Development of a Community Based Participatory Environmental Research Project: Focus Groups to Investigate Fish Consumption Patterns. A Screening Assessment for Metals and Estrogenicity in White Bass and Channel Catfish Caught in the Three Rivers Area of Pittsburgh, Pennsylvania

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Background of the Problem in the Three Rivers Area

Subsistence fishing provides nutrition for some low income residents of the Allegheny County area. Additionally, sports fishing groups increasingly angle in the waters of the Allegheny, Monongahela and Ohio Rivers, known as the Three Rivers Area (TRA), for predatory species including large and small mouthed bass. Information is not generally available regarding the demographics of these fisherman in the TRA; the species of fish caught and preferred by each group for table usage; the sizes of fish retained for the kitchen; the methods used to clean and prepare as well as cook wild fish for eating; and the types and amounts of fish consumed in any given time period of each season. Initial information from both representatives of sport-fishing and meat-fishing groups indicates that the White Bass and the Channel Catfish are important fish for table usage in Western Pennsylvania and Allegheny County, descriptive information obtained from the

Pennsylvania Fish and Boat Commission (PFBC) on each fish is presented in Appendix A.
There is abundant evidence that legacy wastes from the Iron and Steel Industry (ISI) have and continue to contaminate fish habitat through; runoff and leeching from RCRA, Superfund and Brownfield sites; transport from contaminated surface soils, subsurface media and groundwater transport; and residual toxins deposited in river bottom sludge and sediment. Ongoing industrial operations, overflow from municipal wastewater plants and urban runoff adds contaminants including polycyclic aromatic hydrocarbons (PAH), household chemicals and products, estrogenic compounds and heavy metals to each rivers ecosystem.

Upon entering the rivers water contaminants enter a complex food web and can move between trophic levels. These contaminants can also be deposited in river sediment. Plants and animals that are low on the food chain take up contaminants as well as nutrients via bio- concentration and bio- accumulation. Bio-magnification of contaminants can occur as you move up the food chain to predatory fish through processes such as the concentration of many organic pollutants in lipids and the binding of heavy metals to proteins. Bottom feeding fish such as the bullhead catfish and common carp are particularly susceptible to contaminant ingestion during feeding activities in polluted river bottoms. Their bottom stirring activity also leads to resuspension of sediments into the river environment, which can lead to exposure of other species to contaminants in these resuspended particles. Sediments on river bottoms act as a sink and source of contaminant dispersion during high water periods. Additionally, sediments containing contaminants deposit in slow water pools behind dams, built for navigation, these pools happen to be hot spots for anglers.

There exists the potential for human exposure to a variety of contaminants from ingestion of fish caught in the TRA. Detailed studies of the Mahoning River (MR), used for ISI and municipal waste disposal in analogous fashion, to the TRA of Pittsburgh, have shown bottom sediment containing extremely high levels of heavy metals (mercury, lead, zinc, copper, cadmium, silver and iron), grease, oil, organic compounds, PCBs and PAH and pesticides. According to the Pennsylvania Department of Environmental Protection (DEP) Watershed Management tool eMapPA major river segments of both the Three Rivers and major feeder streams are considered impaired under their 305(b) layer. This means that impaired sections of the river and streams are not attaining at least one of the following uses;

- Aquatic Life use attainment - The integrity reflected in any component of the biological community. (i.e. fish or fish food organisms)
- Human Health use attainment - The risk posed to people by the consumption of aquatic organisms (ex. fish, shellfish, frogs, turtles, crayfish, etc.) or the ingestion of drinking water
- Recreational use attainment - The risk associated with human recreation activities in or on a water body. (i.e. exposure to bacteria and other disease causing organisms through water contact recreation like swimming or water skiing)

Particularly disturbing is information from the Pennsylvania Fish and Boat Commission that spots for catching large predatory fish are located at the mouth of streams feeding the Allegheny River, many of which are continuously or intermittently impaired.

It has been reported that the estrogenic activity of effluent-dominated streams is increasing. These estrogenic compounds are theorized to come from pharmaceuticals - especially hormone replacement drugs, cosmetics and creams, animal feedlot estrogenic compounds, building materials and household products and natural products. Since rivers and streams have been channelized causing substantial runoff from urban areas and suburban developments, without sufficient time from swamp, eddy or pooling sedimentation or soil and sub-surface cleansing of water, these estrogenic compounds quickly find there way into large stream bodies and the municipal water supply. There is no treatment for these chemicals at system intakes presently.

