Supporting Information for "Identifying chemicals and mixtures of potential biological concern detected in passive samplers from Great Lakes tributaries using high-throughput data and biological pathways"

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METHODs

Passive sampler construction

For each deployment, SPMD and POCIS passive samplers were constructed at the U.S. Geological Survey, Columbia Environmental Research Center (CERC) according to established protocols (Alvarez et al., 2008). For each site, a protective deployment canister was prepared containing two SPMDs and three POCIS. SPMDs were spiked with a mixture of performance reference compounds (PRCs) including phenanthrene-d_{10}, pyrene-d_{10}, and PCB congeners 11, 29, and 50. Dibenz[*a*,*h*]anthracene-d_{14} was included in the PRC mixture as a photolysis marker to measure the potential photodegradation of sequestered PAHs during the field deployment. For deployment, the passive samplers were removed from packaging, secured to a post at approximately one foot above the streambed and left in place for a duration of 26-60 days. Passive samplers were removed after deployment and shipped overnight to CERC for processing.

Processing and Analysis

Following the field deployment, the SPMDs were processed and analyzed for organochlorine (OC) pesticides, total polychlorinated biphenyls (PCBs), polybrominated diphenyl ether (PBDE) flame retardants, and polycyclic aromatic hydrocarbons (PAHs). The POCIS were analyzed for a suite of organic wastewater-related chemicals (OWCs) including pharmaceuticals, fragrances, plasticizers, surfactants, antimicrobial disinfectants, and flame retardants. In total, 185 chemicals were targeted for analysis between the SPMD and POCIS (SI Table 2).

Methods for the processing and analyses of the SPMDs and POCIS have been previously reported and are summarized below (Alvarez et al., 2008; 2009; 2014). Analytes of interest were recovered from the SPMDs using two 24-hour dialysis periods with hexane. Following dialysis, the samples were filtered and fractionated using size exclusion chromatography (SEC) to isolate the analytes of interest from matrix and non-target chemicals sampled from the field. After SEC, one SPMD from each site was designated for OC pesticides/total PCBs/PBDEs analyses and the other for PAH analyses. The samples for OC pesticides/total PCBs/PBDEs underwent additional cleanup using gravity-flow Florisil® columns followed by fractionation of the total PCBs from

the OC pesticides and PBDEs using gravity-flow silica gel columns. Following silica gel, the SPMD fractions were analyzed using gas chromatography with electron capture detection. The PAH samples post-SEC underwent a final cleanup by passing each sample through a tri-phasic gravity-flow column containing successive layers of acidic, basic, and neutral silica gel. Following this cleanup, the PAH samples were analyzed using gas chromatography/mass spectrometry (GC/MS) run in selected-ion mode for added sensitivity.

The three POCIS deployed at each site were processed separately, each designated for a different analysis. One was extracted with 25 mL of 80:20 dichloromethane:methyl-*tert*-butyl ether and reduced in volume using rotary evaporation and nitrogen blowdown. The extract was quantitatively transferred into an amber GC vial, *p*-terphenyl-d_{14} (500 ng/mL) added as an instrumental internal standard, and then the volume adjusted to 1 mL with 3:1 (v:v) hexane:isopropanol prior to analysis by GC/MS in full-scan mode for a suite of OWCs (Alvarez et al., 2009; Alvarez et al., 2014). The other two POCIS were extracted with 25 mL of methanol each, with one extract designated for pharmaceutical analysis and the other archived for future use. The extracts for pharmaceuticals were reduced in volume using rotary evaporation and nitrogen blowdown prior to being transferred into 2 mL amber ampoules. The final extracts were adjusted to 1 mL of methanol in the ampoules and flame sealed. Pharmaceutical analyses were conducted at the USGS National Water Quality Laboratory using a triple quadrupole liquid chromatography/mass spectrometry (LC/MS) according to method lab codes 8069 (2010 samples) and 8240 (2014 samples).

