In ToxCast We Trust? Building Support for EPA's High-throughput Screening Tool

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January 28, 2010

Potential Advantages of High-Throughput Screening

- Broad look at chemical properties and biological perturbations
- Establish intrinsic potency
- Identify toxicity pathways
- Determine relevant hazard endpoints
- Incorporate systems biology
- Readily modified with newer, different assays
- Identify different chemicals perturbing same pathways
- Identify “green” chemicals with low perturbation profile

Some (NGO) Concerns

- Broader understanding needed of what ToxCast does, and its strengths & limitations
- As a screening tool, ToxCast must be precautionary
- ToxCast should capture emerging science
- Interpretation should minimize false negatives
- Recognize that a large number of false positives could impact screening efficacy
- Complex and technical tools that are difficult to explain to non-technical audiences

ToxCast Proof of Concept

- Select chemicals with robust conventional data sets
- Run ToxCast assays
- Align interpretation of ToxCast assays with known data
- Adjust for new findings (unexpected or novel results)

Is Further Validation Needed?

- Need for GLP, standardization & validation arose from incidents of fraud in testing labs
- Some regulations may require use of standardized assays
- Validation can also address (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability
- Process of standardization & validation can be lengthy, limiting incorporation of the most up-to-date methods
- Replication generally viewed as a legitimate validation method, especially in academia
- Does the Proof of Concept approach fully address the goals of validation?

Three Levels of ToxCast Need Scrutiny:

- Individual assays
- Grouping of assays
- Analysis & interpretation
  - Individual assays
  - Grouping

Individual Assay Standardization & Validation

- Address (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability
- Guidelines provided by OECD, FDA, etc.
- BUT need to avoid ten year+ EDSP-type validation odyssey

EDSP Hormone Assays

- Basis for selection is clearly articulated:
  - Binding assays: “The capacity of an assay to detect estrogen- and androgen-mediated effects by various modes of action including receptor binding (agonist and antagonist) and transcriptional activation, steroidogenesis, and hypothalamic-pituitary-gonadal (HPG) feedback.”
- Assays underwent international validation process, harmonized with OECD TG
  - Validation process took ~ 10 years
- Criticism that validated assays do not reflect current science
  - Tension between regulatory needs & evolving science

Expand Proof of Concept to Compare with Validated Assays?

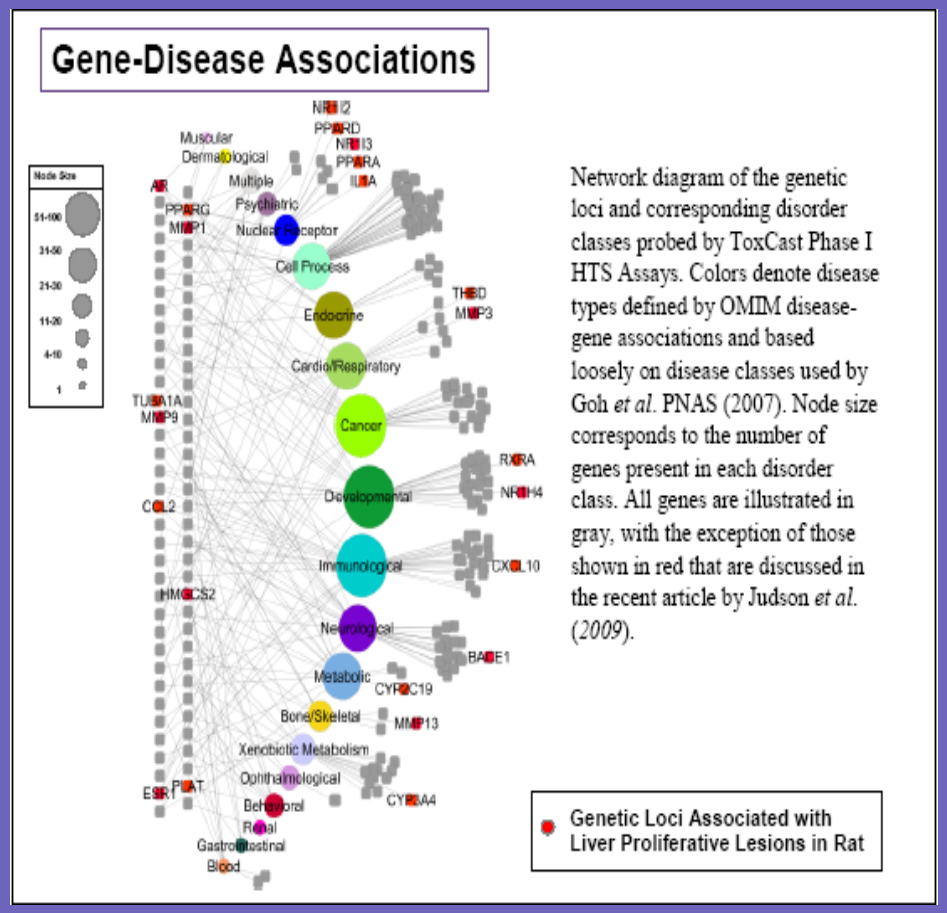
- Leverage the knowledge of EDSP, compare ToxCast assays to EDSP assays
- Identify ToxCast assays that have been validated for other reasons (e.g., FDA)
- Reinforce confidence in accuracy, precision, selectivity, sensitivity

