Towards Risk-Based Environmental Monitoring and Technology Assessment – Toxicogenomics and Data Science

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Contaminants of Emerging Concern (CECs) Threat

**Problem**: Unknown toxicity and risks associated with large and increasing number of contaminants?

- 85,000 chemicals listed in TSCA, most lack of comprehensive toxicological and exposure data
- US EPA ToxCast/ExpoCast program is screening hundreds of chemicals

**In Water**…

- Current treatments not designed to effectively remove CECs
- CECs are widely-spread, present in mixtures
- Harmful effects exert at very low concentrations
- Various, many metabolites and transformation intermediates

**Challenges in establishing sufficient risk assessment framework and regulations**
Problem: Targeted/regulated chemical(s)-based treatment efficacy is not sufficient for risk-reduction/mitigation

- Treatment designed for targeted pollutants may have unintended impact on water matrix
- The target-chemical-based approach does not consider the complex and broader risks that mixtures of contaminants and transformation products, pose to the environment and human health

Challenge: Lacking feasible tools for evaluating overall toxicity and risk reduction through treatment
Paradigm Shift in Toxicity Testing: Tox21

Balance between certainty and cost

<table>
<thead>
<tr>
<th>Human experience</th>
<th>Standard rodent toxicological tests</th>
<th>Alternative animal models</th>
<th>Biochemical- and cell-based in vitro assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 studies/year</td>
<td>10–100/year</td>
<td>100–10,000/year</td>
<td>&gt;10,000/day</td>
</tr>
</tbody>
</table>

Immediate human relevance

Predict

Legacy data

Knowledge

Prioritize

Computational toxicology

System Biology

Critical toxicity pathways

High throughput

Molecular toxicology

Collins et al., Science (2008)
Objectives of This Study

Develop of a new toxicomics-based toxicity assessment platform for toxicity evaluation, screening and classification of contaminants, specifically:

1. Develop methods of applying real time gene/protein expression profiling for toxicity assessment

2. Establish computation methods for quantifying toxicomic information and determine molecular toxicity endpoints

3. Validate the methods by correlating the endpoints from the proposed methods with conventional methods

4. Demonstrate the applications of the methods for assessing, emerging contaminants and for exposure assessment (in water)
What is, and Why Toxicomics?

Toxicomics: biological response to toxicants (sub-cytotoxic levels) involves changes at molecular level, monitor changes in gene/protein expression patterns for toxicology assessment.
Real Time Gene/protein Expression Profiling via Whole-cell-array

<table>
<thead>
<tr>
<th>Cell with GFP infusion</th>
<th>Toxicity assessment assays on parallel reporter strains</th>
<th>Data generation, Gene profiling and clustering</th>
</tr>
</thead>
<tbody>
<tr>
<td>gfp-transformed E. coli. Or Yeast strains for &gt; x1000 genes</td>
<td>Chemical applied on plates, one gene in each well, expression monitored on fluorometer</td>
<td>Chemical-specific gene real-time gene expression profiles generated</td>
</tr>
</tbody>
</table>

Measure: changes in gene expression patterns in exposure to CECs compare to control with no exposure
Part I

- Stress response pathway ensemble-based assay
- Can molecular disturbance/ stress response pathways be quantified and have dose-response model?
What Pathway(s) to Quantify?
Cellular Response Pathways and Toxicity

AOP- Adverse Outcome Pathway

**Chemical**
- Macromolecular Interactions and damages
  - Protein
  - Lipid
  - DNA...

**Stress response**
- *Receptor activation*
- *Signal transduction*
- *DNA repair*
- *Lipid synthesis*
- *Protein repair and degradation*

**Cellular Response**
- Cell stress, dysfunction, apoptosis,...

**Cellular Effects**
- Disrupted homeostasis, physiology, development and function

**Toxicity pathways**
**Mode of action**
- Restore Homeostasis?
  - Yes: Damage repaired
  - No

**Toxicity Effects**
**Adverse outcome**
- Organism/animal:
  - Lethality
  - Impaired Development
  - Impaired Reproduction
  - Cancer....