Quality Control

For each deployment, a series of field blanks, representing a minimum of 10% of the study sites, were prepared and shipped with the samplers. The field blanks were exposed to the ambient air at the study site during both the deployment and retrieval of the passive samplers. The field blanks account for any potential contamination of the passive samplers due to the shipment and field activities involved in the study. Fabrication blanks for both SPMD and POCIS were prepared concurrently with the field-deployed samplers and were stored at <-20 ºC in the laboratory throughout the field deployment period. Fabrication blanks account for potential contamination of the passive samplers from the sampler matrix, construction, and processing of the samplers. All blanks were processed concurrently with the field-deployed samplers. A series of matrix spikes or surrogate recovery standards were used in conjunction with the field-deployed samplers to determine method performance for the targeted chemicals. PRC data from the blank SPMDs were used in the calculation of C_w to reduce bias due to recoveries of PRCs during processing (Alvarez, 2010).

Concentrations of analytes measured in the blanks were used to establish the method detection limits (MDL) and method quantitation limits (MQL) as described by Keith (1991). The MDL was calculated as the mean of the blank detections plus three times the standard deviation of the blanks. The MQL was set at the higher of the values between the lowest instrumental calibration level or the mean plus ten times the standard deviation of the blanks. In cases where there were no blank detections, the MQL was set at the lowest instrumental calibration level and the MDL was set at 20% of the MQL. These limits were then applied to the uptake models to determine what a representative average water concentration would be as described in the next section.

Estimation of time-weighted water concentrations

The estimation of time-weighted average water concentrations (C_w) of chemicals from residues measured in the SPMD and POCIS requires the use of uptake models and either experimentally or theoretically-derived sampling rates. The calculation of C_w from SPMD data uses a series of polynomial regression models which take into account site-specific environmental factors affecting uptake as determined by the loss of the PRCs (Alvarez, 2010). The calculations were performed using versions 5.1 and 5.2 of the SPMD calculation spreadsheet for the 2010 and 2014 data (USGS SPMD Water Concentration Estimator Spreadsheet). A detailed description of the theoretical aspects of uptake and derivation of the models has been described by Huckins et al. (2006). Estimation of C_w from POCIS data was performed using a basic integrative uptake model where C_w equals the concentration in the sampler divided by the chemical specific sampling rate divided by the deployment period (Alvarez, 2010). Sampling rates for chemicals in the POCIS were taken from the literature or estimated as necessary and are listed in Table SI-2.

ToxCast Screening values

Results from version 3.2 of the ToxCast database (US EPA, 2020) were used to evaluate potential biological activities associated with chemicals detected from the passive samplers. Annotations for ToxCast assays describing the intended biological targets are available in the database download (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data) or the U.S. Environmental Protection Agency (US EPA) CompTox dashboard (https://comptox.epa.gov/dashboard).

Analysis of passive sampler data follows previously published methods (Blackwell et al., 2017; Corsi et al., 2019). Descriptions from these publications are included below with several revisions for differences in the suite of chemicals monitored in the present study:

Data from all assay platforms (Kavlock et al., 2012) available in the ToxCast database were considered for the current study with the exception of BioSeek, which had several endpoint values that were anomalously low compared to those from other sources. Considering the nature of the assays and the associated reliability/quality for detecting gain or loss of signal, Attagene "gain" endpoints and Novascreen "loss" endpoints were used, while Attagene "loss" endpoints and Novascreen "gain" endpoints were removed because these assays were not optimized or designed to report for the given assay direction (Blackwell et al., 2017). Chemical/assay combinations that did not result in a significant test response (a "hit call") were also removed from consideration.