EDSP Assay Grouping

- Selection of assays for inclusion in Tier 1 Test battery based on "The degree that *in vitro* and *in vivo* assays complemented one another in the battery as summarized in the table below." and
- "[R]odent and amphibian *in vivo* assays were selected for the proposed battery based on their capacity to detect direct and indirect effects on thyroid function (hypothalamic-pituitary-thyroidal, HPT, feedback). Thus, the robustness of the proposed battery is based on the strengths of each individual assay and their complementary nature within the battery to detect effects on the E, A or T hormonal systems."

ToxCast Assay Grouping

- Conduct survey of Pathway/Process associated with ToxCast HTS assays. Networks, generated with Cytoscape 2.6.1
- Conduct a principle component analysis
- Identify gene-disease associations (hazard endpoints)

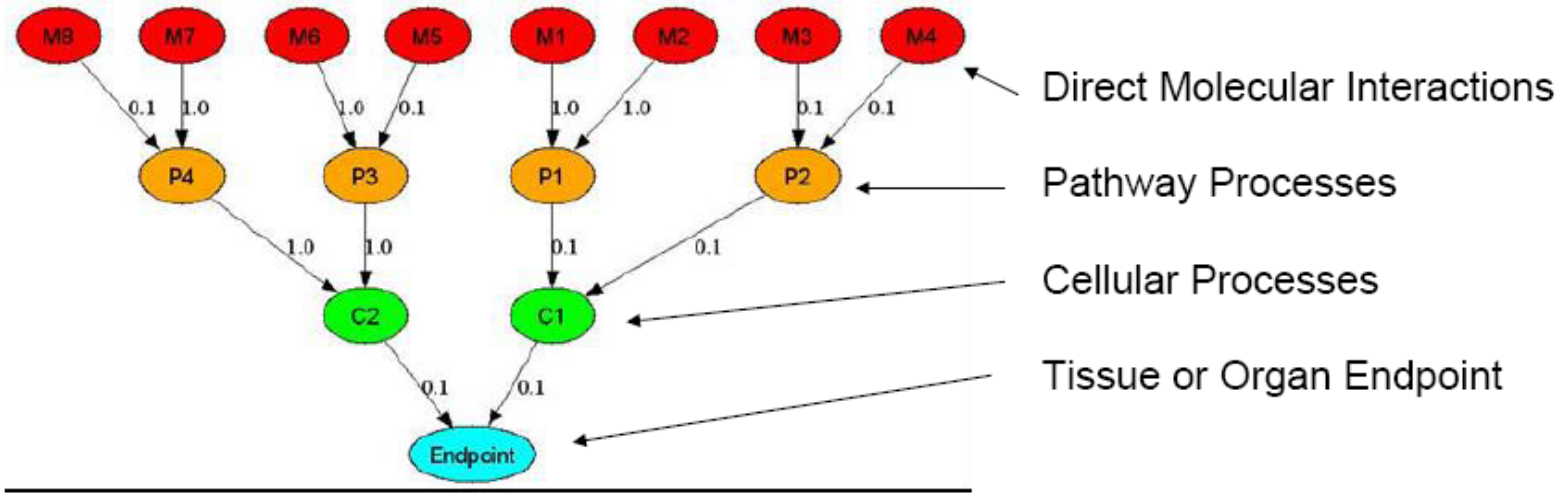


Network diagram of the genetic loci and corresponding disorder classes probed by ToxCast Phase I HTS Assays. Colors denote disease types defined by OMIM disease-gene associations and based loosely on disease classes used by Goh *et al.* PNAS (2007). Node size corresponds to the number of genes present in each disorder class. All genes are illustrated in gray, with the exception of those shown in red that are discussed in the recent article by Judson *et al.* (*2009*).

Mortensen et al. 2009. Evaluating the Boundaries of Toxicity Pathway Space Using High-Throughput Environmental Chemical Data (Poster, but also Judson et al 2010)

Computer-Aided Tools

ToxCast Biological Ontology Used in ToxMiner Predictive Modeling



Each chemical will have a spectrum of activities for M-P-C-E nodes.
Predictive classifiers will include features from multiple data levels.

Would Expert Elicitation be a Useful Addition?

