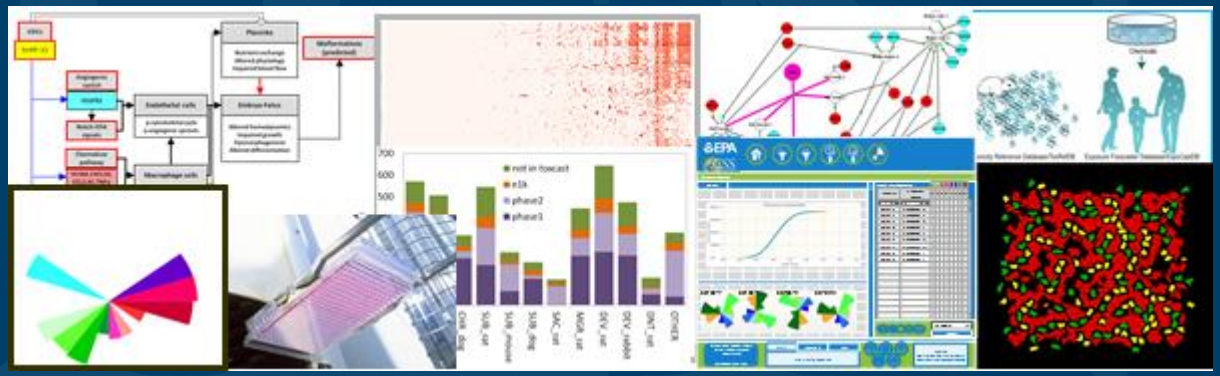
EPA
United States
Environmental Protection
Agency

Enhancing the Application of Alternative Methods Through Global Cooperation

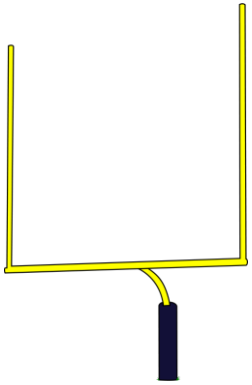


10th Congress on Alternatives and Animal Use in the Life Sciences

August 21, 2017

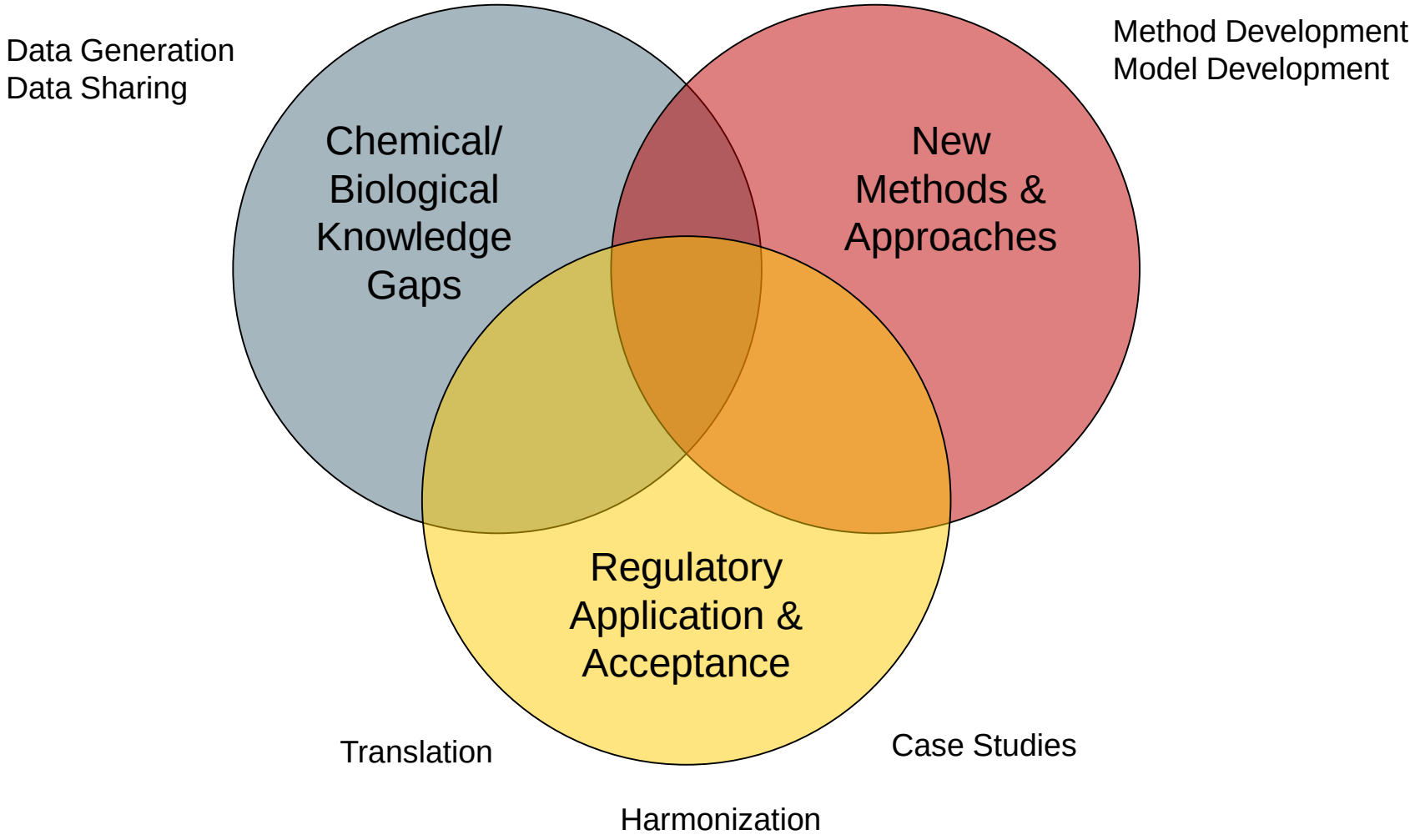
Rusty Thomas
Director
National Center for Computational Toxicology

