Attachment 4

Evidence of Endocrine Disruptors and Immune System Suppressors in the Results of the 2007 Trace Organics Monitoring Project on the North Fork of the Shenandoah River

This document provides an overview of the endocrine system, the way in which certain chemicals can disrupt that system and the evidence for such chemicals being found in the samples collected by the Friends of the North Fork in 2007. This document should be read in conjunction with the report from the Friends entitled "River Health Report: Organic Chemicals in the North Fork and their Relationship to Recent Fish Kills and the Intersex Traits of Smallmouth Bass" and the laboratory report from USGS entitled "Investigation of Organic Chemicals Potentially Responsible for Mortality and Intersex in Fish of the North Fork of the Shenandoah River."

The endocrine system consists of glands and hormones (chemical messengers) that regulate the growth, metabolism, reproduction, and the functioning of many organs of an animal. The major hormones that regulate the reproductive system are estrogens and androgens (e.g., testosterone).

Endocrine disruptor chemicals (EDCs) are synthetic or natural chemicals that can interact with estrogen receptors in animal cells, alter the function of the endocrine system, and adversely affect the animal's health. EDCs mimic natural hormones by virtue of their size and molecular structure. Even at low concentrations they can stimulate, block, or modify reactions in the endocrine system.

It has been reported (Thacker, 2005) that there may be as many as 87,000 chemicals that can act as endocrine disruptors. However, the vast majority of these chemicals have not yet been adequately tested to determine whether they are ***known*** or merely suspected EDCs. Much more research is needed to establish mechanisms of disruption, dose/response relationships, and the effects of mixtures of EDCs (Kavlock et al., 1996; National Research Council, 1999). Known natural EDCs are found in some plants (phytoestrogens) or in fungi (mycotoxins) on plants, but in general these are benign to animals.

Synthetic chemicals that are EDCs include: dioxins; PCBs; benzo(a)pyrene; phthalates; bisphenol A; alkylphenols; many insecticides, herbicides, and fungicides; heavy metals, and synthetically produced hormones identical to natural hormones, such as those in human oral contraceptives and certain animal feed additives (National Research Council, 1999; European Commission for the Environment Research website http://ec.europa.eu/research/endocrine/background_disruption_en.html

Evidence that endocrine disruption has occurred in the North Fork comes from the work of Dr. Vicki Blazer of the U.S. Geological Survey and her colleagues. They found that all 8 male smallmouth bass they collected from the North Fork in the fall of 2004 displayed intersex characteristics. The male fish had precursor egg cells within their reproductive organs (testicular oocytes); this phenomenon is suggested as a biological marker for endocrine disruption (Blazer et al., 2007). Importantly, endocrine disruption has also been shown to compromise or modulate the immune systems of several fish species (Aaltonen et al., 1997; Baier-Anderson and Anderson, 2000; Dunier et al., 1995). Immunomodulation in the North Fork has been suggested by the finding of bacteria, fungi, and parasites as causes of skin lesions on the fish (Blazer et al., 2007). Whether such immunomodulation has allowed normally warded-off diseases to prevail, resulting in fish kills, is a major topic of investigation by researchers working with the Shenandoah River Fish Kill Task Force.

We now turn our attention to the specific chemicals that were found in the river during the 2007 sampling program. As reported in the USGS report, a number of organochlorine pesticides and PCBs were measured in the samples collected with the Semi-Permeable Membrane Device (SPMD). Concentrations greater than the method quantification limit (MQL) were found for the pesticide trifluralin, the pesticide/fungicide hexachlorobenzene, the insecticide chlorpyrifos, the now-banned pesticides trans-chlordane and cis-chlordane, related metabolite oxychlordane, and related constituent trans-nonachlor. DDT metabolites o,p'DDE and p,p'DDE were also found, as were heptachlor epoxide (formed from the banned pesticide heptachlor) and dieldrin (used in termite control and as a wood preservative). PCBs were detected but were at concentrations below the MQL. All of these chemicals are known or suspected EDCs.

Similarly, other agricultural herbicides and insecticides were found in the Polar Organic Chemical Integrative Sampler (POCIS) sampler. Concentrations greater than the MQL were found for atrazine, desethylatrazine (a degradation product of atrazine), prometron, simazine, and carbaryl. All of these are known or suspected EDCs

Analyses for three natural hormones and one synthetic hormone detected only 17 α-ethynylestradiol, the synthetic estrogen hormone found in birth control pills, above the MDL in the POCIS sampler at Mt. Jackson deployed in the late spring period.

Yeast estrogen screen assays completed by the USGS on both the SPMD and POCIS extracts show that relative estrogenicities of the mixture of chemicals from POCIS are many times more than for SPMD at equivalent deployments. In fact, the SPMD levels were viewed as typical for background levels while the POCIS levels were particularly high. Seemingly, this indicates that intersex consequences should be more attributable to polar chemicals that are collected in the POCIS. However, further investigation could show this not to be the case. The non-polar lipophilic chemicals in the SPMD will bioaccumulate to a much greater extent than will polar hydrophilic POCIS chemicals. Biomagnification of SPMD chemicals as they move up the food chain to the top predators, especially smallmouth bass, may be an important factor.

The number of chemicals sampled by SMPD and POCIS and carefully analyzed in this North Fork study is indeed impressive. However, because of the vast number of known or suspected EDCs, it cannot be comprehensive for EDCs. Some of the known EDCs not analyzed in this study include bisphenol A (lacquer for lining food cans), nonylphenol and alkylphenol ethoxylates (surfactants, plasticizers), phthlates (plasticizers), dioxins, polybrominated diphenyl ethers (flame retardants), and heavy metals. One chemical that should be looked at closely is arsenic. Arsenic is a known EDC (Watson and Yager, 2007). The state has observed arsenic in some fish from the area. One potential source of arsenic in the river is roxarsone, an arsenic containing compound that is added to some poultry feed and can end up in poultry litter that is used as a fertilizer. It is now under study for its environmental effects (Schreiber et al., 2004).

This trace organics monitoring study has verified and quantified the presence of a number of endocrine disrupting chemicals in the North Fork of the Shenandoah River. There may be more. This study is a vital part of the investigations to determine the connection between EDCs, intersex condition in fish, fish kills, and the health of the river.

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