.00), with a probability of 0.97. Mercury levels in Cheswick, Freeport and Ford City are not significantly different from each other.

3.2   REGRESSSION ANALYSES

3.2.1   Arsenic – Whole Sample

Models were constructed stepwise, as shown in Table 2. Location was entered last to allow for the effect of any other variable to appear.

Table 2 – Stepwise Regression for log(arsenic)

Step	Model	Degrees of Freedom	Type I Sum of Squares	F	p
1	Centered Std. Length	1, 83	0.82093958	1.28	0.2607
2	Centered Std. Length	1, 82	0.82093958	1.27	0.2634
	(Centered Std. Length)^2	1, 82	0.06335485	0.10	0.7552
3	Centered Weight	1, 82	3.11561306	5.08	0.0269*
4	Centered Weight	1, 81	3.11561306	5.16	0.0258*
	(Centered Weight)^2	1, 81	1.38190377	2.29	0.1343
5	Sex	2, 80	0.52021833	0.42	0.6603
	Centered Weight	1, 80	3.0379980	4.87	0.0301*
6	**Centered Weight**	**1, 79**	**3.11561306**	**7.32**	**0.0083***
	Location	**3, 79**	**16.69820230**	**13.08**	**<.0001***
7	Centered Weight	1, 76	3.11561306	7.08	0.0083*
	Location	3, 76	16.69820230	12.64	<.0001*
	Centered Weight*Location	3, 76	0.05379435	0.12	0.9467*

   * significant value

Weight and location are the only significant predictors of Log(As), with location explaining the majority of the variance, controlling for weight. However, weight does not explain significant variance when controlling for location ($F = 0.48$, $p = 0.4912$). The parameter estimates also reveal that the contribution of weight is not significant when location is in the model.

```
                                 Standard
   Parameter              Estimate      Error    t Value   Pr > |t|
   Intercept           -3.643116857 B  0.11405936   -31.94     <.0001
   centwgt              0.000118515    0.00017137     0.69     0.4912
   Location  Cheswick   0.955778375 B  0.25648910     3.73     0.0004
   Location  Ford City  1.177341838 B  0.20411402     5.77     <.0001
   Location  Freeport   0.776553536 B  0.20210355     3.84     0.0002
   Location  Pittsburgh 0.000000000 B      .           .         .
```

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The ANOVA contrast findings were upheld with weight in the model. Pittsburgh is significantly different from the other sites ($F = 35.01$, $p < .0001$) and the Allegheny River sites are homogenous ($F = 1.74$, $p = 0.1826$). Residuals were normally distributed.

3.2.2 Arsenic – Pittsburgh and the Allegheny River Group Separately

Because of the importance of location in predicting arsenic level, the Pittsburgh group and the Allegheny River group were analyzed separately for the effect of weight. Initial regression of Log(As) on weight in the Pittsburgh group showed no significant impact.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
centwgt	1	0.22776072	0.22776072	0.36	0.5532

Residual plots, Cook's Distance and DFBeta's were examined for influential points that may have washed out any differences. Two points were found with high Cook's Distance, indicating that they may have undue influence. When these two points are removed from the dataset, the effect of weight on arsenic in the Pittsburgh group becomes significant.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
centwgt	1	1.94927771	1.94927771	4.64	0.0384

There is no reason to believe that these data points are incorrect, however, so the initial finding of no significance stands.

For the Allegheny River group, weight was not a significant predictor of arsenic level. Residual plots, Cook's Distance and DFBeta's were examined for influential points that may have obscured any effect, but none were found.

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3.2.3 Mercury – Whole Sample

Models were constructed stepwise, as shown in Table 3. Location was entered last to allow for the effect of any other variable to appear.

