Supplemental Material for EHP9334ART Ryan et al. Biomarkers

The following material is supplemental to the manuscript Using Biomarkers to Inform Cumulative Risk Assessment by Ryan PB, Burke TA, Cohen Hubal EA, Cura JJ, and McKone TE. It contains the Section entitled **Endocrine Disruptors – An Ecological Point of View** and Supplemental Figures 1 and 2 that describe the Framework Applied to Neurobehavioral Endpoints Case Study and the Framework Applied to Endocrine Disruption Endpoints Case Study.

Endocrine Disruptors – An Ecological Point of View

Background

Ecosystems may offer sentinel effects with respect to ultimate human outcomes and may offer an improved understanding of the relationship between multiple sources of numerous contaminants, and the myriad of effects they can produce. Certain contaminants, because of their environmental persistence and slow degradation, are dispersed widely in the environment. Ideally, a biomarker should discriminate among populations from various regions and account for this widespread dispersion. There is ample evidence of persistent organic pollutants (POPs), such as PCBs, in organisms from developed countries. There is also evidence of food chain accumulation from animals and contaminated media in remote ecosystems. Marine animals and fish are contaminated with PCBs, organochlorine (OC) pesticides, and heavy metals in the Canadian Arctic (Muir et al. 1990), while in a Himalayan snowpit, there are organic compounds indicative

of petroleum residues such as automobile and diesel exhaust (Xie et al. 2000). In the Puget Sound and North Pacific Ocean Harbor, PCBs have been detected in seals, sockeye salmon, and albatross (Ross 2004). Anthropogenic chemicals may spread throughout the environment through ingestion of migrating prey (Ross 2004), direct uptake, the transport of volatile chemicals through the atmosphere, or the transport of ionic and polar compounds dissolved in water (Ross 2004).

Ecosystem Exposure to Multiple Contaminants

Environments are often subject to exposure from multiple contaminants (See Supplement Figure 2.) Ideally, suites of biomarkers could be used to identify the many chemicals present. In many field studies, aquatic organisms have been exposed to a complex mixture of organochlorines, heavy metals, and other various contaminants (Kuzyk et al. 2003). Ecosystem contamination as a result of multiple chemicals affects a species differently than contamination by a single chemical. The effects of mixtures usually exceed those of the most active constituents alone (Faust et al. 2004). For example, in a study assessing the effects of cadmium and zinc on rainbow trout, an exposure to a mixture of the metals resulted in a greater biological response than from exposure to each individual metal (Lange 2002).

Contaminants affect the biota throughout an ecosystem in different ways and can elicit various effects, each of which may be detected by a biomarker. In one case, marine gastropods were exposed to multiple organotins such as tributyltin (TBT) and triphenyltin (TPT) and, as a result, the organisms suffered genotoxicity, inhibition of ATP synthesis,

and inhibition of CYP enzymes (Fent 2004). The responses to the same contamination are often species-specific in organisms ranging from bacteria and green algae (Ma et al. 2004) to fish (Lemly 1997).

Effects of contamination are not only species-specific, but can vary according to specific chemical interactions as well. Timing and mode of exposure of pollutants, compound toxicity, and age of subjected organisms are factors responsible for differential sensitivity to
chemicals across species (Ottinger et al. 2005). In a study examining the toxicity of arsenic and cadmium, the metals were found to cause different toxic interactions depending on the sequence of the exposure (Hochadel and Waalkes 1997). The interactions among contaminants may substantially add, suppress, or multiply the effects of single components.

Ecosystem Biomarkers

Biomarkers are quantitative measures of the biological response of an organism to a chemical and can often be more valuable to environmental assessment than chemical analysis. Biomarkers reflect the concentration of contaminant that is biologically available, giving a true sense of how much of the contaminant is affecting the biota. Biomarker monitoring is often less expensive and more effective than using traditional chemical analysis. The biological responses of organisms to stressors are long-lasting and biomarkers can reveal episodes of contamination that intermittent chemical monitoring would miss (Handy et al. 2003).

