Dover Chemical – Dover, Ohio

Sugar Creek

Fish, Health Assessment, Contaminant Exposure Analysis
Work Plan

April 21, 2015

Objectives

1. Evaluate exposure of natural resources (fish) to selected hazardous substances released from the Dover Chemical facility to Sugar Creek.

2. Confirm the spatial extent of hazardous substances potentially released from the Dover Chemical facility to Sugar Creek in fish tissue.

3. To the extent possible, establish relationships between exposure to hazardous substances and biological injury in Sugar Creek through fish health assessments.

Sampling Activities

Sampling will be conducted in June 2015

Fish Tissue Concentrations

Whole body fish tissue concentrations will be assessed at 3 locations in Sugar Creek. Fish will be captured using static fyke nets and other trapping methods including electrofishing. Sampling locations are listed in Table 1. Physical habitat of Sugar Creek will be recorded at each fish sampling site and will be used in the overall environmental assessment of the stream

Fish Health Assessment

Evaluate biological health indicators related to exposure to hazardous substances present in contaminated water and sediments, (*e.g.*, physical anomalies, immunosuppression, and reproductive effects). Fish will be collected from each sample location and analyzed for the parameters in Table 2. Fish health assessment will include species, length and weight recorded for each fish, as will the date and time of collection. Fish species (Table 3) used in fish health assessment (Table 4) will be held live in aerated coolers until anesthetized with MS222 and necropsied. Each fish for contaminant analysis will be labeled with a unique sample number, placed in a plastic ziplock bag and held on ice until they can be shipped to the analytical laboratory. Larger fish will be analyzed as single fish, while smaller individuals may be composited based on analytical laboratory requirements.

Results

The results of the data collected will provide information on the level of chemical contaminants in fish tissue samples from Sugar Creek in the vicinity of Dover Chemical. The fish health assessment data will be used to potentially link injuries to hazardous substances that have been released into the environment in the vicinity of Dover Chemical.

Quality Assurance/ Sampling Methods

Ohio EPA Manuals

All physical and biological, field, data processing, and data analysis methods and procedures adhere to those specified in the Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices (Ohio Environmental Protection Agency 2009), Biological Criteria for the Protection of Aquatic Life, Volumes II - III (Ohio Environmental Protection Agency 1987b, 1989a, 1989b, 2006a, 2006b). Fish tissue sampling protocols follow the Ohio EPA Fish Collection Guidance Manual (Ohio EPA 2004).

Fish Health Assessment

- Necropsy-based assessment – A full necropsy will be completed. Any grossly visible external or internal abnormalities will be documented. Presence of external and internal parasites will be documented. Raised white or reddened lesions (presumptive papillomas or squamous cell carcinomas), abnormal barbels, raised black lesions (presumptive melanomas), discolored skin areas (melanistic spots) and ulcerated areas will be removed and preserved for later histopathological diagnosis.

- Blood analyses – Blood will be drawn from each fish. Blood smears are made immediately for later genotoxic assays. Plasma will be collected for thyroid hormone, reproductive hormones and vitellogenin.

- Condition indices – Each fish will be weighed, measured, bled and scales/spines/otiliths taken for aging. In addition, during the necropsy, liver, spleen and gonad will be weighed. From this data, condition factor, size at age, hepatosomatic, splenosomatic and gonadosomatic indices can be calculated.

- Immune function assays – Portions of head kidney will be harvested aseptically and shipped to the Leetown laboratory for immune function assays. These will include macrophage, lymphocyte and natural killer cell functions.

- Histopathology – Liver, spleen, kidney, gill, gonad and any gross external abnormalities will be fixed and prepared for histopathological analyses (as described in Appendix #3). A variety of histologic biomarkers, including (but not limited to) gill cartilage deformities, hepatocyte vacuolization, altered cell foci, bile duct proliferation, neoplastic changes, nephron regeneration and macrophage aggregates in spleen and liver will be documented and in some cases, quantified.

- Reproductive biomarkers – The reproductive hormones 17β estradiol, and 11 ketotestosterone) will be measured in plasma, as will vitellogenin. Gonad histology will be used to confirm sex, determine reproductive stage, and detect the presence of atresia, intersex, neoplasia, ceroid deposits, Sertoli cell proliferation and other abnormalities.

- Genotoxic markers – a pair of blood smears from each fish will be used to measure micronuclei frequency.

- Xenobiotic-metabolizing enzyme activity (EROD) – During the necropsy, pieces of liver will be rapidly frozen and used to measure EROD activity.

Table 1. List of sampling locations (fish - F, Sugar Creek, 2015)

River Mile	Sampling Media	Location	Purpose
Sugar Creek			
3.4	F	Upstream Dover Chemical	Background site
1.9	F	Adj. Dover Chemical	Near-field/adjacent conditions
1.3	F	Downstream Dover Chemical	Far-field/downstream

Table 2. List of chemical parameters to be analyzed fish samples Sugar Creek, 2015.