Assay results with the following data quality flags in the ToxCast database were removed from consideration for the present study: "Borderline active", "Only highest conc above baseline, active", "Gain AC50 < lowest conc & loss AC50 < mean conc", "Biochemical assay with < 50% efficacy", and "AC50 less than lowest concentration tested". Dose-response curves for chemical-assay combinations remaining after this selection process were also examined manually. In addition, all Tanguay_ZF_120hpf_ActivityScores were removed because this endpoint is an aggregation of activity from other zebrafish assays that are all represented

separately in the ToxCast data set. Dose-assay response curves were examined manually for the remainder of the chemical-assay combinations after this selection process to identify unreliable endpoints that were not filtered out through this process. Forty dose-assay response curves were found to be of questionable quality based on anomalous values or lack of response and removed from analysis (Table SI-4). With these various exclusions, 321 total ToxCast assays were suitable for computations for the chemical data set in the present study (Table SI-3).
The ToxCast data analysis pipeline provides several summary metrics modeled from chemical dose-assay response curves: the activity concentration at cutoff (ACC), the half maximal activity concentration (AC50), and the top value (T), or efficacy (Filer et al., 2016). The ACC is an assay-specific metric determined as a multiplier of the baseline median absolute deviation of measured activity in the assay that provides an indication of the concentration at which the bioactivity measured first exceeds the baseline concentration. More thorough descriptions of its derivation are provided elsewhere (Filer et al., 2016; Judson et al., 2009). Because this metric is indexed to a standard response threshold, its use has been favored in recent applications of ToxCast data over the other metrics (Blackwell et al., 2017; Corsi et al., 2019; Fay et al., 2018). Thus, the ACC (parameter name modl acc in the ToxCast database) was used as the final endpoint for comparison with passive sampler data.

Screening for potential pathway-based effects

Extrapolating exposure-activity ratio (EAR) information for application to environmental concentrations can be complex. For example, current information does not include correction for chemical partitioning in the assay system (e.g., free versus bound chemical in a test well), so actual biological activity in vitro or in situ may differ from this. However, it does provide a value that effectively normalizes for relative concentration detected in the environment and relative potency to elicit a specific biological effect. Thus, the EAR value is suitable for relative ranking and prioritization. Still, it is recognized that not all ToxCast assays used in this analysis are likely to be relevant to ecological species even though many of them target biological activities that are conserved among species. A complete evaluation of ToxCast assays for ecological relevance is currently not available, so the EAR approach used here is conservative in this respect.

