Application of a Genome Wide Live Cell Array system in Ecotoxicity Assessment

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1) What kinds of ADVERSE EFFECTS can chemicals cause?

2) What is the MECHANISMS of the adverse effects caused by the chemicals?

3) What is the THRESHOLD of chemical to cause the adverse effects?

4) Which BIOMARKERS can be used to indicate the adverse effects/Chemical exposure?
Chemical Safety Assessment
• Environmental Quality Standards
• Chemical Risk Assessment

Contaminants Identification
• Toxicity Identification & Evaluation
  • Effect-directed Analysis

Mixture Toxicity Assessment

Conventional Toxicity Testing
• Animal based tests
• Time, Labor, Cost
• Poor mechanistic information
• Demands not met

Predictive Toxicology
• In vitro based tests
• High through-put
• Genomic, system Biology, bioinformatics
• Mechanistic information
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Toxicity = Exposure(Concentration) \times \text{Time}
Genome Wide Live Cell Reporter Array

**Reporter Gene Assay---**
A Successful Tool for Chemical Toxicity Assessment

- **TF**
- **Promoter**
- **Luciferase/GFP**
- **Activation**
- **Gene Expression**
  (Transcript/Protein)
Cell pre-culture, over night

Cell innoculation, 2-3 hour

Chemical dosing, 2-5 min

Incubation & Fluo/OD measurement, 3-6 hour

Data Analysis

Cytotoxicity Assay (Alamar Blue)

1800 strains/ 70% genome

one strain each well

Culture

Measurement
Naphthenic Acids

ZnO nano-particles

Time: 0 --------------3---------------6h

Zhang et al EST 2009
A) Altered Gene Expression
Concentration-dependent relationship

Fold Change >2.0

Fold Change >1.5

Zhang et al EST 2011
B) Concentration-dependent response of transcription

Transcriptional Response

Cytoxicity

Concentration

NOTEC – no observed transcriptional effect concentration

Zhang et al EST 2011
C) Fold change vs # gene altered

D) Ecotoxicity data plot (TPTC)

NOTEC – no observed transcriptional effect concentration

Zhang et al EST 2011
E. Coli Transcriptional Regulatory Network
Mechanism of Toxicity

Naphthenic Acids (NAs)

Transcriptional network database: Regulon DB  
Network analysis platform: Cytoscape  
Active modules identification: jActiveModules
Examples: BDEs and derivatives

High Concentration

BDE-47

Natural Source

6-MeO-BDE-47

Human Blood

6-HO-BDE-47
Cytotoxicity

Inhibition profile of *E. coli* growth by 6-HO-BDE-47 at different concentrations
Real-time gene expression profiles of differentially expressed genes in *E. coli*.

Su et al EST 2011 (Accepted)
Pathways altered by OH-BDEs
BDE-47, HO-BDE-47, MeO-BDE-47
Discussion & Future application

• A novel genomic tools for toxicity assessment
  – High throughput technology coupled with genomic information
  – Affordable to most lab
  – Real time monitoring of gene expression
  – Increased resolution of biological responses

• Tools for hypothesis testing in Predictive toxicology
  – Mechanism based Chemical classification
  – Aids in quantitative structure & activity relationship analysis
  – Mixture effects: mode of action

• Application and future research
  – Chemical (eco)toxicity test
  – Contamination identification by the approach of effect-directed analysis
  – Mammalian cell based reporter array system
Thank you!

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