Focusing on Common Goals

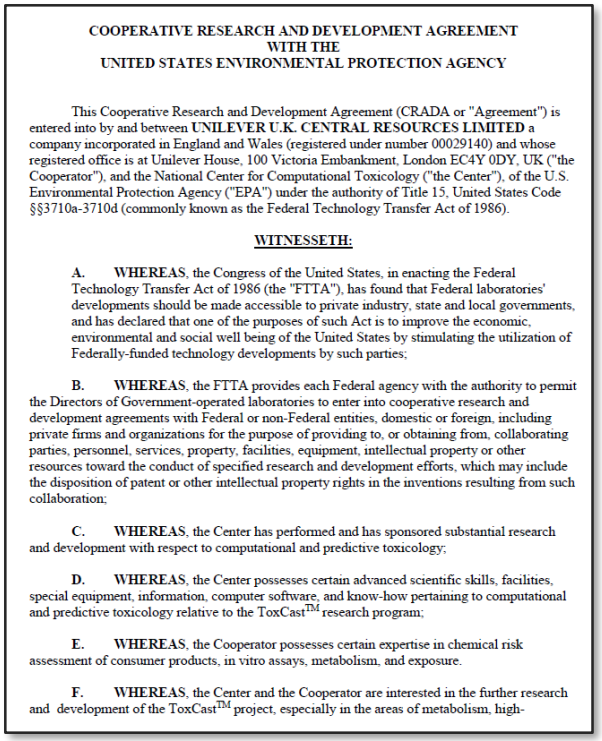


- Progress towards the development and translation of alternative testing methods is a common goal that crosses organizational, stakeholder, and international boundaries.
- The challenge is that organizations have different missions, different regulatory frameworks, and need to apply alternative methods to different decision contexts.
- Advancing the development and application of alternative methods will require focusing on common goals that address key challenges in advancing toxicology testing in the 21st century and provide common benefit across organizations and international boundaries.

Global Collaboration Strategy



EPA-Unilever Collaboration to Advance Development of Alternatives



COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT WITH THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

This Cooperative Research and Development Agreement (CRADA or "Agreement") is entered into by and between **UNILEVER U.K. CENTRAL RESOURCES LIMITED** a company incorporated in England and Wales (registered under number 00029140) and whose registered office is at Unilever House, 100 Victoria Embankment, London EC4Y 0DY, UK ("the Cooperator"), and the National Center for Computational Toxicology ("the Center"), of the U.S. Environmental Protection Agency ("EPA") under the authority of Title 15, United States Code §§3710a-3710d (commonly known as the Federal Technology Transfer Act of 1986).

WITNESSETH:

A. **WHEREAS**, the Congress of the United States, in enacting the Federal Technology Transfer Act of 1986 (the "FTTA"), has found that Federal laboratories' developments should be made accessible to private industry, state and local governments, and has declared that one of the purposes of such Act is to improve the economic, environmental and social well being of the United States by stimulating the utilization of Federally-funded technology developments by such parties;

B. **WHEREAS**, the FTTA provides each Federal agency with the authority to permit the Directors of Government-operated laboratories to enter into cooperative research and development agreements with Federal or non-Federal entities, domestic or foreign, including private firms and organizations for the purpose of providing to, or obtaining from, collaborating parties, personnel, services, property, facilities, equipment, intellectual property or other resources toward the conduct of specified research and development efforts, which may include the disposition of patent or other intellectual property rights in the inventions resulting from such collaboration;

C. **WHEREAS**, the Center has performed and has sponsored substantial research and development with respect to computational and predictive toxicology;

D. **WHEREAS**, the Center possesses certain advanced scientific skills, facilities, special equipment, information, computer software, and know-how pertaining to computational and predictive toxicology relative to the ToxCast™ research program;

E. **WHEREAS**, the Cooperator possesses certain expertise in chemical risk assessment of consumer products, in vitro assays, metabolism, and exposure.

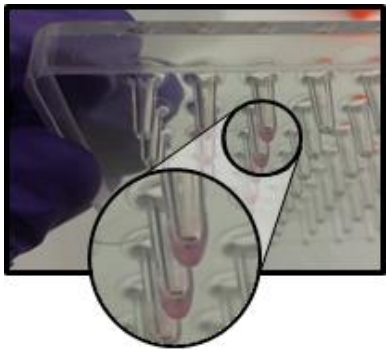
F. **WHEREAS**, the Center and the Cooperator are interested in the further research and development of the ToxCast™ project, especially in the areas of metabolism, high-

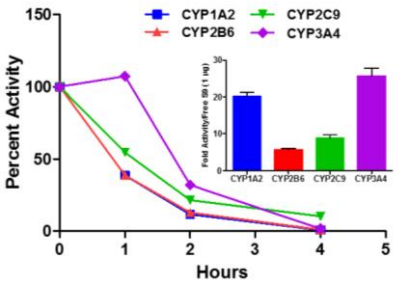
- Collaborative Research and Development Agreement (CRADA) between Unilever and U.S. EPA in July 2015
- Goal: Perform research and development on three chemical screening methods and translation of the results into risk assessment for use by private and public entities.
  - ToxCast and toxicokinetic assays
  - High-throughput transcriptomic assay
  - Universal retrofit of high-throughput screening assays with metabolic competence

Developing Methods to Address Metabolic Competence

"Extracellular" Approach

Chemicals metabolism in the media or buffer of cell-based and cell-free assays

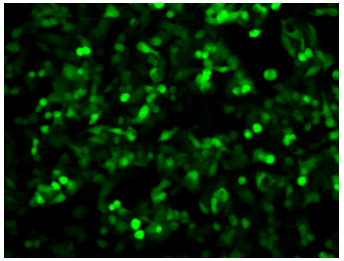


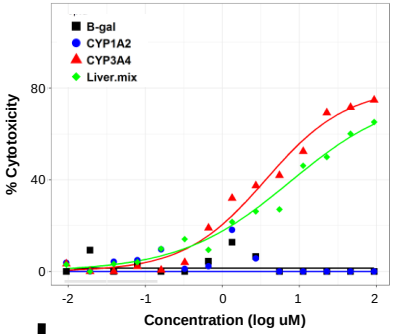


More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Approach

Capable of metabolizing chemicals inside the cell in cell-based assays





More closely models effects of target tissue metabolism

Integrated approach to model *in vivo* metabolic bioactivation and detoxification