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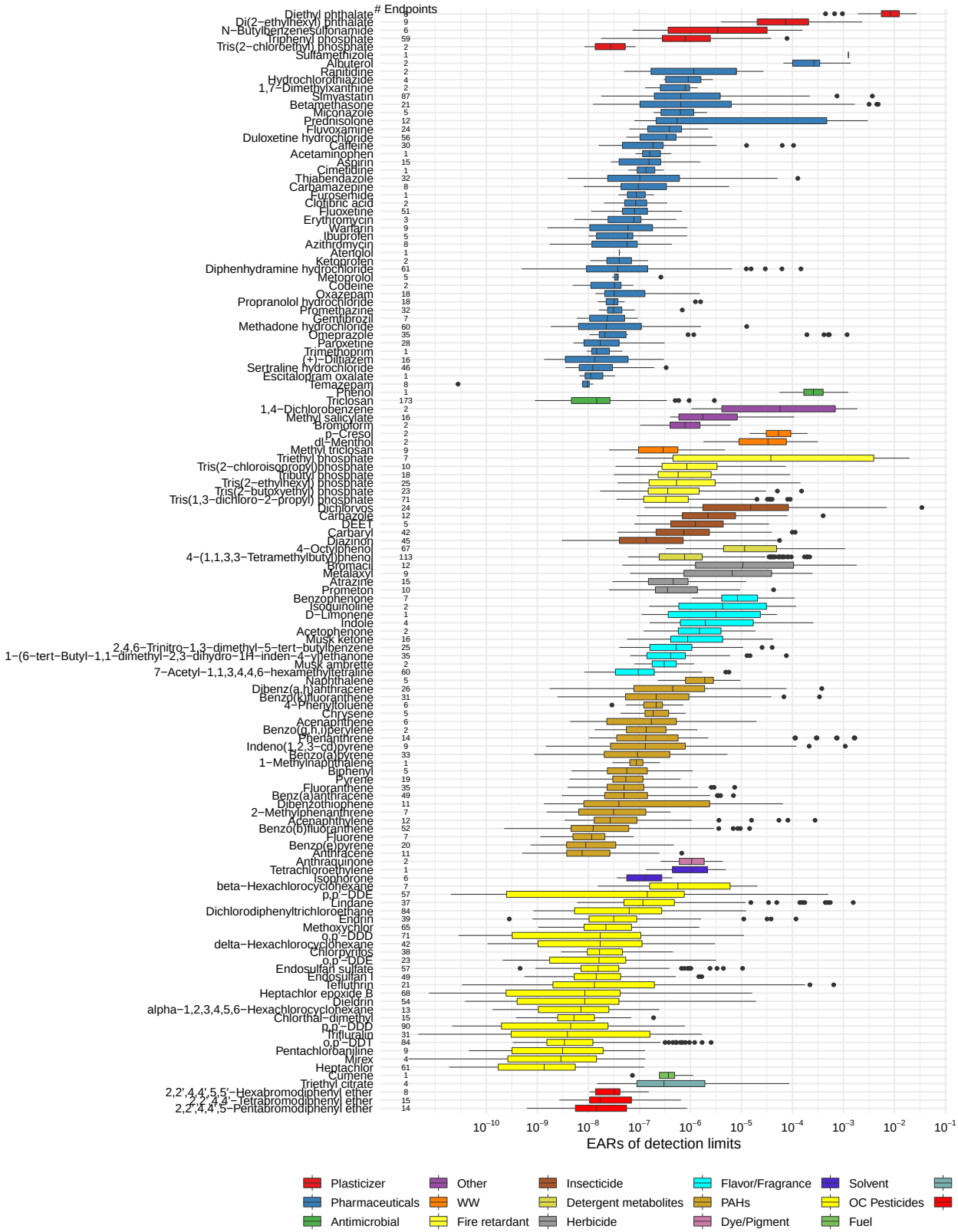

Figure SI 1: Exposure activity ratios (EARs) at the detection level for chemicals monitored with passive samplers in Great Lakes tributaries, 2010-2014.