- Invite experts in specific hazard endpoints (and stakeholders) to engage in a focused, facilitated review of the selection of assays for inclusion in the evaluation of hazard endpoints.
- Request feedback on scope and depth of coverage, interpretation of results, and recommendations for additional assays.
- Evaluate associations with disease.
- Develop recommendations for updating assays as new science emerges

Interpretation – Single Assay

- Are the assays appropriately sensitive and specific?
- What level of perturbation is biologically significant?
  - Scientific and policy considerations
  - Benefit from expert + stakeholder discussion

Interpretation – Grouped Data

- Are the selected assays adequate in scope and depth to predict potential hazard endpoints?
- Are they sufficiently sensitive to minimize false negatives?
- What might be missing?
- What is on the horizon?

Addressing Vulnerability & Variability

- National Research Council, Science and Decisions (2008) identified as important considerations
  - Population vulnerability
    - Co-morbidities
    - Non-chemical stressors
  - Age, life-stage, genetic variability
  - Cumulative exposures
  - Non-chemical stressors
- Useful to discuss how ToxCast can (or cannot) address these issues

Benefits of Expert Elicitation

- Obtain constructive feedback on scope, depth, and interpretation
- Establish stronger linkages to disease endpoints
- Develop recommendations for robust analyses including population vulnerability & variability
- Published analyses could boost stakeholder confidence
- Generate plan for continuous improvement

Examples of ToxCast Hazard Evaluations

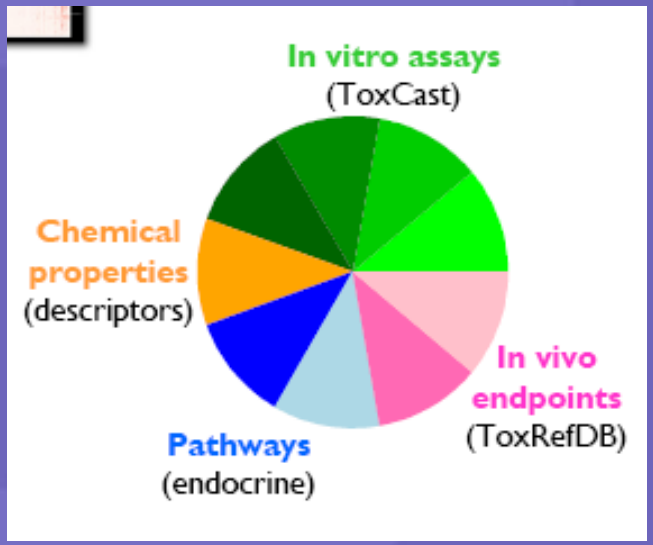
- Different types of analyses
- Different displays of information
- Approach taken could facilitate stakeholder engagement