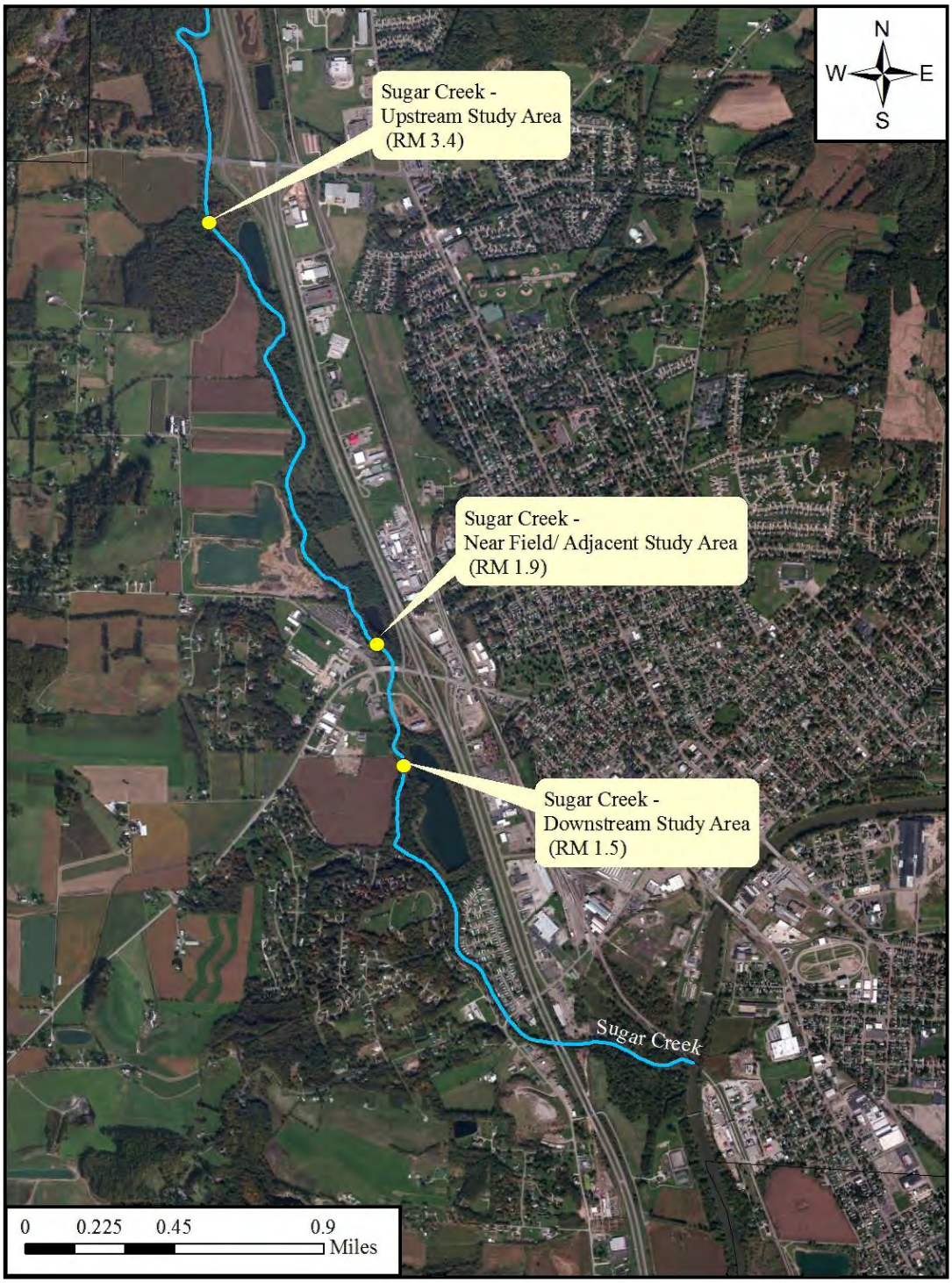
Parameters	Method	Fish Tissue
polychlorinated dibenzo-p- dioxins	USEPA 1613B	All samples
polychlorinated dibenzo furans	USEPA 1613B	All samples
polychlorinated biphenyls (congeners)	USEPA 1668A-X or 8082X	All samples
chlorinated pesticides (including hexachlorobenzene)	USEPA 1656A or 8081A	All samples
percent lipid	gravimetic	All samples

Table 3. Targeted Fish Species Collected for Fish Health Assessment

Fish species	Trophic Level	Contaminants Analysis	Fish Health Assessment
smallmouth bass	carnivore	whole body	Smallmouth bass
sand shiner, spotfin shiner, bluegill, northern hog sucker,	insectivore	whole body	bluegill
central stoneroller	herbivore	whole body	
Carp, white sucker	omnivore	whole body	White sucker

Table 4. General Outline of the Fish Health Study 2015

Media	Parameters	Purpose
Fish	Necropsy	General health
Fish	Condition indices	General health
Fish	Macrophage, lymphocyte and natural killer cells functions	Immune function
Fish	Histopathology – liver, spleen, kidney, gill, external abnormalities	General Health, Biomarkers of contaminant exposure
Fish	17β estradiol 11 ketotestosterone vitellogenin	Reproductive status and health
Fish	Gonad histology	reproductive health



Sugar Creek, Tuscarawas County, Dover, Ohio. 2014 Surface Water and Fish Sampling Locations

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