Historically, biomarkers have been used to identify contaminated areas and

potential stressors (Adams 2003; Adams et al. 2001), but are currently being incorporated into the regulatory framework with varying degrees of success. Scientists offer differing solutions using biomarkers in environmental risk assessment. Adams (Adams 2003; Adams et al. 2001) recommends that an experimental framework of biomarker studies should be developed that applies to each situation and that this general framework should conform to the basic guidelines suggested in the Framework for Ecological Assessment (U.S. EPA 2005). The generalized field-biomonitoring framework consists of a weight of evidence approach, applying line of evidence at several different levels of biological organization. The appropriate biomarker would then be selected based on the information available (Adams et al. 2001). Adams, et al. (Adams et al. 2001) also recommend the identification of modifying factors that influence the interpretation of biomarker responses to stresses. Handy et al. (Handy et al. 2003) believe that the major drawback of biomarkers is the temporal and spatial variability between biological responses. To overcome the variability, Handy et al. recommend careful selection of reference sites, suites of biomarkers for multivariate analysis, and the introduction of sentinel organisms to calibrate the temporal shift.

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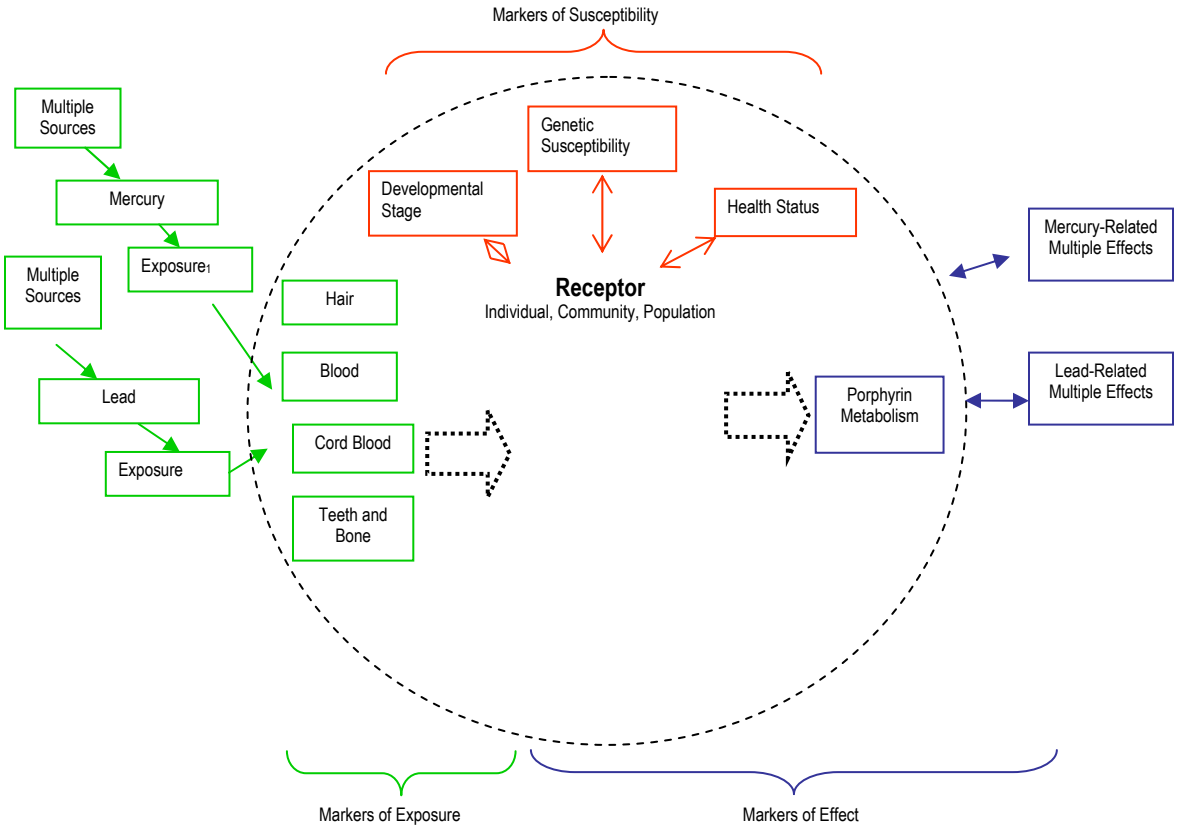
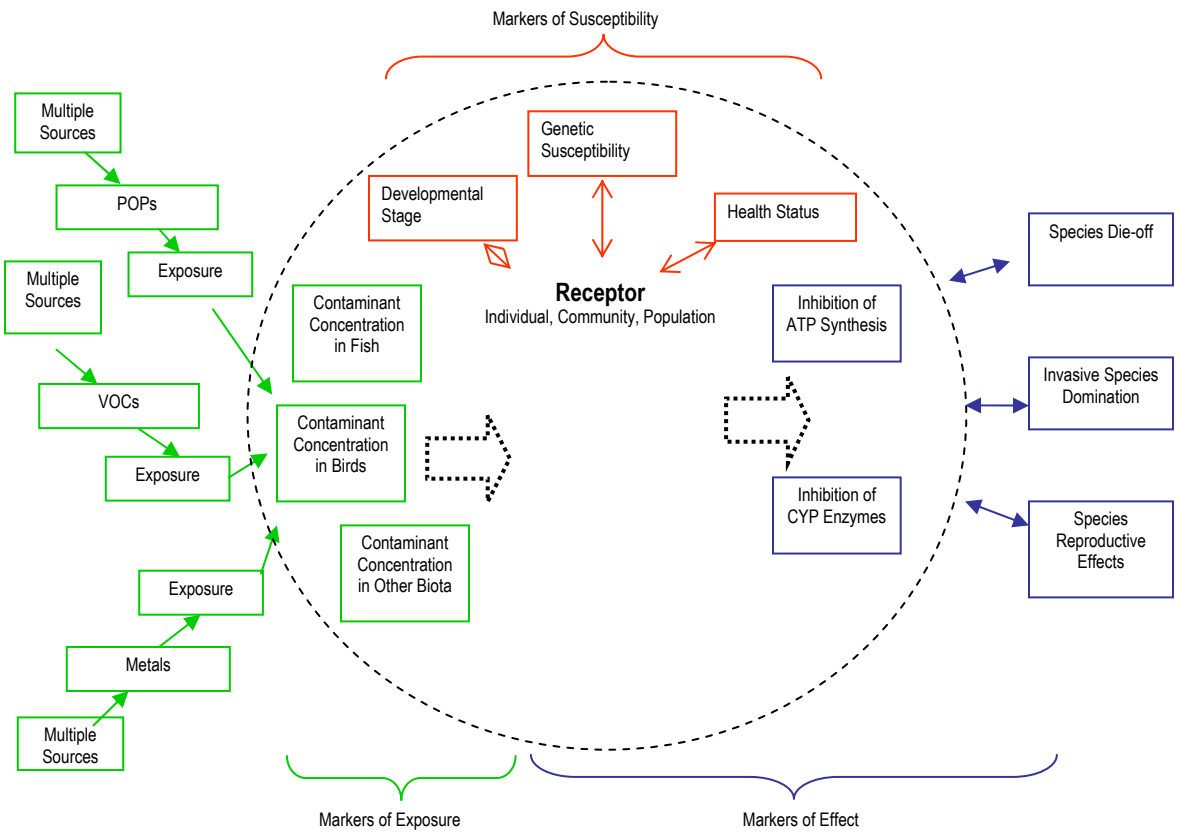
Supplemental Figure Legends

Supplemental Figure 1. Framework Applied to Neurobehavioral Endpoints Case Study

Supplemental Figure 2. Framework Applied to Endocrine Disruption Endpoints Case Study. The primary impact here is upon ecological communities.

Supplemental Figure 1



Supplemental Figure 2