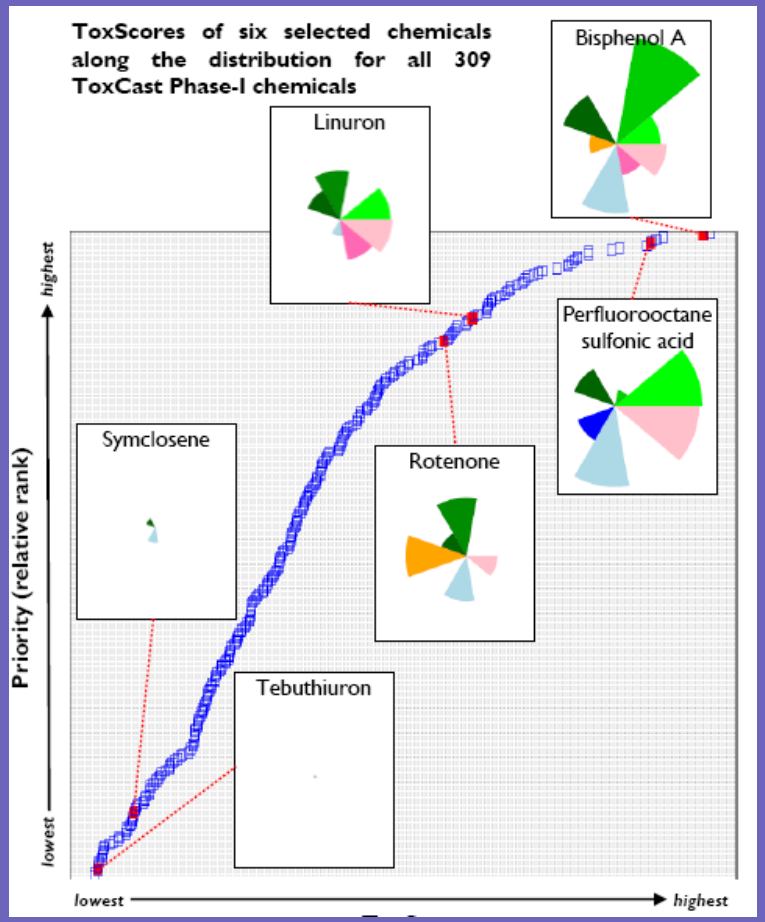
Analysis of Endocrine Disruptors

Each chemical profile/signature/fingerprint gives a priority score (ToxScore) that can be ranked along any domain

ToxScore = f(In vitro assays + Chemical properties + Pathways + In vivo endpoints)