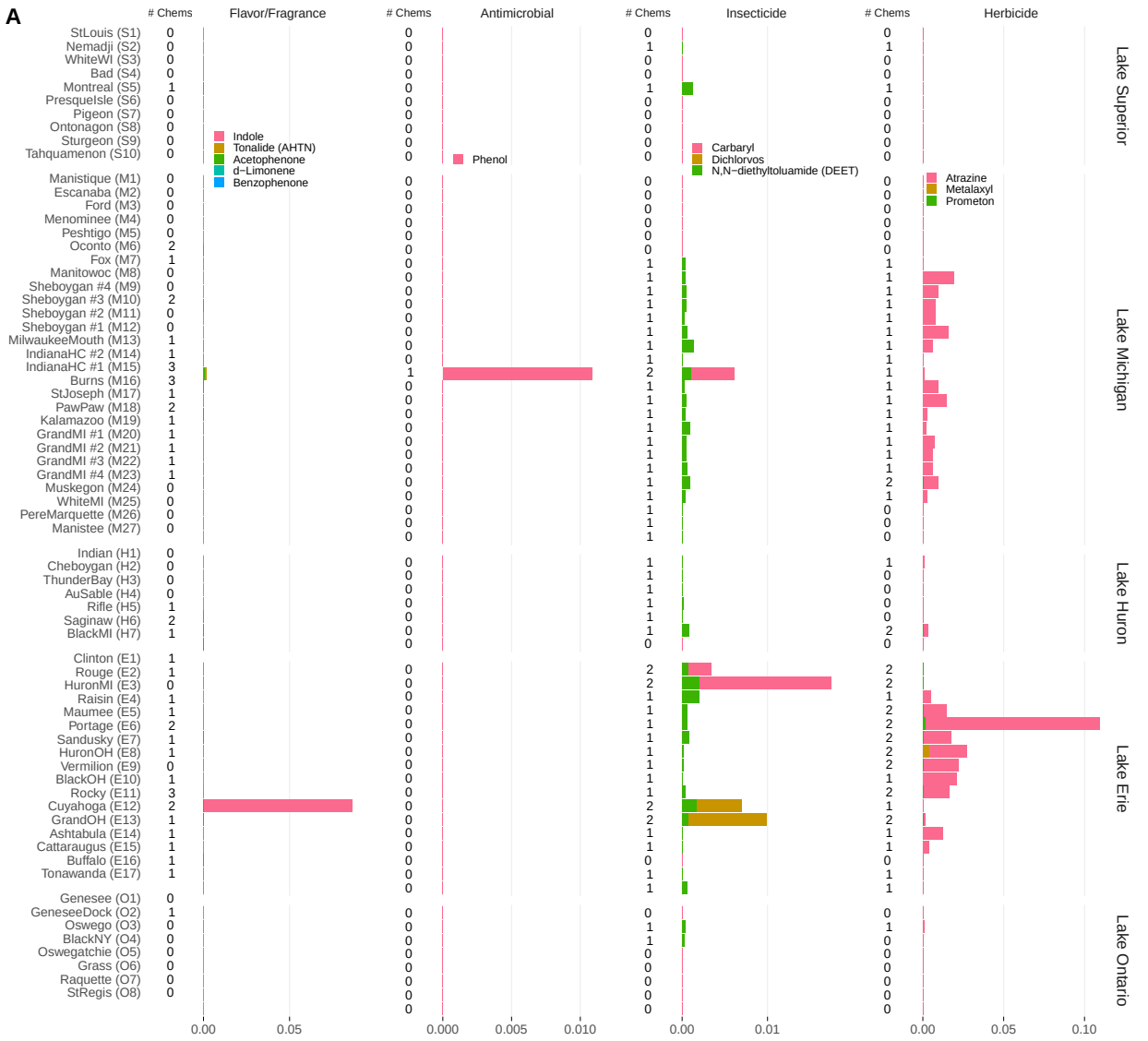

Figure SI 2-A: Maximum exposure-activity ratios (EAR) for flavor/fragrance, antimicrobial, insecticide, herbicide computed from passive sampler chemistry data from 69 Great Lakes tributaries collected in 2010 and 2014 for chemicals included in the ToxCast database. [Site names are followed parenthetically by the map names from Figure 1; # Chems, number of chemicals with computed EAR values; EAR$_{chem}$, exposure activity ratio]")