Screening for estrogenic effects in male fish in Germany, Norway and the United States has shown highly elevated levels of blood plasma vitellogenin concentrations in male fish. Vitellogenin is a protein used for egg yolk production and would not be expected to be found in high concentrations in male fish. The British Royal Society released a report in June, 2000 which states that all sewage effluents in the UK have enough estrogenic activity to feminize fish, further they have discovered that some fish in ALL rivers of the United Kingdom are intersex. The Royal Society study found that in rivers with high levels of effluent that all male fish were intersex to varying degrees. Similar findings have been described in the Upper and Lower Potomac River by the United States Geological Survey (USGS). Fish in the Upper Potomac were being studied in order to determine if there was a link to higher than normal human cancer rates in the area.

Store bought fish consumption is also an important component of the diet of anglers and non-anglers in the Pittsburgh area. Their exists no data on their fish purchasing and consumption patterns or methods of cooking. Data are also missing on the level of contaminants in store bought fish in the Pittsburgh area, of particular interest are those fish labeled organic in stores both White Bass (or equivalent trophic level) and Channel Catfish are commercially available.

Study Objectives

1. Identify fishing groups to partner with for the study and explore the TRA for fishing holes to identify groups of meat-fishers and low income fishers. Conduct

two (2) focus group meetings with identified groups in order to better understand qualitatively – group demographics, attitudes to river water quality, the locations of fishing, types of fish taken, sizes preferred, number of fish meals eaten per week, the serving size eaten at each meal, and the methods used to clean and cook each type of fish. Build community environmental capacity within these groups to include an education program regarding contaminants in fish so that groups can actively participate in the initial screening and possible downstream research studies and understand the risks associated with wild fish consumption.

2. In conjunction with Objective #1, obtain nautical maps of the TRA for the purpose of establishing the boundaries of the study. Maps will be digitized so that the locations of fishing spots and all catches may be geo-referenced. Additionally, the locations of sewer outflows, water intakes, active and inactive industries, and brownfield, CERCLA and RCRA sites will be shown on finished maps.
3. Catch 40 White Bass and 40 Channel Catfish by rod and reel (the way that fishers catch their fish so that more accurate risk assessments of the fish can be made. The study will attempt to achieve a balanced design so that 10 fish of each species come from the Allegheny, Monongahela and Ohio Rivers. Additionally, 10 fish of each species will be caught in the upper reaches of the Allegheny and Monongahela Rivers and 10 fish of each species will be bought commercially.
4. Prepare all fish for analysis including division of fish into two fillets- one for metals analysis and one for estrogenic activity. Each organism's kidneys, gonads, heart and liver will be archived.
5. Screen all fish for metal contamination. Fish will be analyzed by ICP-MS for at least mercury, cadmium, arsenic, manganese, lead, chromium, copper, zinc, selenium and cobalt. Report measures of central tendency and the variability of each contaminant in each fish species in the TRA and Upper River areas. Evaluate the metal concentrations between all sampling sites to the extent possible given the small sample sizes.
6. Screen all fish for estrogenic activity using two assays (See methods below). Evaluate the efficacy of testing the fish extracts in 3 breast cancer cell lines.
7. Prepare a report detailing the results of the focus group work, analyze fish consumption patterns qualitatively. Report the results of and analyze the metals and estrogenicity fish screens. Include all maps generated during the study. Develop a detailed list of recommendations for further study of TRA fish consumption patterns and fish at each tropic level including their habitat and comment on the policy implications of findings.
8. Preliminarily assess health risk from eating locally and commercially caught White Bass and Channel Catfish.

Methods

The methods of the study can be divided into four major parts;

1. **Focus Groups**- At least two focus groups will be convened to obtain in-depth qualitative information on fish consumption patterns, size and species preferences, favorite fishing areas, and methods of cleaning and cooking. One

focus group will be made-up of spots fishing group members and another from subsistence style fishers. These focus groups will be informal and participants will be encouraged to talk about fishing experiences, perceived needs, and observations and perceptions of water and fish quality. Each session will last approximately 90 minutes and the group will not contain more than 10 members to encourage interaction between members. One objective of the focus groups is to gain information to develop a Community Based Participatory (CBP) approach to to help define the research objectives for further research on the TRA.