Table 3 – Stepwise Regression for log(mercury)

Step	Model	Degrees of Freedom	Type I Sum of Squares	F	p
1	Centered Std. Length	1, 83	1.92796070	2.02	0.1591
2	Centered Std. Length	1, 82	1.92796070	2.00	0.1612
	(Centered Std. Length)^2	1, 82	0.16491867	0.17	0.6803
3	Centered Weight	1, 82	4.72297213	5.12	0.0263*
4	Centered Weight	1, 81	4.72297213	5.17	0.0256*
	(Centered Weight)^2	1, 81	1.62798179	1.78	0.1857
5	Sex	2, 80	0.67758845	0.36	0.6959
	Centered Weight	1, 80	5.26508156	5.66	0.0197*
6	**Centered Weight**	**1, 79**	4.72297213	**8.44**	**0.0047***
	Location	**3, 79**	10.48132172	**18.74**	**<0.0001***
7	Centered Weight	1, 76	4.72297213	8.19	0.0054*
	Location	3, 76	10.48132172	18.17	<0.0001*
	Centered Weight*Location	3, 76	0.11207535	0.19	0.9000

* significant value

Weight and location are the only significant predictors of Log(Hg), with location explaining the majority of the variance, controlling for weight. However, weight does not explain significant variance when controlling for location (F = 0.64, p = 0.4262). Again, the parameter estimates also reveal that the contribution of weight is not significant when location is in the model.

```
                                       Standard
       Parameter               Estimate      Error    t Value    Pr > |t|
       Intercept           -2.130867367 B   0.13076479   -16.30     <.0001
       centwgt              0.000157142     0.00019647     0.80     0.4262
       Location  Cheswick    1.449859336 B   0.29405517     4.93     <.0001
       Location  Ford City   1.332073978 B   0.23400909     5.69     <.0001
       Location  Freeport    1.404142955 B   0.23170417     6.06     <.0001
       Location  Pittsburgh  0.000000000 B      .             .         .
```

The ANOVA contrast findings were upheld with weight in the model. Permutation tests were necessary since the residuals were not normally distributed. Pittsburgh is significantly different from the other sites (F = 55.15, max F from permutations = 16.74) and the Allegheny River sites are homogenous (F = 0.11, range from permutations = 0.00-16.70), with a probability of 0.84.

3.2.4 Mercury – Pittsburgh and the Allegheny River Group Separately

Again, the Pittsburgh group and the Allegheny River group were analyzed separately for the effect of weight. Initial regression of Log(Hg) on weight in the Pittsburgh group showed no significant impact.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
centwgt	1	0.38312952	0.38312952	0.73	0.3975

Residual plots, Cook's Distance and DFBeta's were examined and one point was found with high Cook's Distance. It was one of the same points that stood out in the arsenic analysis. When this point is removed from the dataset, the effect of weight on mercury in the Pittsburgh group becomes significant.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
centwgt	1	2.24144282	2.24144282	7.20	0.0111

Again, however, there is no reason to believe that this data point is incorrect, so the initial finding of no significance stands.

For the Allegheny River group, weight was not a significant predictor of mercury level. Residual plots, Cook's Distance and DFBeta's were examined for influential points that may have obscured any effect, but none were found.

3.3   RISK ASSESSMENTS

Risk assessments for human consumption of contaminated fish tissue are based on criteria put forward by the US EPA (USEPA, 2000). As part of the Clean Water Act the EPA has developed recommended criteria for concentrations of pollutants in water. These recommendations also include concentrations of pollutants in fish. For arsenic, the recommended concentration for fish tissue is 0.014 mg/kg (parts per million). In the current study 78% of the fish had concentrations above the EPA criterion. See table 4 for the breakdown by location.

For methylmercury, the recommended concentration for fish tissue is 0.3 mg/kg. In the current study, no fish from Pittsburgh had concentrations above the EPA criterion, but 35% of the Allegheny River fish did (see Table 4).

Table 4 – Comparison to EPA Criteria for Fish Tissue

		As (EPA criterion = 0.014 mg/kg)		*Hg* (EPA criterion = 0.3 mg/kg)	
Location	N	# above	% above	# above	% above
Pittsburgh	39	22	56	0	0
Cheswick	8	8	100	4	50
Freeport	17	16	94	5	29
Ford City	29	27	93	10	34
Total	93	73	78	19	20

3.3.1 Calculating Average Daily Doses

In addition to the recommendations, the EPA also publishes reference doses for human ingestion of toxins. These doses are research-based and represent levels at which no adverse effects would be expected for people who ingest toxins at the reference dose over a lifetime.

