NAMs for Estrogenicity

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National Academies Committee on Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for use in Human Health Risk Assessment

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Key Points

- Community Needs
  - People are exposed to thousands of chemicals
  - *In vitro* NAMs can be used to rapidly test many chemicals
- Variability and Concordance
  - There is variability across *in vitro* methods, and within *in vivo* methods (lab-to-lab)
  - *In vitro* to *in vivo* concordance is approximately the same as lab-to-lab *in vivo*
- *In Vivo* vs. *In Vitro* Adversity
  - *In vitro* assays are good predictors of the uterotrophic response
  - Metabolic activation / deactivation is challenging, but NAM methods to address this are being developed
- Data for Benchmarking New Approaches
  - A large collection of reference chemicals are available
  - 1800+ chemicals have been evaluated *in vitro*, although few have corresponding *in vivo* data
  - Very little direct human data

Community Needs

- Over a lifetime, people can be exposed to many thousands of chemicals
  - Few of these have been thoroughly tested for toxicity, including estrogenicity
- EPA is required to test for potential endocrine disrupting chemicals through the Food Quality Protection Act (FQPA) and other statutes
  - Origin of the Endocrine Disruptor Screening Program (EDSP)
  - There are ~10,000 chemicals on the EDSP queue (pesticides, chemicals potentially found in drinking water)
- *In vitro* NAMs have been used to test 1,800 chemicals and more are in queue
- *In silico* NAMs (QSAR models) have tested all defined chemicals in the EDSP Universe

https://www.epa.gov/endocrine-disruption

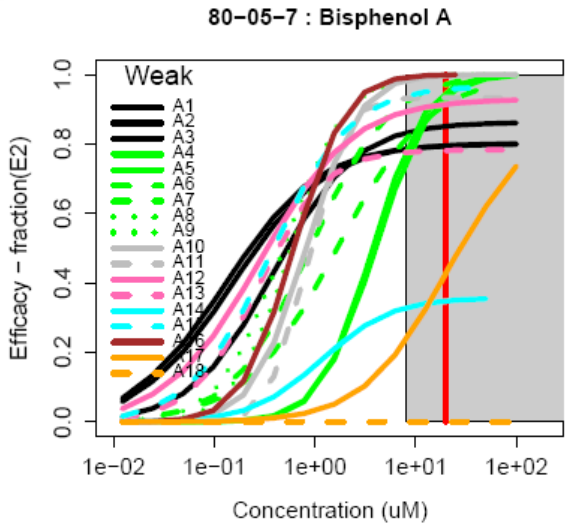
Variability and Concordance

- We have tested 1800 chemicals for estrogenicity in 18 separate *in vitro* assays
  - Different points on the biological pathway
  - Different cell types
  - Different readout technologies
- Key learnings:
  - All *in vitro* (and probably all *in vivo*) assays can show false positive results through "assay interference" – the assay is positive for reasons unrelated to the target the assay is supposed to assess
  - Best to use "orthogonal" assays – if multiple different technologies give the same results, confidence in result (positive or negative) is increased.
  - Quantitative results (potency) may still be variable

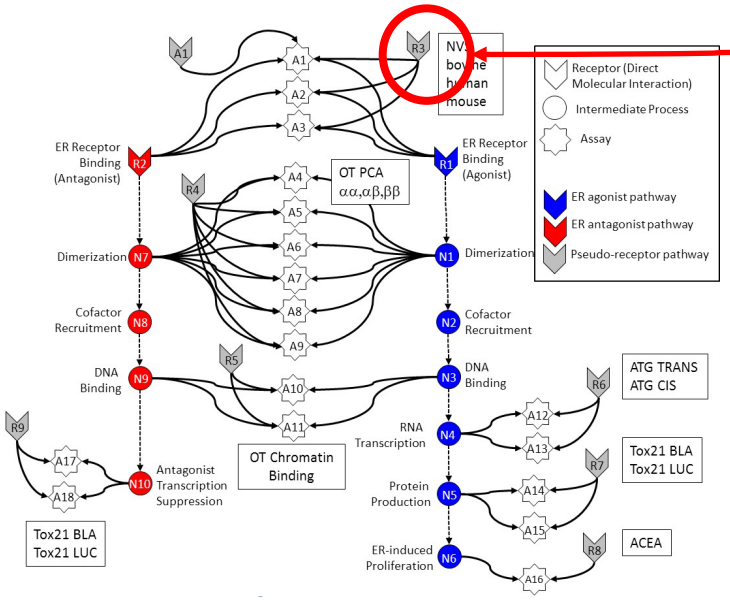
Judson et al., Tox.Sci. doi: 10.1093/toxsci/kfw092 (2016)
Judson, et al., ToxSci 148 (1) pp 137-154 (2015)
Browne et al., Environ. Sci. Technol. 2015, 49, 8804−8814, DOI: 10.1021/acs.est.5b02641