Collaboration with Unilever

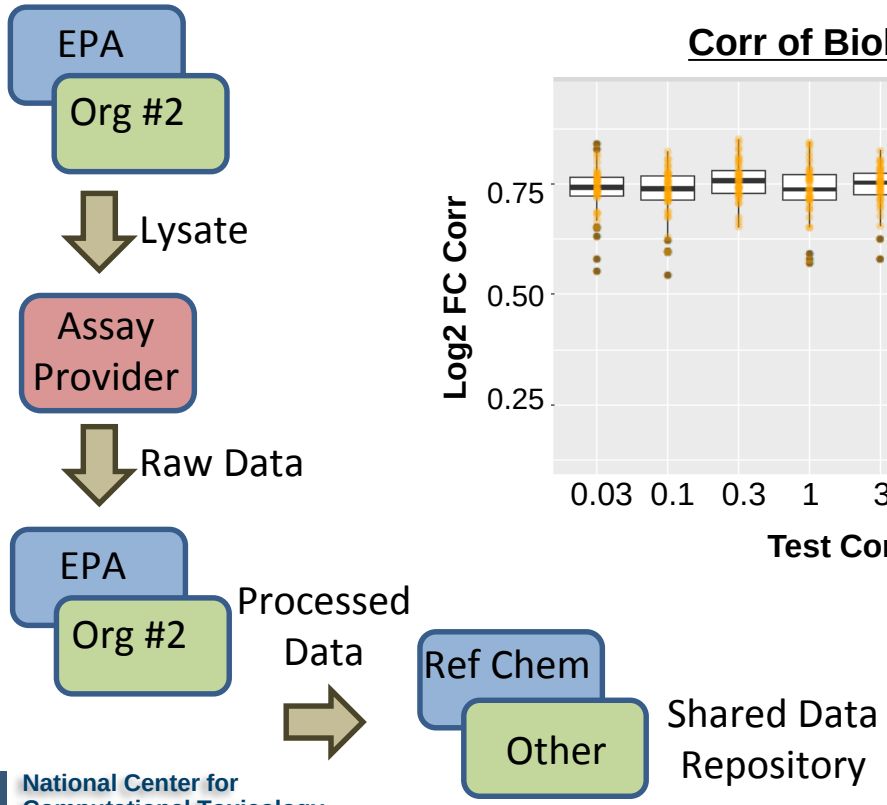
Developing Methods to Broadly Capture Potential Biological Effects

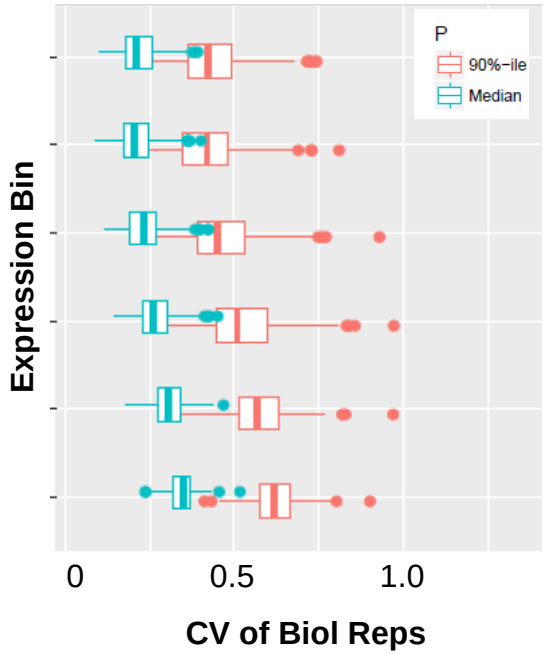
United States Environmental Protection Agency

High-Throughput Transcriptomic Screen

- TempOSeq whole transcriptome assay
- Low cost
- 384-well, cell lysate compatible
- Automatable

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	MAQC-A (Us)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	non-treated
B	MAQC-A (Us)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	non-treated
C	MAQC-B (Us)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	non-treated
D	MAQC-B (Us)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	DMSO
E	Bulk Lysate (DMSO)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	DMSO
F	Bulk Lysate (DMSO)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	DMSO
G	Bulk Lysate (Trichostatin)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	DMSO [No Label]
H	Bulk Lysate (Trichostatin)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Trichostatin (1 µM)
I	Lysis Buffer (Us)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	Trichostatin (1 µM)
J	Lysis Buffer (Us)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	Trichostatin (1 µM)
K	MAQC-A (Them)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	Genistein (10 µM)
L	MAQC-A (Them)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Genistein (10 µM)
M	MAQC-B (Them)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Genistein (10 µM)
N	MAQC-B (Them)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	Sirolimus (0.1 µM)
O	Lysis Buffer (Them)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Sirolimus (0.1 µM)
P	Lysis Buffer (Them)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Sirolimus (0.1 µM)





Collaboration with Unilever

Collaborative Estrogen Receptor Activity Prediction Project (CERAPP)

A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1510267.

Research

CERAPP: Collaborative Estrogen Receptor Activity Prediction Project

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Background: Humans are exposed to thousands of man-made chemicals in the environment. Some chemicals mimic natural endocrine hormones and, thus, have the potential to be endocrine disruptors. Most of these chemicals have never been tested for their ability to interact with the estrogen receptor (ER). Risk assessors need tools to prioritize chemicals for evaluation in costly *in vivo* tests, for instance, within the U.S. EPA Endocrine Disruptor Screening Program.

Objectives: We describe a large-scale modeling project called CERAPP (Collaborative Estrogen Receptor Activity Prediction Project) and demonstrate the efficacy of using predictive computational models trained on high-throughput screening data to evaluate thousands of chemicals for ER-related activity and prioritize them for further testing.