In order to assess the risk to consumers of fish from the Pittsburgh and Allegheny River locations, the Average Daily Dose (ADD) must be calculated. The formula is:

$$ADD = \frac{Concentration * Intake * Frequency * Duration}{Weight * AveragingTime}$$

Concentration is the concentration of toxins in fish flesh in mg/kg. *Intake* is the amount of fish consumed in one meal. The EPA recommends using 228 grams (8 oz.) for adults and 114 grams (4 oz.) for children (under age 16). *Frequency* is the number of fish meals per day; 1 meal per day is used in these calculations. *Duration* is the number of days per week a fish meal is consumed. Surveys of subsistence and semi-subsistence anglers in the Pittsburgh region indicate these consumers eat an average of 4 meals of fish per week. *Weight* is the average weight in kg of the consumer. EPA recommends using the national averages 22kg for children age 3-8, 45 kg for children 9-15, 64kg for women of childbearing age and 70kg for other adults. *Averaging time* is 7 days (1 week).

The ADD can then be divided by the reference dose (RfD) to acquire the Hazard Quotient, the excess risk to consumers of these fish.

95% confidence intervals around the mean were calculated for Log(As) and Log(Hg) for each location and then exponentiated back to the original scale. Thus, a range of ADD and Hazard Quotients can be calculated.

For arsenic, the table of average daily doses is presented in Table 5. Dividing by the EPA reference dose of 0.0003 provides the corresponding hazard quotients in Table 6.

Table 5 -- Average Daily Doses of Arsenic

Concentration / Group	Pittsburgh		Allegheny River	
	Lower CI = 0.021mg/kg	Upper CI = 0.034 mg/kg	Lower CI = 0.056 mg/kg	Upper CI = 0.076 mg/kg
Children 3-8	0.000062	0.000101	0.000165	0.000225
Children 9-15	0.000030	0.000049	0.000081	0.000110
Women of Childbearing Age	0.000043	0.000069	0.000114	0.000155
Other Adults	0.000039	0.000063	0.000104	0.000141

Table 6 -- Hazard Quotients for Arsenic

Group	Pittsburgh		Allegheny River	
	Lower	Upper	Lower	Upper
Children 3-8	0.207	0.336	0.562	0.750
Children 9-15	0.101	0.164	0.270	0.367
Women of Childbearing Age	0.143	0.231	0.380	0.516
Other Adults	0.347	0.472	0.130	0.211

For mercury, the hazard quotients are presented in Table 7, omitting the intermediate table of ADD. The EPA reference dose for methylmercury is 0.0001 mg/kg/day.

Table 7 -- Hazard Quotients for Mercury

Concentration / Group	Pittsburgh		Allegheny River	
	Lower CI = 0.098mg/kg	Upper CI = 0.155mg/kg	Lower CI = 0.385mg/kg	Upper CI = 0.587mg/kg
Children 3-8	2.90	4.59	11.40	17.38
Children 9-15	1.42	2.24	5.57	8.50
Women of Childbearing Age	2.00	3.16	7.84	11.95
Other Adults	1.82	2.88	7.17	10.93

3.3.2   Arsenic and Cancer Risk

Hazard Quotient refers to non-carcinogenic risk. Inorganic arsenic is associated with multiple cancers, so separate calculations must be made to assess those risks. Again the US EPA has provided guidelines. The critical metric for cancer risk is Target Cancer Risk (TR). For inorganic arsenic, the EPA has set the Target Cancer Risk at 0.000001, or one in one million. The formula to calculate Target Cancer Risk is:

$$TR = \frac{Concentration * Intake * CSF * Frequency * Duration_{Carc}}{Weight * AveragingTime_{Carc}}$$

Intake and *Weight* are the same variables used in calculating average daily dose. *Concentration* here is the concentration of inorganic arsenic. Inorganic arsenic was not directly measured for the fish in this study, but 10% is an EPA-utilized estimate of inorganic arsenic from total arsenic (USEPA, 2003).

Because most cancers develop over a long period of time, the time elements of this formula are different than the ones used to calculate average daily dose. Here *Frequency* refers to meals per year. 4 meals per week becomes 208 meals per year. *Duration$_{Carc}$* is 30 years, an average length of time for exposure. *AveragingTime$_{Carc}$* is the average American lifespan, 70 years, expressed in days, 25,550. Obviously, these time frames can not be simply applied to risk assessments for children. The cancer risks calculated here apply to adults only.