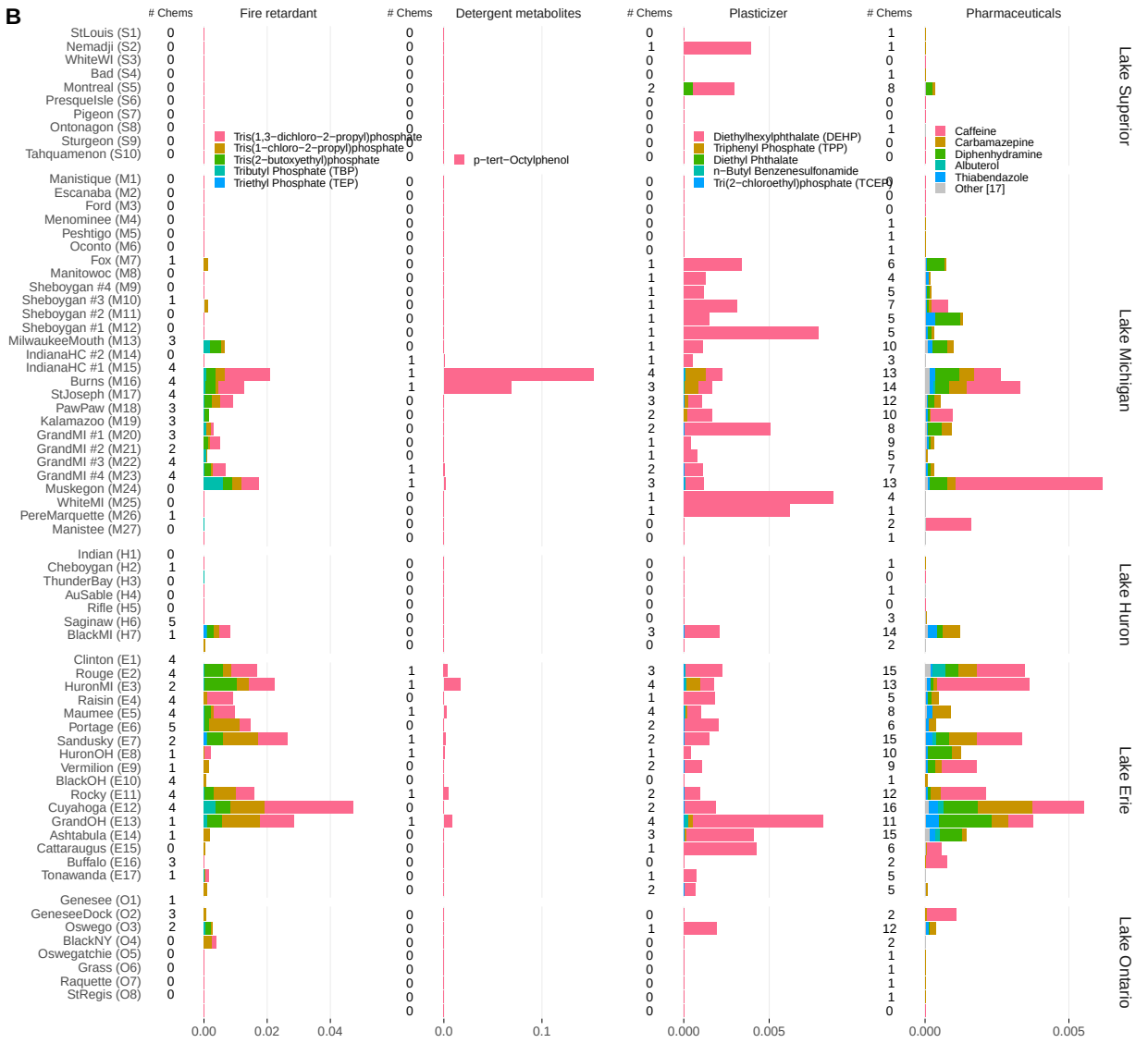

Figure SI 2-B: Maximum exposure-activity ratios (EAR) for fire retardant, detergent metabolites, plasticizer, pharmaceuticals computed from passive sampler chemistry data from 69 Great Lakes tributaries collected in 2010 and 2014 for chemicals included in the ToxCast database. [Site names are followed parenthetically by the map names from Figure 1; # Chems, number of chemicals with computed EAR values; EAR$_{chem}$, exposure activity ratio]")