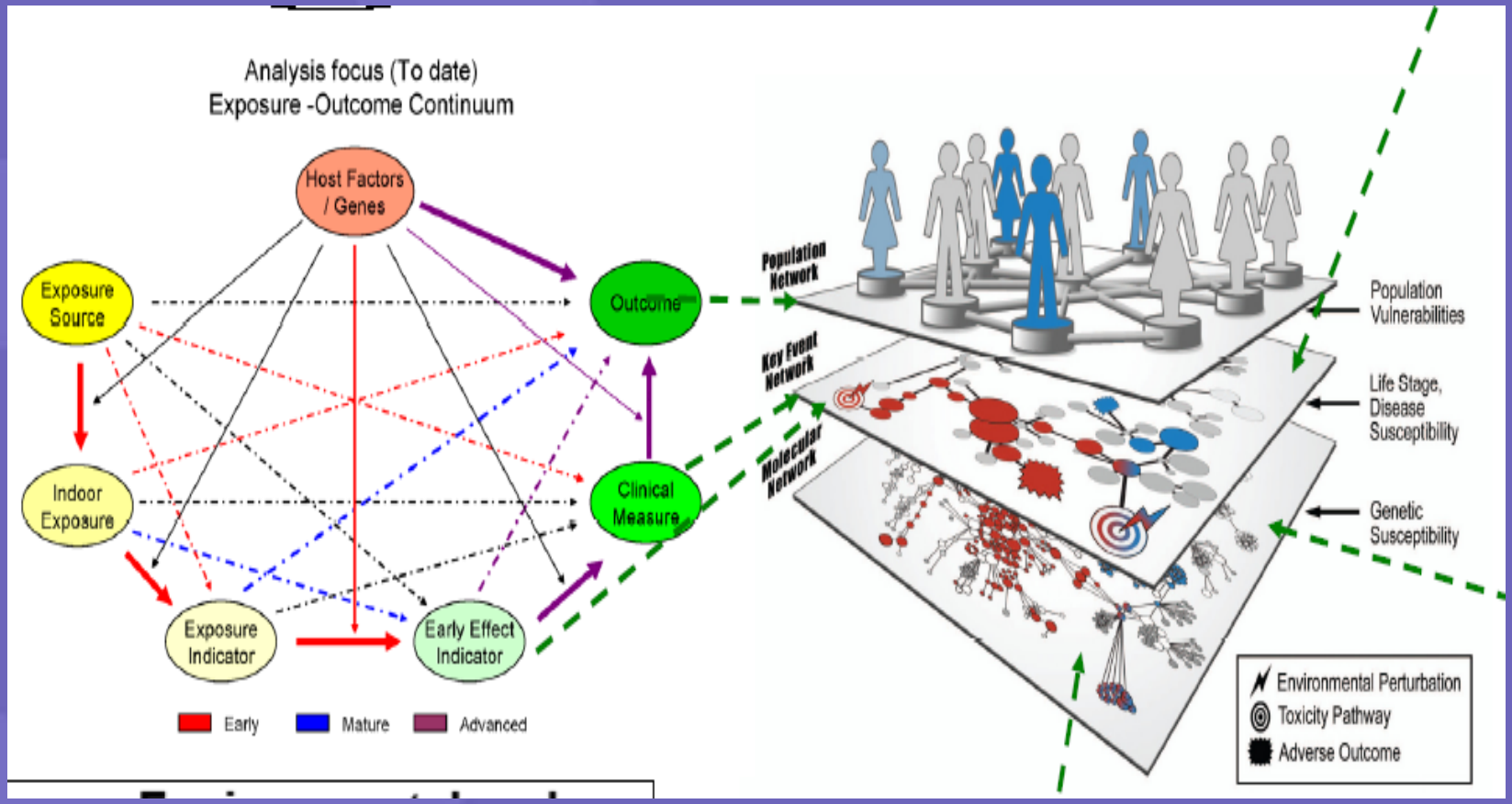
$$\text{ToxScore} = \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{P} w_p * \text{pathway}_p + \sum_{1}^{E} w_e * \text{endpoint}_e$$





Reif et al. 2009. Endocrine Profiling and Prioritization Using ToxCast Assays. (Poster Presentation, BOSC Meeting)