There is an additional variable in this formula – *CSF* or Cancer Slope Factor. Unlike reference dose, which assumes a "safe" level of exposure, cancer risk assessment makes no such assumptions. Cancer risk is assumed to be proportional to exposure, with even a small exposure creating a small increase in cancer risk. CSF is an estimate of the likelihood of cancer per unit

intake of a chemical over a lifetime, calculated from animal experiments or epidemiological studies. The CSF for inorganic arsenic is 1.5 mg/kg/day.

95% confidence intervals for 10% of total arsenic were calculated. Table 8 shows the resulting cancer risks. These should be compared to the EPA target of 0.000001.

Table 8 -- Cancer Risk from Inorganic Arsenic

	Pittsburgh		Allegheny River	
	Lower CI = 0.0021mg/kg	Upper CI = 0.0034mg/kg	Lower CI = 0.0056mg/kg	Upper CI = 0.0075mg/kg
Adults	0.00000251	0.00000406	0.00000668	0.00000895

As the table shows, there is approximately a two-fold to 4-fold increase in cancer risk among regular consumers of Pittsburgh fish, and a 7- to 9-fold increase for people who consume fish from the Allegheny River locations.

3.3.3 Calculating "Safe" Consumption Limits

These hazard quotients and excess cancer risks indicate that current consumption rates among subsistence and semi-subsistence anglers and their families are unsafe. It is possible to use this data to calculate "safe" amounts to eat by using the reference dose and target cancer risk.

The greatest threat, cancer from inorganic arsenic, will be assessed first. The people who consume these fish do not have any direct way of affecting the concentration of inorganic arsenic that is in their food. Nor is it reasonable to expect them to simply eat smaller pieces of fish. The variable that is most easily changed is the frequency with which they eat the fish. A "safe" frequency can be calculated by algebraically modifying the target cancer risk formula:

$$freq = \frac{TR * Weight * AveragingTime_{Carc}}{Concentration * Intake * CSF * Duration_{Carc}}$$

Table 9 shows the "safe" frequencies using the EPA TR value of 0.000001 and the upper confidence limits for inorganic arsenic (to be extra conservative). Meals per month is meals per year divided by 12, always rounded down.

Table 9 -- "Safe" Consumption Frequencies based on Inorganic Arsenic Content

	Pittsburgh (Upper 95% CI = 0.0034)		Allegheny River (Upper 95% CI = 0.0075)	
	meals/yr	meals/month	meals/yr	meals/month
Adults	51.27	4	23.24	1

For "safe" methylmercury frequencies, the average daily dose formula is modified, using the reference dose (RfD) of 0.0001 in place of ADD.

$$freq = \frac{RfD * Weight * AveragingTime}{Concentration * Intake * Duration}$$

Again using the upper confidence limits, Table 10 shows the "safe" frequencies. The formula provides meals per day; multiplying by 30 (and again, rounding down) gives meals/month.

Table 10 -- "Safe" Consumption Frequencies based on Mercury Content

Concentration / Group	Pittsburgh (Upper 95% CI = 0.155)		Allegheny River (Upper 95% CI = 0.587)	
	meals/day	meals/month	meals/day	meals/month
Children 3-8	0.22	6	0.06	1
Children 9-15	0.45	13	0.12	3
Women of Childbearing Age	0.32	9	0.08	2
Adults	0.35	10	0.09	2

4.0   DISCUSSION

The current study found significant differences between the Pittsburgh site and the Allegheny River sites in terms of both arsenic and mercury levels. The Allegheny River sites were shown to be homogenous in terms of both arsenic and mercury levels. No other factors were shown to be significantly associated with mercury or arsenic levels. This was somewhat surprising as mercury is known to bioaccumulate and it was expected that larger fish would contain more mercury. However, previous research has varied as to the correlation between metal levels and fish size (Burger, et al., 2007) so perhaps these results are not out of the norm. No such association was expected for arsenic as it does not bioaccumulate in the same way.