Methods: CERAPP combined multiple models developed in collaboration with 17 groups in the United States and Europe to predict ER activity of a common set of 32,464 chemical structures. Quantitative structure–activity relationship models and docking approaches were employed, mostly using a common training set of 1,677 chemical structures provided by the U.S. EPA, to build a total of 40 categorical and 8 continuous models for binding, agonist, and antagonist ER activity. All predictions were evaluated on a set of 7,522 chemicals curated from the literature. To overcome the limitations of single models, a consensus was built by weighting models on scores based on their evaluated accuracies.

Results: Individual model scores ranged from 0.69 to 0.85, showing high prediction reliabilities. Out of the 32,464 chemicals, the consensus model predicted 4,001 chemicals (12.3%) as high priority actives and 6,742 potential actives (20.8%) to be considered for further testing.

Conclusion: This project demonstrated the possibility to screen large libraries of chemicals using a consensus of different *in silico* approaches. This concept will be applied in future projects related to other end points.

Citation: Mansouri K, Abdelaziz A, Rybacka A, Roncaglioni A, Tropsha A, Varnek A, Zakharov A, Worth A, Richard AM, Grulke CM, Trisciuzzi D, Fourches D, Horvath D, Benfenati E, Muratov E, Wedebye EB, Grisoni F, Mangiatordi GF, Incisivo GM, Hong H, Ng HW, Tetko IV, Balabin I, Kancherla J, Shen J, Burton J, Nicklaus M, Cassotti M, Nikolov NG, Nicolotti O, Andersson PL, Zang Q, Politi R, Beger RD, Todeschini R, Huang R, Farag S, Rosenberg SA, Slavov S, Hu X, Judson RS. 2016. CERAPP: Collaborative Estrogen Receptor Activity Prediction Project. Environ Health Perspect 124:1023–1033; http://dx.doi.org/10.1289/ehp.1510267

Introduction

There are tens of thousands of natural and synthetic chemical substances to which humans and wildlife are exposed (Dionisio et al. 2015; Egeghy et al. 2012; Judson et al. 2009). A subset of these compounds may disrupt normal functioning of the endocrine system and cause health hazards to both humans and ecological species (Birnbaum and Fenton 2003; Diamanti-Kandarakis et al. 2009; Mahoney and Padmanabhan 2010; UNEP and WHO 2013). Endocrine-disrupting chemicals (EDCs) can mimic or interfere with natural hormones and alter their mechanisms of action at the receptor level, as well as interfere with the synthesis, transport, and metabolism of endogenous hormones (Diamanti-Kandarakis et al. 2009). Exposure to EDCs can lead to adverse health effects involving developmental, neurological, reproductive, metabolic, cardiovascular, and immune systems in humans and wildlife (Colborn et al. 1993; Davis et al. 1993; Diamanti-Kandarakis et al. 2009).

The estrogen receptor (ER) is one of the most extensively studied targets related to the effects of EDCs (Mueller and Korach 2001; Shanle and Xu 2011). This concern about estrogen-like activity of man-made chemicals is because of their potential for negatively affecting reproductive function (Hileman 1994; Kavlock et al. 1996). The emergence of concerns about EDCs has resulted in regulations requiring assessment of chemicals for estrogenic activity [Adler et al. 2011; U.S. Environmental Protection Agency (EPA) 1996; U.S. Food and Drug Administration (FDA) 1996]. There are numerous *in vitro* and *in vivo* protocols to identify potential endocrine pathway-mediated effects of chemicals, including interactions with hormone receptors (Jacobs et al. 2008; Rotroff et al.

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Supplemental Material is available online (http://dx.doi.org/10.1289/ehp.1510267).

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The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency or the U.S. Food and Drug Administration.

The authors declare they have no actual or potential competing financial interests.

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- International research project involving 17 groups in US and Europe
- Goal: Develop community consensus (Q)SAR models to predict ER binding and agonist/antagonist activity and prioritize chemicals for additional bioactivity screening
  - Evaluated a total of 40 categorical and 8 continuous models for predicting binding, agonist, and antagonist ER activity
  - Train models using ToxCast data for a set of 1677 compounds
  - Test predictions using an evaluation set of 7522 chemicals curated from the literature
  - Constructed consensus model using weighted performance scores
  - Predicted ER activity for 32,000 chemicals

Developing Models to Predict Potential Endocrine Activity

Table 1. Methods adopted by the participant groups (alphabetic order) in the modeling procedure.