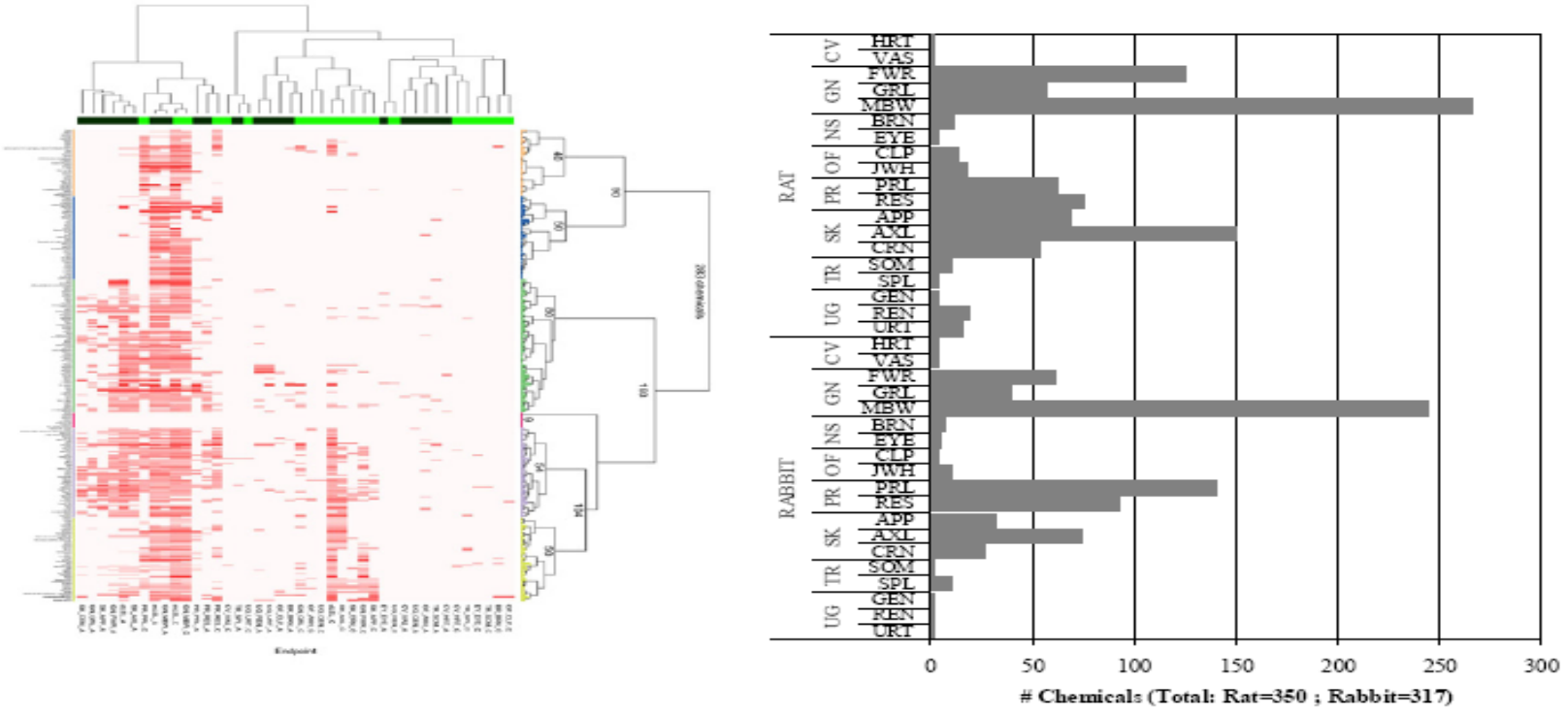
Systems Biology Research on Childhood Asthma



Gallagher et al. 2009. Mechanistic Indicators of Childhood Asthma (MICA): A Systems Biology Approach for the Integration of Multifactorial Environmental Health Data. (Poster Presentation, BOSC)

Reproductive & Developmental Toxicity

Predictive Modeling for Prioritization



Martin et al. 2009. Characteristics and Applications of the ToxRefDB In Vivo Datasets from Chronic, Reproductive and Developmental Assays (Poster Presentation, BOSC)

No Shortage of Hot Topics

- Pathway analyses could facilitate evaluation of:
  - Metabolic syndrome
  - Breast cancer
    - Confer with the California Breast Cancer Research Program?
  - Prostate cancer
  - Autism
  - Complex modes of action
  - Chemical mixtures

Summary Suggestions

- Continue Proof of Concept testing to demonstrate that assays predict known hazards
- For assays with “validated” counterpart, compare results
- Develop “validation” documents that address accuracy, sensitivity, etc. and include case studies
- Facilitate expert elicitation to evaluate assay evaluation and grouping, as well as interpretation
  - Publish analyses
  - Disease linkages
  - Stimulate research interests
- Provide summaries for “engaged layperson” stakeholders
  - Case study analyses
  - Update the FAQs
    - Why is each assay included? What does it “represent”?
    - How can hazard endpoints be characterized by grouping assay results?
- Establish framework for updating assays, analyses
- Consider applying ToxCast in non-regulatory applications to demonstrate utility and build confidence (green chemistry, alternatives analysis)