**Supplemental Appendix S4-- Metadata Ecoregion 69 and 70: Step-by-Step Calculation and Spreadsheet Tools for Predicting Stressor Levels that Extirpate Genera and Species**

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# METADATA

 Data is provided as an Excel® file (Appendix\_S5-Data\_69-70.csv). The data used in the case example are from a large field data set, the West Virginia Department of Environmental Protection (WVDEP’s) in‑house Watershed Assessment Branch database (WABbase). Chemical and biological samples are from 1996−2011 and 1997−2010, respectively. The WABbase contains data from Level III Ecoregions 66, 67, 69, and 70 in West Virginia (USEPA 2010; Omernik 1987; Woods et al. 1996). The example dataset includes 3,734 paired biological and specific conductivity samples throughout Ecoregion 69 and 70.

A wide range of SC levels were sampled, which is useful for modeling the response of organisms to different levels of ionic concentration. Several data filters that excluded low pH ≤6 and high proportion of chloride ions ([HCO3−] + [SO42−] ≤ [Cl−]) were applied prior to finalization of the data set. Additional criteria were used to identify macroinvertebrates for inclusion in the example extirpation concentration distribution: occurrence at reference sites and occurrence in 25 or more samples. A total of 176 macroinvertebrate genera were selected which occurred at 25 or more sampling locations. SC ranged from 15−11,646 μS/cm which allowed the response of organisms to be modeled for a wide range of SC levels.

# REFERENCES

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USEPA (Environmental Protection Agency). 2010. Primary distinguishing characteristics of Level III Ecoregions of the Continental United States. Available online at: ftp://ftp.epa.gov/wed/ecoregions/us/Eco\_Level\_III\_descriptions.doc.

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