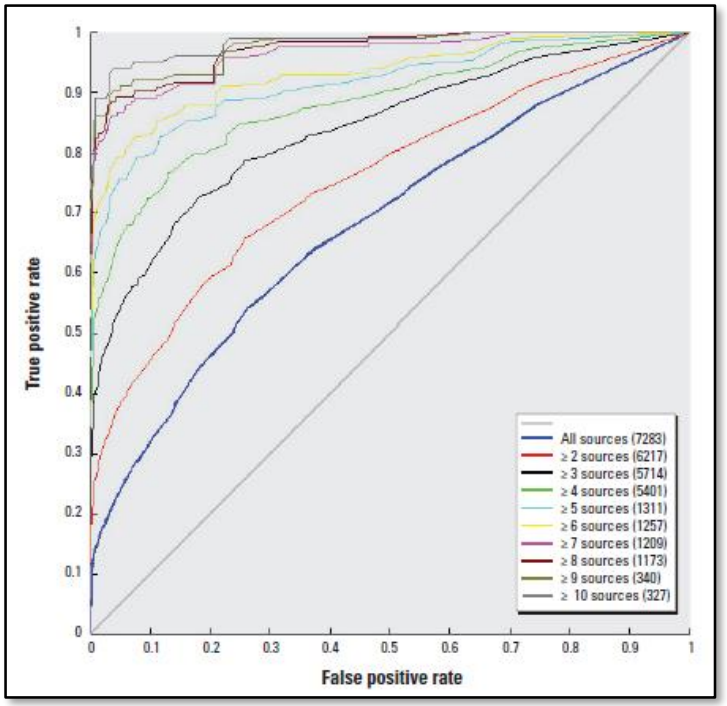
Model name	Calibration method	Descriptors software/type	Training set (No. of chemicals)	Predictions type
DTU	PLS/fragments	Leadscope	METI (595,481)/ToxCast™ (1,422)	Categorical
EPA_NCCT	GA + PLSDA	PADEL	ToxCast™ (1,529)	Categorical
FDA_NCTR_DBB (Ng et al. 2014)	DF	Mold2	ToxCast™ (1,677)	Categorical
FDA_NCTR_DSB	PLS	3D-SDAR	ToxCast™ (1019)	Categorical
ILS_EPA (Zang et al. 2013)	SVM + RF	Qikprop	ToxCast™ (1,677)	Categorical
IRCCS_CART (Roncaglioni et al. 2008)	CART-VEGA	2D descriptors	METI (806)	Categorical
IRCCS_Ruleset	Ruleset	SMARTS	ToxCast™ (1,529)	Categorical
JRC_Ispra (Poroikov et al. 2000)	PASS	MNA	—	Categorical
Lockheed Martin	kNN	Fingerprints	ToxCast™ (1,677)	Categorical + continuous
NIH_NCATS	Docking	AutoDock score	—	Categorical
NIH_NCI_GUSAR (Filimonov et al. 2009)	RBF-SCR	MNA, QNA	ToxCast™ (1,677)	Categorical
NIH_NCI_PASS (Poroikov et al. 2000)	PASS	MNA	ToxCast™ (1,677)	Categorical
OCHEM (2015)	*Consensus*	11 Descriptor types	ToxCast™(1,660)	Categorical + continuous
RIFM	SVM	Fingerprints	ToxCast™ (1,677)	Categorical
Umeå (Rybacka et al. 2015)	ASNN	DRAGON	METI + (Kuiper et al. 1997; Taha et al. 2010)	Categorical
UNC_MML	SVM+RF	DRAGON	ToxCast™ (120)	Categorical
UNIBA (Trisciuzzi et al. 2015)	Docking	GLIDE score	ToxCast™ (1,677)	Categorical
UNIMIB	kNN	DRAGON + fingerprints	ToxCast™ (1,677)	Categorical
UNISTRA (Horvath et al. 2014)	SVM	ISIDA	ToxCast™ (1,529)	Categorical + continuous

Predictions type: A categorical model is one that provides an active/inactive call for each chemical, whereas a continuous model provides a prediction of the potency (in μM) for each active chemical. Calibration methods: PLS (partial least-squares), PLS-DA (partial least-squares discriminant analysis), SVM (support vector machines), RF (random forest), DF (Decision forest), kNN (*k* nearest neighbors), ASNN (associative artificial neural networks), PASS (algorithm derived from Naïve Bayes classifier), RBF-SCR (self-consistent regression with radial basis function interpolation).

Table 5. Statistics of categorical *consensus* predictions for binding on ToxCast™ and literature data.

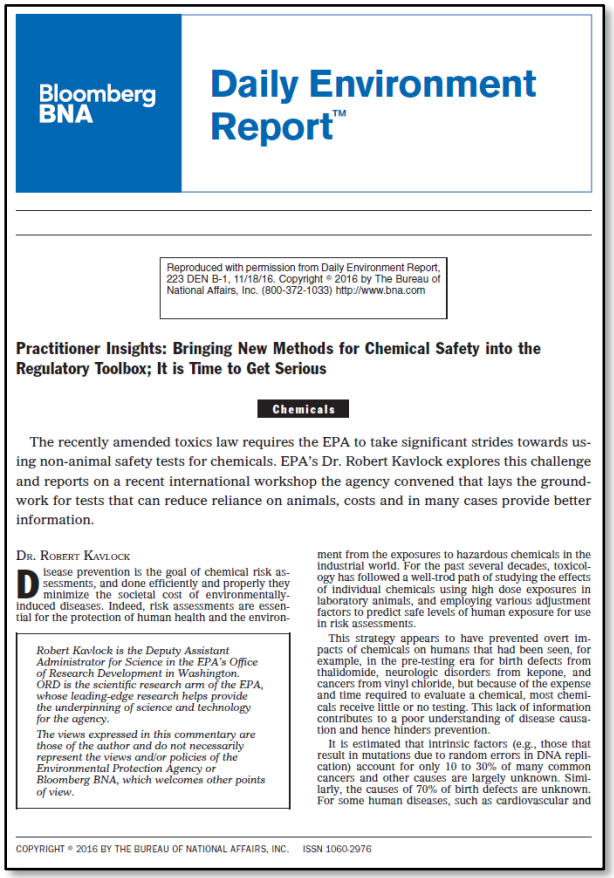
Statistics/used data	ToxCast™ data	Literature evaluation set (all: 7,283)	Literature evaluation set (> 6 sources: 1,257)
Sensitivity	0.85	0.23	0.85
Specificity	0.98	0.95	0.97
Balanced accuracy	0.92	0.59	0.91

The literature data with more than six sources represents the most consistent part of the evaluation set.



Mansouri *et al.*, EHP, 2016

Case Study for Regulatory Application of Quantitative *In Vitro* Screening



Bloomberg BNA

Daily Environment Report™

Practitioner Insights: Bringing New Methods for Chemical Safety into the Regulatory Toolbox; It Is Time to Get Serious

Chemicals

The recently amended toxics law requires the EPA to take significant strides towards using non-animal safety tests for chemicals. EPA's Dr. Robert Kavlock explores this challenge and reports on a recent international workshop the agency convened that lays the groundwork for tests that can reduce reliance on animals, costs and in many cases provide better information.

DR. ROBERT KAVLOCK

Disease prevention is the goal of chemical risk assessments, and done efficiently and properly they minimize the societal cost of environmentally-induced diseases. Indeed, risk assessments are essential for the protection of human health and the environment from the exposures to hazardous chemicals in the industrial world. For the past several decades, toxicology has followed a well-trod path of studying the effects of individual chemicals using high dose exposures in laboratory animals, and employing various adjustment factors to predict safe levels of human exposure for use in risk assessments.