- Organ response:
  - Disrupted homeostasis, physiology, development and function
Stress Response Pathways Ensemble Based Stress Response Library

Genes/pathways that are related to stress responses

- **Redox stress**
  - oxyR
  - soxR

- **Detoxify**
  - sodB, sodC

- **DNA Damage**
  - lexA, recA

- **Lipid damage, Drug Resistance**
  - cmr, emrA

- **Protein damage**
  - entC, cueR

- **Receptor activation**

**Basic cellular toxicity mechanism**

**Toxic effect/response characterization**

3-D Toxic Stress Response profiling

Simultaneous measurements of altered gene/protein expression patterns with temporal resolution yield 3-D toxic response pathway ensemble profiles.

- **Gene**
  - sbmC
    - Hg(50)
    - H2O2(5)
    - nAg(50)
  - Exposure Time (mins)
  - Gene expression fold change
  - Time

- **Gene**
  - sulA
    - 4NNP(5)
    - nTiO2_a(10)
    - MMC(30)
  - Exposure Time (mins)
  - Gene expression fold change
  - Time

High dose

Low dose

Gene

Time

Altered Gene expression Level
A New TELI Index For Quantifying Molecular Response and Pathway Activities

TELIF = Transcriptional Effect Level Index or PELI considers 3-dimensional data that include:
-Magnitude of gene/protein response.
-Temporal pattern and cumulative effects
-Extent of cellular pathway(s) response

$$TELI(gene) = \frac{\int_{t=0}^{t=2hr} (e^{\ln(I)} - e^{\ln(1)})}{\text{Exposure Time}}$$  \hspace{1cm} (1)

$$TELI(total) = \int_{\text{gene}(i=1)}^{\text{gene}(i=n)} Wi \ast (TELI_{gene})$$  \hspace{1cm} (2)

Gou and Gu, 2011,2014 ES&T
Lan et al., 2014,2015,ES&T
Gene Enrichment Analysis To Identify Toxicity Mechanism

Modified gene set enrichment analysis (GSEA) technique for *time series* toxicogenomics data analysis

Toxicant-induced expression profiles are *time*, *concentration* and *chemical*-dependent

1) To consider temporal patterns/effects:
   * Propose TELI index, time series modeling

2) To consider different dose concentrations:
   * *common principal components analysis* (CPCA) with different ranking matric (Gao et al., 2015)
Time-dependent analysis results

MMC (0.5 ng/L)-model genotoxicant

Ranked by TELI values

• Mechanism profile is dynamic, time-dependent

• Single “snap shot” at one time point may be biased

• Temporal variability is just as important as expression level changes

Gao et al., 2015 ES&T
Gene Enrichment Analysis – concentration effects

Pb- 0.125 µg/L

- Ranked by CPCA score
  - Ranking profiles vary with dose concentrations
  - Single concentration result may not be comprehensive

Pb- 6 different concentrations

- CPCA may reflect more “conserved” mechanism for a given chemical?

Gao et al., 2015 ES&T
Dose-response Curves Based On New TELI/PELI

Dose-response relationship of TELI exist and they can quantify toxicity pathway response

Gou at al., 2011; Lan et al., 2014. ES&T
Part II Phenotype Anchoring

- Do molecular effect-based endpoints correlate with cell/organism level phenotypic endpoints?
- DNA-damage and repair pathways-based PELI correlated with phenotypic endpoints
DNA Damage Related AOP

DNA damaging agent(s) Reacts with DNA

- Single strand break
- Double strand break
- Bulky adducts
- Base alkylation, ...

DNA damage repair

- Double strand break repair
  - Base excision repair
  - Homologous recombination
  - Non-homologous end-joining
  - Nucleotide excision repair
  - Mismatch repair
  - Direct repair & Base excision repair

Repair failed

- Unrepaired DNA
  - Cell killing
  - Mutations
  - Tumor formation
  - Lethality; Impaired Development; Cancer

Ames

Comet

PCR

HPLC/MS

Immuno-slot-blot assay

DNA damaging agent(s) Reacts with DNA

- DNA damage
- DNA repair

Lethality; Impaired Development; Cancer
Prediction of Genotoxicity

Lan et al., 2014, 2015 ES&T
## Prediction of Genotoxicity

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Yeast assay in this study</th>
<th>Genotoxicity assay</th>
<th>In vivo carcinogenesis assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>Mammalian cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ames test 68-74</td>
<td>Comet test 57, 74-81</td>
</tr>
<tr>
<td>4-NQO</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzo [a] pyrene (with S9)</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lead (II) nitrate</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Atrazine</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N-nitrosodimethylamine (NDMA)</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Bromodichloromethane</td>
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<tr>
<td>Chlorodibromomethane</td>
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<tr>
<td>Formaldehyde</td>
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<tr>
<td>Tetraycline hydrochloride</td>
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<td>-</td>
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<td>Aspirin</td>
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<td>-</td>
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</tr>
<tr>
<td>Erythromycin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Lan et al., 2014, 2015 ES&T
Part III Application for Water Quality Monitoring