The differences between Pittsburgh and the Allegheny River sites seem to indicate differing levels of pollutants in the water. One caveat to this finding is the factor of time. The Pittsburgh fish were all caught in 2005, while the Allegheny River fish were all caught in 2008. There is no evidence to suggest a drastic change in contaminant levels between 2005 and 2008, but the time difference must be noted. In a previous study, however, fish from further up the Allegheny River in Kittaning (see Figure 8) caught in 2005 were also shown to have significantly higher contaminant levels than Pittsburgh fish caught at the same time (Liu, 2007).

Subsistence and semi-subsistence anglers and their families are at increased risk of adverse health effects from both mercury and arsenic from consuming these catfish. The calculations presented in this paper are generally conservative. Intake estimates assume that all

arsenic and mercury consumed is absorbed. This is a reasonable assumption for methylmercury, but the metabolism of arsenic is complex. 100% absorption is likely an over-estimation. Exposure to these toxins from sources other than fish may add further risk. Dangerous levels of mercury are not expected to be commonly found in sources other than fish. Arsenic however may be present in drinking water and in soil and those sources may contribute additional exposure.
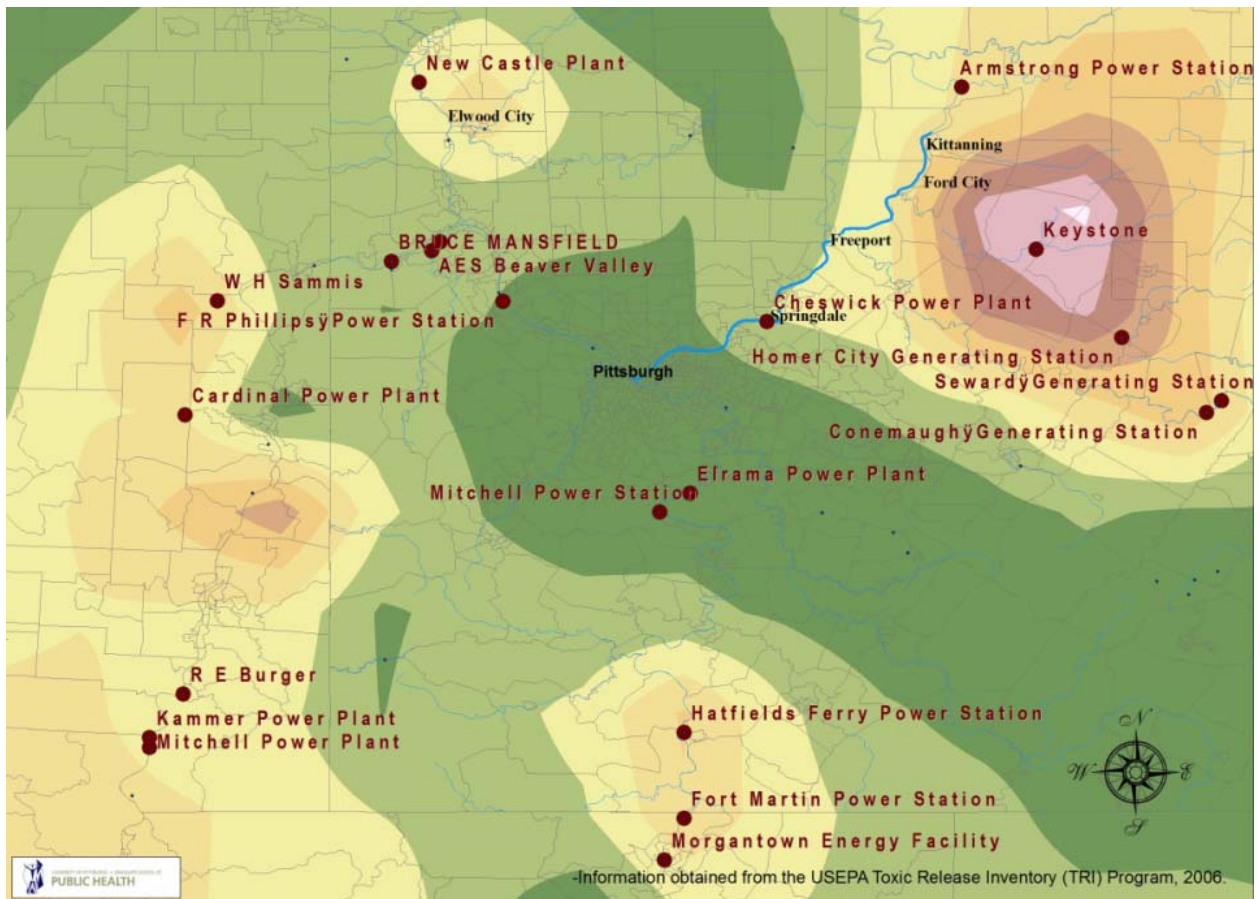