This strategy appears to have prevented overt impacts of chemicals on humans that had been seen, for example, in the pre-testing era for birth defects from thalidomide, neurologic disorders from kepone, and cancers from vinyl chloride, but because of the expense and time required to evaluate a chemical, most chemicals receive little or no testing. This lack of information contributes to a poor understanding of disease causation and hence hinders prevention.

It is estimated that intrinsic factors (e.g., those that result in mutations due to random errors in DNA replication) account for only 10 to 30% of many common cancers and other causes are largely unknown. Similarly, the causes of 70% of birth defects are unknown. For some human diseases, such as cardiovascular and

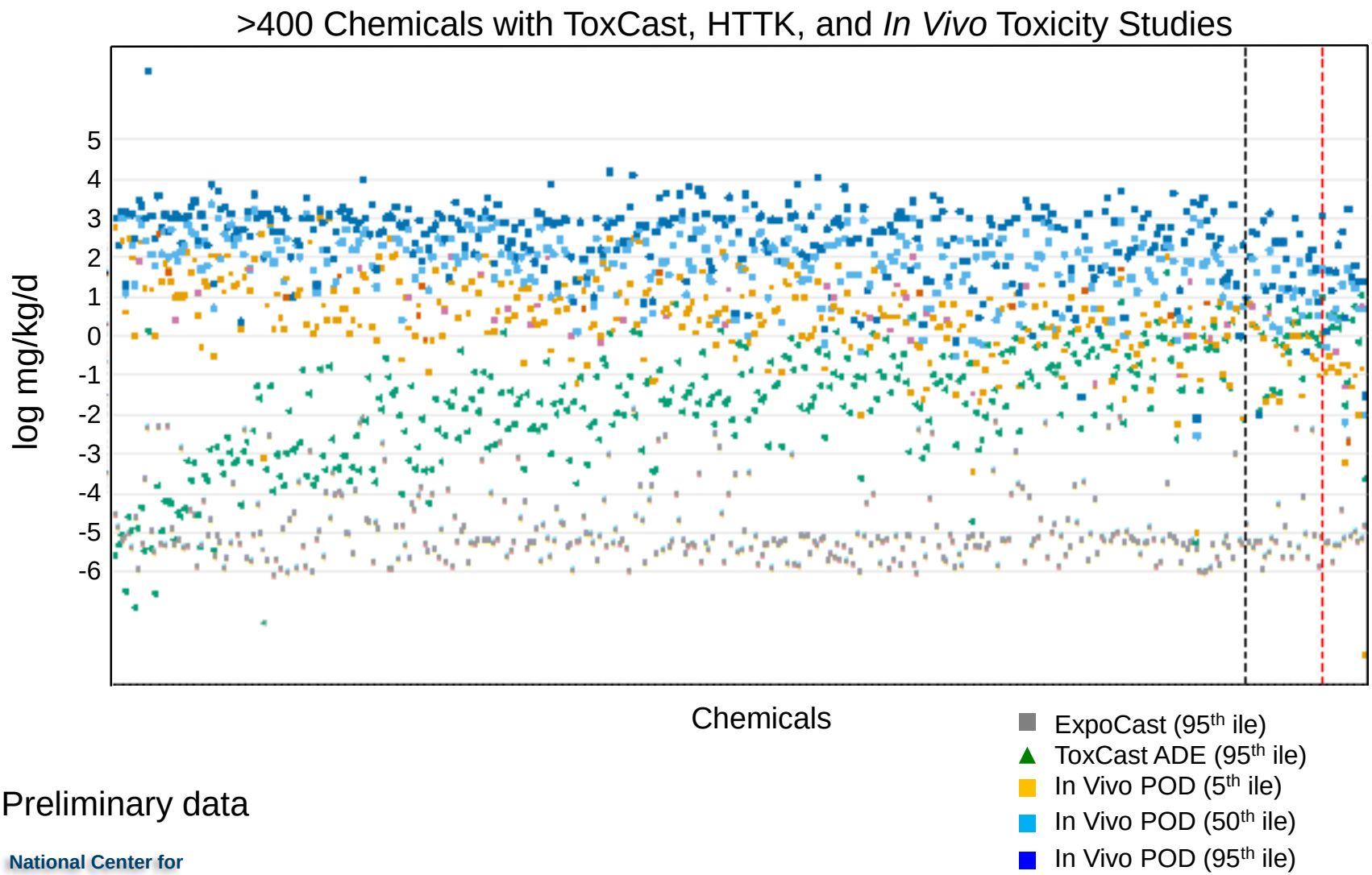
Robert Kavlock is the Deputy Assistant Administrator for Science in the EPA's Office of Research Development in Washington. ORD is the scientific research arm of the EPA, whose leading-edge research helps provide the underpinning of science and technology for the agency.

The views expressed in this commentary are those of the author and do not necessarily represent the views and/or policies of the Environmental Protection Agency or Bloomberg BNA, which welcomes other points of view.