Subsistence type and sports fishermen will be reached by river bank and power boat surveys on the Three Rivers. Launch areas will be targeted. It is anticipated that sports fisherman can be reached through regular meetings of their groups while the subsistence group will be primarily contacted while fishing.

2. Fish Catch

Fish will be caught from shore and from boats by GSPH and UPCI researchers. All researchers will follow the regulations of the Pennsylvania Fish and Boat Commission. GSPH and UPCI researchers will be assisted in fishing spot locations, and fish identification by Venture Outdoor Director, Sean Burke. The weight, sex and standard length of all fish species caught will be noted. The GPS coordinates of all White Bass and Channel Catfish will be recorded. Each fish will have a unique specimen number and will include the initials of the river the fish was caught in, the date, the fisher's initials, and the tissue type. The heart, gonads, liver and kidneys of each fish will be archived for analysis in subsequent studies. Dissections will be performed in the field where possible. Other species of interest, caught by researchers, which can be legally taken will be sampled and archived for analysis in subsequent studies.

3. Analysis of Fish for Metals- One fillet of each fish will be used for metals analysis. Amounts over those necessary to perform the analysis will be achieved for latter use. Tissues will be 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 enable us to routinely prevent background contaminants from entering the samples.

Samples will be analyzed using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) for a suite of 29 metals including As, Cd, Cr, Mn, Pb, Se, Co, Cu, Fe, Ni, Zn. Mercury (Hg) will be measured by a Cold Vapor Technique.

4. Estrogenic Activity/General-One half of each fish including the skin of each fish will be retained for the estrogenicity study A one inch square, plus replicate, will be taken from each fish. The square will be centered on the lateral line and will extend one-half inch above and below the lateral line. Fish parts would be extracted by organic solvent extraction as determined in methods development, and samples would be tested in two assays to determine estrogenicity. A screening assay, the competitive cER-α assay, will be used to screen all samples. It is less expensive, and generally predicts interactions with estrogen receptors. Depending on the results of this assay, we predict that we would screen about 20% of the same samples using the cER-ß assay, which is more expensive but can detect other types of estrogenic substances. After analysis of these data, we would evaluate the efficacy of testing the fish extracts in the breast cancer cell lines. We predict that 20% of the samples would be tested in our standard assay

Competitive <u>in vitro</u> cytosolic estrogen receptor (cER) assay: This assay determines the ability of agents to interact with the estrogen receptor and generally predicts estrogenicity. Details of our specific assay have been published (Eagon et al. 1996). Briefly, aliquots of cytosol prepared from livers of ovariectomized female rats (a source of ER-α) or cytosol from ventral prostate of male rats (a source of ER-ß) are incubated with 5nM [^{3}H]-E_2 in the absence (control) and presence of test substances. Estrogens E_2 and diethylstilbestrol (DES), the potent and nonaromatizable androgen dihydrotestosterone (DHT), and the phytoestrogens genistein and/or coumestrol will be used in concentrations of 5nM-50mM, which represent a range of 1X-10,000X the concentration of labeled E_2. The individual **FISH** extracts will be tested from dilutions of 1/20th to full-strength, or more dilute as needed. The mixtures are incubated at 4°C overnight, and bound ligand is separated from free by use of spin columns made of P6 resin (BioRad Inc.) (2) or by dextran-coated charcoal treatment (3). The ER binding assay can be used to estimate an "E_2 equivalence" of the crude extracts by comparing the competition of the extract with a dose-response curve generated using either E_2 or DES as a standard competitor. All competition is expressed relative to the results for control binding (100%) in which there is no competitor present.

Breast cancer cell lines- We use a number of breast cancer cell lines that vary in their expression of ERs (MCF-7, HTB-129 (also known as T47D), BT-20) and normal breast cells (HMEC line). The MCF-7 cell line expresses primarily ER-α, HTB-129 express primarily ER-ß, and BT20 cells are ER-negative.