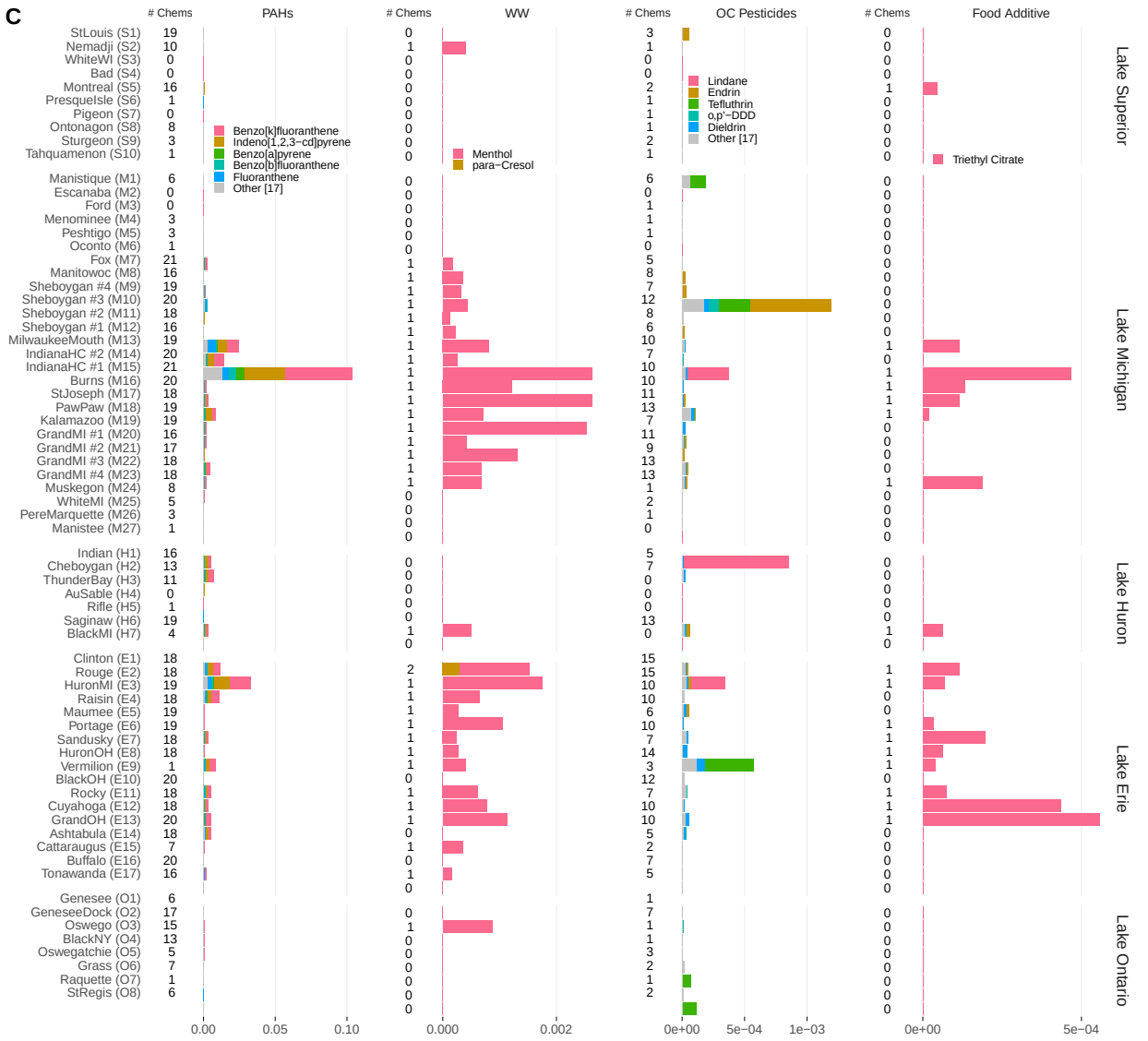

Figure SI 2-C: Maximum exposure-activity ratios (EAR) for pahs, ww, oc pesticides, food additive computed from passive sampler chemistry data from 69 Great Lakes tributaries collected in 2010 and 2014 for chemicals included in the ToxCast database. [Site names are followed parenthetically by the map names from Figure 1; # Chems, number of chemicals with computed EAR values; EAR$_{chem}$, exposure activity ratio]")

Sum of Maximum EAR_{chem} Values

Figure SI 2-D: Maximum exposure-activity ratios (EAR) for food additive, other, dye/pigment, solvent, pbdes computed from passive sampler chemistry data from 69 Great Lakes tributaries collected in 2010 and 2014 for chemicals included in the ToxCast database. [Site names are followed parenthetically by the map names from Figure 1; # Chems, number of chemicals with computed EAR values; EAR_{chem}, exposure activity ratio]")