 ISSN 1060-2976

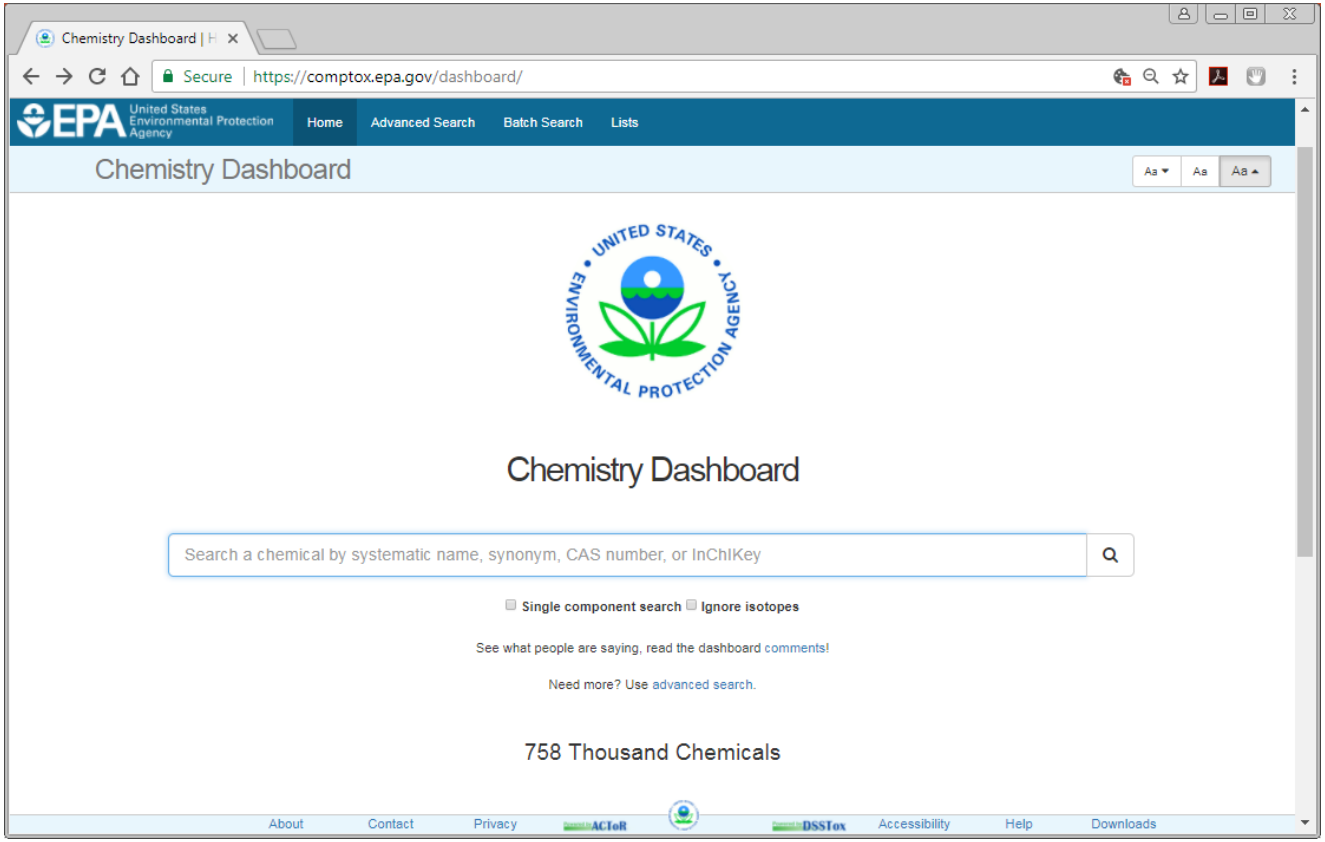
- International case study stemming from 2016 intergovernmental workshop
- Participants include EPA, Health Canada, ECHA, EFSA, and A*STAR
- Goal: Determine whether *in vitro* bioactivity from broad high-throughput screening studies (e.g., ToxCast) can be used as a conservative point-of-departure and when compared with exposure estimates serve to prioritize chemicals for future study or as lower tier risk assessment.