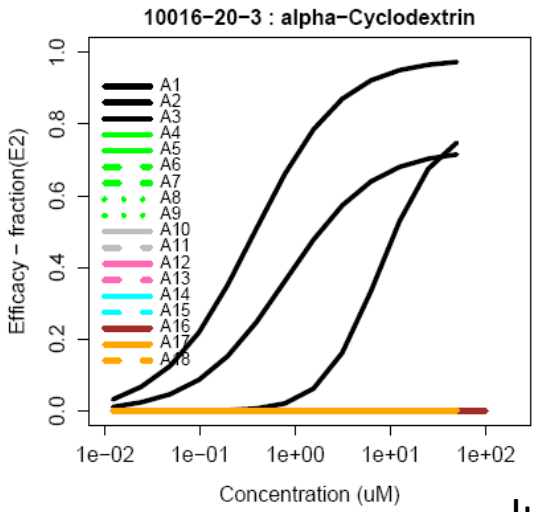
Example of Quantitative Uncertainty

True Agonist



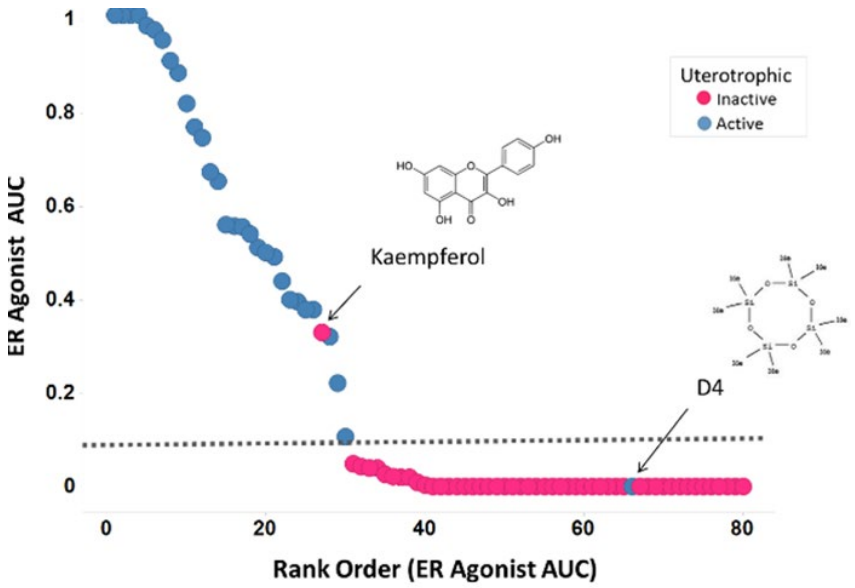
Assay Interference Example "R3"

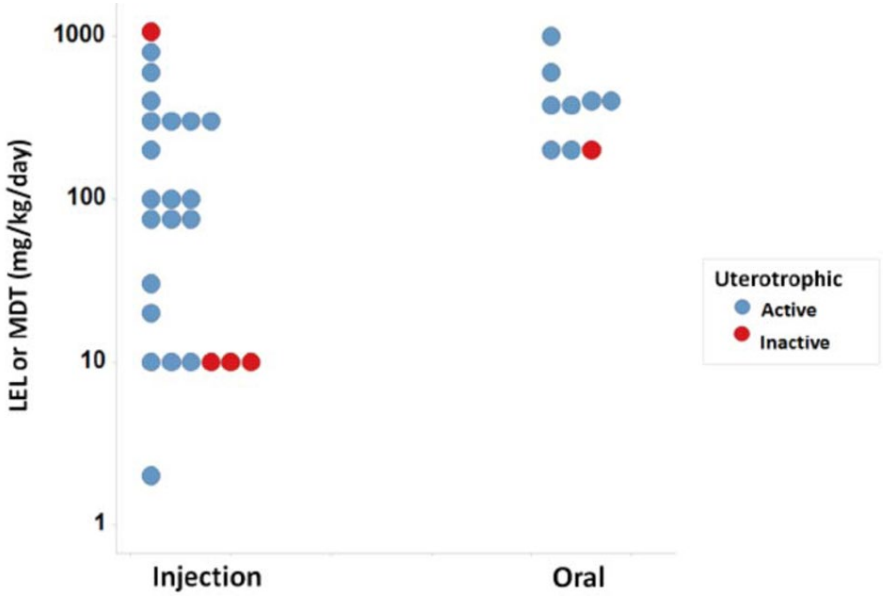




Judson, et al., ToxSci 148 (1) pp 137-154 (2015)

In Vitro to *In Vivo* Concordance





42 Chemicals with *in vitro* and *in vivo* data
1 false negative – chemical was volatile *in vitro*
1 false positive – chemical is metabolically deactivated *in vivo*

Variability in published *in vivo* uterotrophic data for Bisphenol A
Potency spans ~3 orders of magnitude

Browne et al. Environ. Sci. Technol. 2015, 49, 8804−8814, DOI: 10.1021/acs.est.5b02641

In Vivo and *In Vitro* Adversity

- NAMs can model some of the *in vivo* effects of estrogenic chemicals
- Estrogen receptor (ER) *in vitro* assays answer the following questions
  - Does the chemical interact with the ER?
  - Is the interaction in an agonist or antagonist mode?
  - Does the chemical cause ER-dependent cell proliferation?
  - What blood concentration is required to have an ER-related effect?
  - Assays test the human ER
- In vitro toxicokinetics assays and models answer the following question
  - What human oral dose is required to reach the blood level that is required to have an ER-related effect?
- Exposure NAM models answer the following question
  - Are humans likely to be exposed at levels that cause ER-related effects?
- Cannot directly predict what adverse phenotype will be seen

Data for Benchmarking New Methods

- Does a chemical interact with ER?
  - Good reference chemicals and published assays to compare against (see Browne et al. (2016))
- Is the chemical estrogenic through a non-genomic mechanism (GPR30)?
  - Reference chemicals and methods are sparse
  - Not clear how important this is for environmental chemicals
- Does the chemical affect estrogen signaling through the steroidogenesis pathway?
  - Chemicals can block the production of estrogen
  - Reference chemicals and methods exist (see Haggard et al. (2017))

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