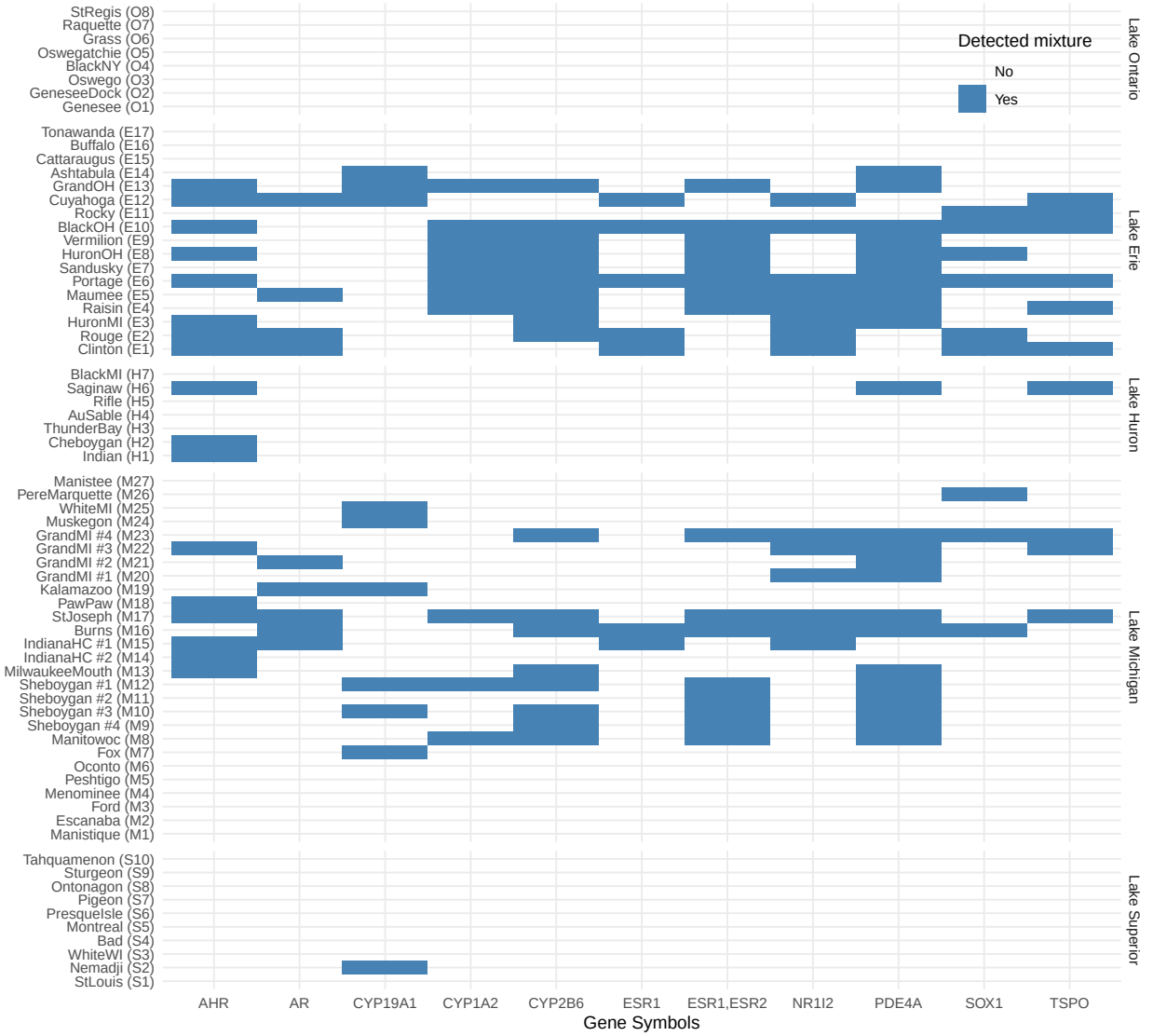

Figure SI 3: Monitoring sites with exposure activity ratios > 10-3 at 10% or more of monitored sites for gene targets and associated chemical mixtures as defined in Table 1.