In Vitro Bioactivity as a Conservative Point of Departure Case Study



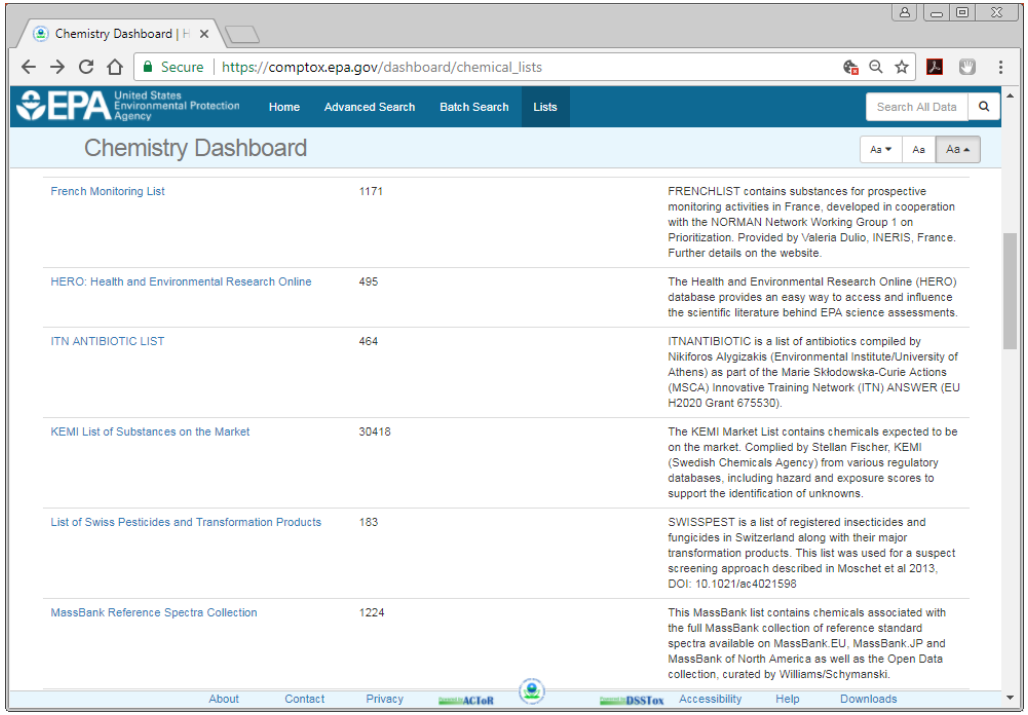
Preliminary data

CompTox Dashboard Integrates Phys-Chem, Hazard, Fate and Exposure



https://comptox.epa.gov/dashboard/

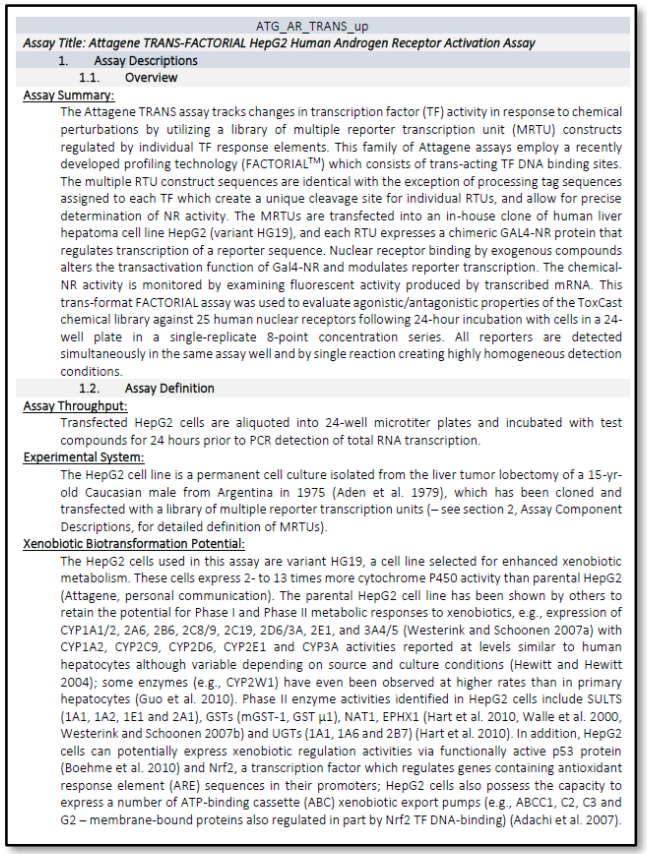
CompTox Dashboard to Share and Harmonize Chemical Information



https://comptox.epa.gov/dashboard/

- Chemical information and lists shared by international collaborators
- Examples include
  - INERIS
  - Norman Network
  - EU Massbank
  - ETH
  - Luxembourg Center for Systems Biomedicine
  - Helmholtz Zentrum München
  - QSARDB

ToxCast Annotations in OECD 211 Format to Harmonize Assay Information



ATG_AR_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Androgen Receptor Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

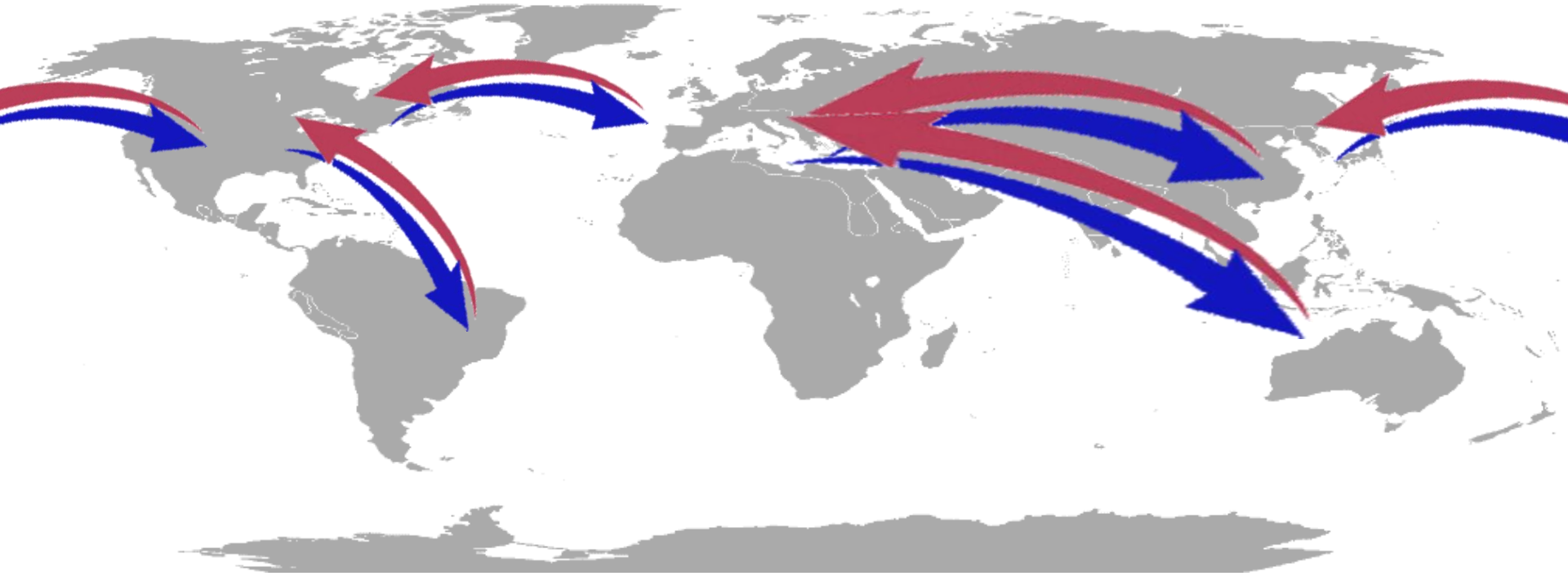
The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 (Aden et al. 1979), which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 (Westerink and Schoonen 2007a) with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions (Hewitt and Hewitt 2004); some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes (Guo et al. 2010). Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST μ1), NAT1, EPHX1 (Hart et al. 2010, Walle et al. 2000, Westerink and Schoonen 2007b) and UGTs (1A1, 1A6 and 2B7) (Hart et al. 2010). In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein (Boehme et al. 2010) and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) (Adachi et al. 2007).

- OECD project with Validation Management Group – Non-Animal (VMG-NA)
- Goal: Evaluate the strengths and limitations of the OECD Guidance Document No. 211 while also providing international community with ToxCast/Tox21 assay annotations in a harmonized template
  - Completed endocrine-related assays
  - Expanding effort to include entire ToxCast/Tox21 assay set

Chemicals and Potential Safety Concerns Cut Across Boarders…



Safety information, data, and cooperation on new testing methods should be shared the same way.

Thank You for Your Attention!

EPA's National Center for Computational Toxicology

We Want to Collaborate With You!