* Application for Water Toxicity
* Technology Assessment
* Water Quality Monitoring
Approach overview – Pilot WWTP with parallel disinfection and oxidation treatments

Influent → Bar Screen → Oxidation Ditch → Clarifier → WWTP A

Sampling (SPE enriched)

Chemical analysis → Toxicity test

- UV/H2O2
- UV
- O3
- NH2Cl
- Cl2

In collaboration with Shane Snyder et al., unpublished
- Technology-dependent effluent toxicity profiles
- Certain process seemed to generate toxic products
- Treatment parameters affect efficacy

In collaboration with Shane Snyder et al., unpublished
Toxicity Evolution During CEC Degradation

--Advanced Oxidation (Electro-Fenton) Process for CEC degradation
---Treatment efficacy based on temporal toxicity level and profiles

Gene involved in different stress response categories

<table>
<thead>
<tr>
<th>Stress Type</th>
<th>Bisphenol A</th>
<th>Triclosan</th>
<th>Ibuprofen</th>
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</thead>
<tbody>
<tr>
<td>Oxidative Stress</td>
<td><img src="image1" alt="Gene Expression" /></td>
<td><img src="image2" alt="Gene Expression" /></td>
<td><img src="image3" alt="Gene Expression" /></td>
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<tr>
<td>Protein Stress</td>
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<td><img src="image5" alt="Gene Expression" /></td>
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<tr>
<td>Membrane Stress</td>
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<td><img src="image8" alt="Gene Expression" /></td>
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<tr>
<td>DNA Stress</td>
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<td><img src="image11" alt="Gene Expression" /></td>
<td><img src="image12" alt="Gene Expression" /></td>
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<tr>
<td>General Stress</td>
<td><img src="image13" alt="Gene Expression" /></td>
<td><img src="image14" alt="Gene Expression" /></td>
<td><img src="image15" alt="Gene Expression" /></td>
</tr>
</tbody>
</table>

Gou at al., 2014, ES&T, Yuan et al., 2013 Chemosphere
Toxicity Evolution During CEC Degradation

--- Advanced Oxidation (Electro-Fenton) Process for CEC degradation

--- Treatment efficacy based on temporal toxicity level and profiles

--- Identify causal intermediates

- Optimize treatment strategy and condition

Gou at al., 2014, ES&T, Yuan et al., 2013 Chemosphere
Case Study- Dan River Spill

- The third-largest coal ash spill of U.S. occurred at Eden, N.C on Feb 2\textsuperscript{nd}, 2014
- ~39,000 tons of coal ash and 27 million gallons of wastewater spilled into Dan River

In collaboration with Madeline E. Schreiber (VT), Brian Williams (DRBA) (unpublished)
Methods—Overview

- 15 surface water samples
- 14 sediment samples
- leachate

Chemical Analysis
- Total organic carbon (TOC)
- Dissolved organic matter (DOM-EEM)
- Trace elements (ICP-MS)
- Toxicogenomics Assay
- Toxicity test in human cells

Toxicity Assessment
Results Highlights

- Temporal and spatial trends of metals, molecular toxicity
- Insights of toxicity profiles in water and sediment
- Statistical and correlation analysis, as well as “iceberg” metal mixtures to examine the potential contribution of metal mixtures
- Explored the correlation between organic matters, metals with toxicity effects detected

In collaboration with Madeline E. Schreiber (VT), Brian Williams (DRBA) (unpublished)
Conclusions

• A quantitative toxicomics-enabled toxicity assessment platform has been explored and developed (preliminarily).

• Fundamental and quantitative understanding of molecular perturbation and correlation with phenotypic toxicity has been explored

• Allow high rate, feasible and economical mechanistic screening of CECs, mixture and exposure assessment

• The technology applicable to exposure assessment, monitoring, technology efficacy evaluation
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