Figure 9. Map of Power Plants and Mercury Emissions in the Pittsburgh Region
(Michanowicz, 2009)

4.1.1 So Where Does This Mercury and Arsenic Come From?

Both mercury and arsenic are released by numerous industrial processes. In addition mercury and arsenic are both present in coal and are released when coal is burned. A: is the number one source of electrical power in southwestern Pennsylvania. There are numerous coal burning power plants in the area which may contribute to mercury and arsenic in the Allegheny River, as shown in the map above.

Mercury and arsenic are present in smoke stack emissions where they can be borne by the wind to locations quite distant from the actual sources. In addition mercury and arsenic can be contained in wastewater emitted from power plants. Many power plants remove gaseous contaminants from their smokestacks via the use of scrubbers. Scrubbers work by injecting a spray of water and an alkaline absorbent (often powdered limestone) into the smoke produced when coal is burned. That produces a chemical reaction that pulls pollutants out of the airborne emissions but produces a liquid sludge (Schobert, 2002). The harmful chemicals are not eliminated – they are just converted from gaseous to semi-solid form. Analysis of this waste has found toxins including arsenic, mercury, chromium, cadmium, lead, selenium and boron (Hopey, 2007). Finally, solid power plant wastes known as fly ash are often stored in such ways that they can leak into groundwater supplies and thus also end up in local rivers.

4.1.2 Further Concerns about Arsenic

In assessing the impact of inorganic arsenic this study used the general assumption that 10% of the total arsenic contained in fish bodies is inorganic. This assumption may not be correct. Estimates of inorganic arsenic concentrations in freshwater fish range widely

(Lorenzana, et al., 2009). In contaminated areas studies have found that that up to 40% of the total arsenic is inorganic (Buchet, et al., 1996). Speciated analysis of the arsenic in Allegheny River catfish was beyond the scope of this study but may be necessary to provide a true estimate of the risk to people eating this catfish.

Recent studies have also shown that certain forms of organic arsenic may have carcinogenic effects. Monomethylarsonic acid (MMA V) and dimethylarsinic acid (DMA V) are created as the body metabolizes inorganic arsenic, and are thus part of the carcinogenic activity. MMA V and DMA V also exist independently in nature and are found in some fish (Schoof & Yager, 2007). DMA V has been shown to induce bladder cancer in rats but the mechanism is not well understood and may not translate to humans (Cohen, et al., 2006). The EPA has recommended further study.

4.1.3   Recommendations

The current fish advisory for Pittsburgh of no more than 1 channel catfish meal per month is sufficient. Arsenic should be added to the contaminant watch list, which currently only covers PCB's mercury and chlordane.

The fish advisory for the Allegheny River should be strengthened. Currently there is no advisory regarding channel catfish from the upper Allegheny River, other than the statewide recommendation of no more than one wild caught fish meal per week. A local advisory of no more than one meal per month should be instituted.

Sources of these contaminants need to be located and measures put in place to reduce emissions. In addition, reducing the use of coal as a power source should be a priority. This can

be accomplished by both reducing electricity consumption and by shifting more demand to cleaner resources such as solar and wind energy.

4.1.4 Conclusion

Deterring people from eating fish in general is not the aim of this study. Concerns about contaminants do not negate the health benefits of eating fish. Fish should still be part of a balanced diet. For most populations, the health benefits provided by fish outweigh the hazards. Consumption of wild caught fish from Pittsburgh and the Allegheny River and any other contaminated areas, however, should be limited in order to reduce the risks of adverse health effects.

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