Initial end points measured will be changes in cell number and viability. The proliferation assay is performed in phenol red free RPMI medium supplemented with 10% charcoal/dextran-stripped fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells will be seeded into 96 well plates at a concentration of 5000 cells/well. Twenty-four hours post plating, the cells will be treated with either **FISH** extract, estradiol ($1x10^{-9}$) *or both*. The plates are allowed to incubate for 72 hours, at which time the proliferation index relative to untreated cell controls and to the E_2-treated controls (10nM) will be

determined using a commercial assay kit, MTS Proliferation Assay (Promega). Other treatment controls will include the *phytoestrogens genistein and coumestrol* (each at several doses, 1-100nM). An increase in proliferation index will be taken as an estrogenic response. In contrast, a reduction in the E_2-stimulted proliferation due to the presence of the extract will be taken as evidence that the extract may contain antiestrogenic substances. Use of the ER-negative BT-20 cells allows us to detect any cell toxicity that is not related to hormonally active components of the extracts. To confirm the findings that cell proliferation is mediated by ERs, another set of cells treated with extract will be simultaneously treated with the antiestrogenic tamoxifen or ICI 182,780. **FISH** extracts will be tested over a broad range of concentrations, to be determined by preliminary experiments.

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Background Literature

Risk for neurodevelopmental and reproductive toxicity and endocrine disruption as well as immunosuppression and cancer in humans from dietary exposure to organohalogen substances (OHS) has received increasing attention. OHS include polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (4,4´-DDT), polybrominated diphenyl ethers (PBDEs) and pentachlorophenol (PCP). An investigation of elementary schoolchildren supports the hypothesis that PCBs can have a mutable or even detrimental effect on levels of thyroid hormones, with lower triiodothyronine (T_3) and an increase in thyroid-stimulating hormone TSH. The investigators found a statistically significant positive association between the mono-*ortho* congener PCB 118 and TSH as well as statistically significant negative relationships of PCBs 138, 153, 180, 183, and 187 to T_3. There is an association between *in utero* PCB exposure and impairments of memory during infancy and childhood, studies also indicate PCB exposure during adulthood is associated with impairments in memory and learning. Consumption of fish from contaminated water bodies is an important source of exposure to OHS.
Recent studies have shown effects of fish consumption and PCB exposure on thyroid hormones. Thyroid hormones are necessary for the development of brain function and cell growth; because of this, appropriate levels of peripheral thyroxine (T_4) and triiodothyronine (T_3) are especially important in childhood. Deficiency of thyroid hormones can subsequently result in a serious delay in neurological development. Serum PCB level and consumption of Great Lakes fish are associated with significantly lower levels of thyroxine (T_4) and free thyroxine index (FTI) in women and with significantly lower levels of T_4 in men. Fish consumption, but not PCB level, is significantly and inversely associated with triiodothyronine (T_3) in men. Among men, there are significant inverse associations of both PCB and fish consumption with sex hormone-binding globulin (SHBG)-bound testosterone. Another study has reported that there is a significant relationship between consumption of Great Lakes fish and learning and memory impairments in Michigan residents, ages 49-86.

In a study published in Environmental Health Perspectives in 2004 investigators examined the association between Great Lakes sport-caught fish consumption and breast

cancer incidence as part of an ongoing population-based case-control study. The investigators identified breast cancer cases 20-69 years of age who were diagnosed in 1998-2000 (*n* = 1,481) from the Wisconsin Cancer Reporting System. Female controls of similar age were randomly selected from population lists (*n* = 1,301). Telephone interviews were performed to gauge consumption of Great Lakes sport fish, total fish and breast cancer risk factors. The researchers found consumption of fish from the Great Lakes region was associated with elevated breast cancer risk in premenopausal women (RR = 1.70; 95% CI, 1.16-2.50).

Most American's are exposed to methylmercury through contaminated fish including subsistence and sports fishing. Methylmercury, most mercury in fish is in this form, affects reproduction, the cardiovascular system and most particularly the brain and central nervous system. Methylmercury levels found in both salt and freshwater fish have been found to pose a serious health risk to the public. Epidemiological studies of children exposed to low or moderate levels of mercury before birth show an association with neurological and development impairment. Fetal methylmercury poisoning was found in 1958 in Minimata, Japan from ingestion of contaminated fish and shellfish. Examination of these children revealed a high incidence of mental retardation, cerebellar ataxia, primitive reflex, dysarthria, seizure, and pyramidal signs.