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- Previous studies have focused the efforts of environmental monitoring of surface waters on specific point sources, particularly scrutinizing municipal and industrial wastewater effluents as sources of bioactive contaminants in the environment.^{1,2,3}
- Food production-related effluents have received little attention despite being a probable source of bioactive contaminants and being widespread throughout the US.
- The current study sought to serve as a screening level reconnaissance of surface waters impacted by wastewater from food production, including fish, meat, fruit and vegetable, dairy, and brewery and distillery operations, to better characterize their potential contribution to contaminant loading in surface waters.
- Food production effluent samples were collected from 23 facilities from 17 states across the US and analyzed for more than 530 target organics, 40 inorganics, and microbial indicators. Additionally, effluent extracts were screened for bioactivity of approximately 70 endpoints using Attagene Factorial assays.

- Assess the impact of food production-related effluents on receiving surface waters.
- Examine the relationship between chemical occurrence and biological activity.

- Effluent samples were collected directly from the point of discharge as the wastewater exited the outfall using established U.S. Geological Survey (USGS) protocols for the collection of water-quality samples.

- Chemical concentration data were produced by the USGS following a variety of previously defined procedures for a variety of chemical classes. Each sample was analyzed for 576 individual chemicals (37 antibiotics, 53 hormones and hormone conjugates, 14 natural plant phytotoxins, 255 pesticides/pesticide degradates, 108 pharmaceuticals, 85 volatile organic compounds (VOCs), and 34 per- and polyfluoroalkyl substances (PFAS)).⁴⁻¹¹
- Chemistry data were analyzed utilizing toxEval to relate chemical concentration data to the USEPA ToxCast database by generating exposure-activity ratios (EARs), which are defined below.¹²

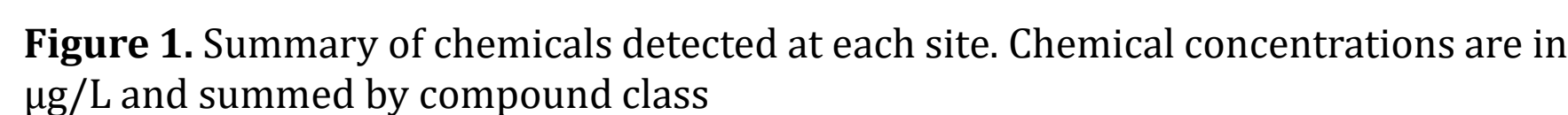
$$\text{EAR mix (unitless)} = \sum \frac{\text{Exposure (concentration, } \mu\text{M)}}{\text{Activity (ACC } \mu\text{M)}}$$

- Effluent extracts were assessed using Attagene cis-FACTORIAL™ and Attagene trans-FACTORIAL™.¹ Extracts in methanol were screened at a 100-fold dilution (50-fold enrichment factor relative to surface water).¹³

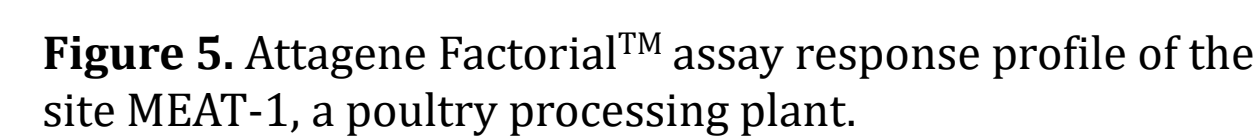
*No bioassay data available for this site

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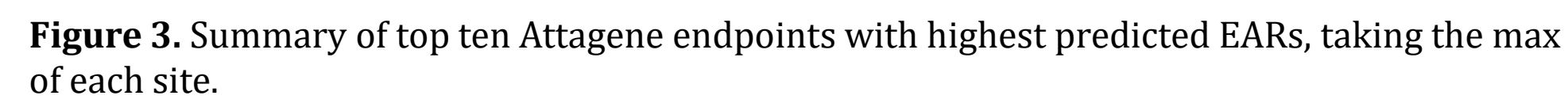
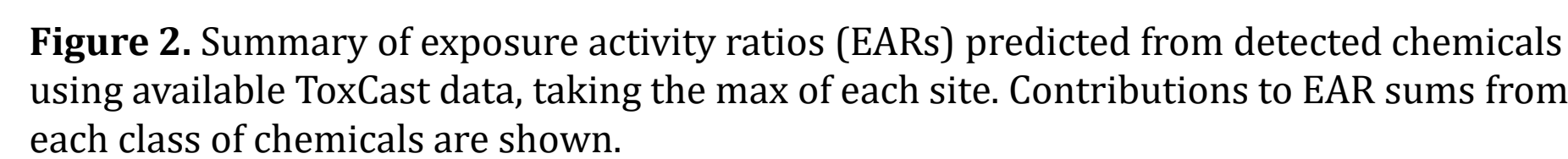
- 194 chemicals out of the 576 target analytes were detected at the sites analyzed. Phytotoxins detected were estrogenic and subsequently grouped with hormones for chemistry and ToxEval summaries.
- Detection of chemicals at individual sites ranged from six total detects (MI-2) to 91 total detects (IA-1). Summed concentrations ranged 0.3436- 233.877 µg/L (Figure 1).



- Pregnane X receptor (PXR), aryl hydrocarbon (Ahr), and estrogen response element (ERE) were the most commonly activated endpoints, showing some activity at every site analyzed for bioassay response (Figure 4).
- ERE and estrogen receptor alpha (Era) endpoints were active at site MEAT-1, correlating with the estrogenic compounds detected at that site and predicted to cause bioactivity (Figure 5).



- Of the 194 detected chemicals, 170 were present in the ToxCast database and were thus analyzed for predicted EAR values utilizing toxEval.
- Hormones and pesticides were the dominant classes driving the overall response in EARs (Figure 2).
- Estrogen receptor (ER), aryl hydrocarbon receptor (AhR), and pregnane X receptor (PXR) were among the highest predicted activity in the Attagene assays, though EARs were all below 0.1 (Figure 3).



- Pesticides were the most frequently detected chemical class (22 of 23 sites), followed by pharmaceuticals (20 of 23) (Figure 1).
- Despite low measured concentrations of hormones, these compounds were the primary driver of elevated EARs due to their high biological potency.
- A majority of measured bioactivity was not predicted by EAR analysis and appears to be driven either by chemicals that were not measured or not currently in the ToxCast database.
- Bioassays identify sites and biological pathways of interest that are not captured based on chemical analysis alone. Including both chemical and biological analyses in environmental monitoring is crucial for capturing a complete view of chemical occurrence and potential biological effects.

- Employ additional statistical approaches to identify relationships between chemical presence and bioactivity.

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