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Modulation of Xenobiotic Metabolizing Enzyme and Transporter Gene Expression in Primary Cultures of Human Hepatocytes Modulated by ToxCast Chemicals

Society of Toxicology annual meeting March 15-19, 2009 in Baltimore MD

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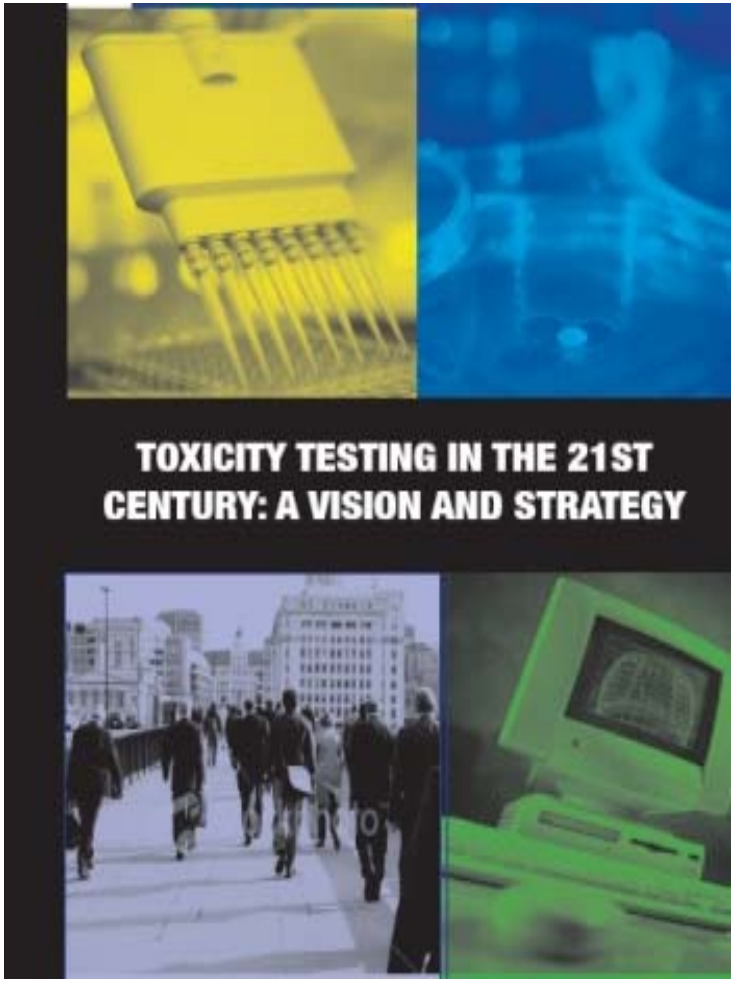
Office of Research and Development
National Center for Computational Toxicology

EPA United States Environmental Protection Agency

Objectives:

- What is ToxCast?
- How does this project fit in with the goals of ToxCast?
- How does the technology work?
- Experimental Design
- What were the results?
- What do the results mean?

Transforming Toxicology



POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

Francis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,

Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

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*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

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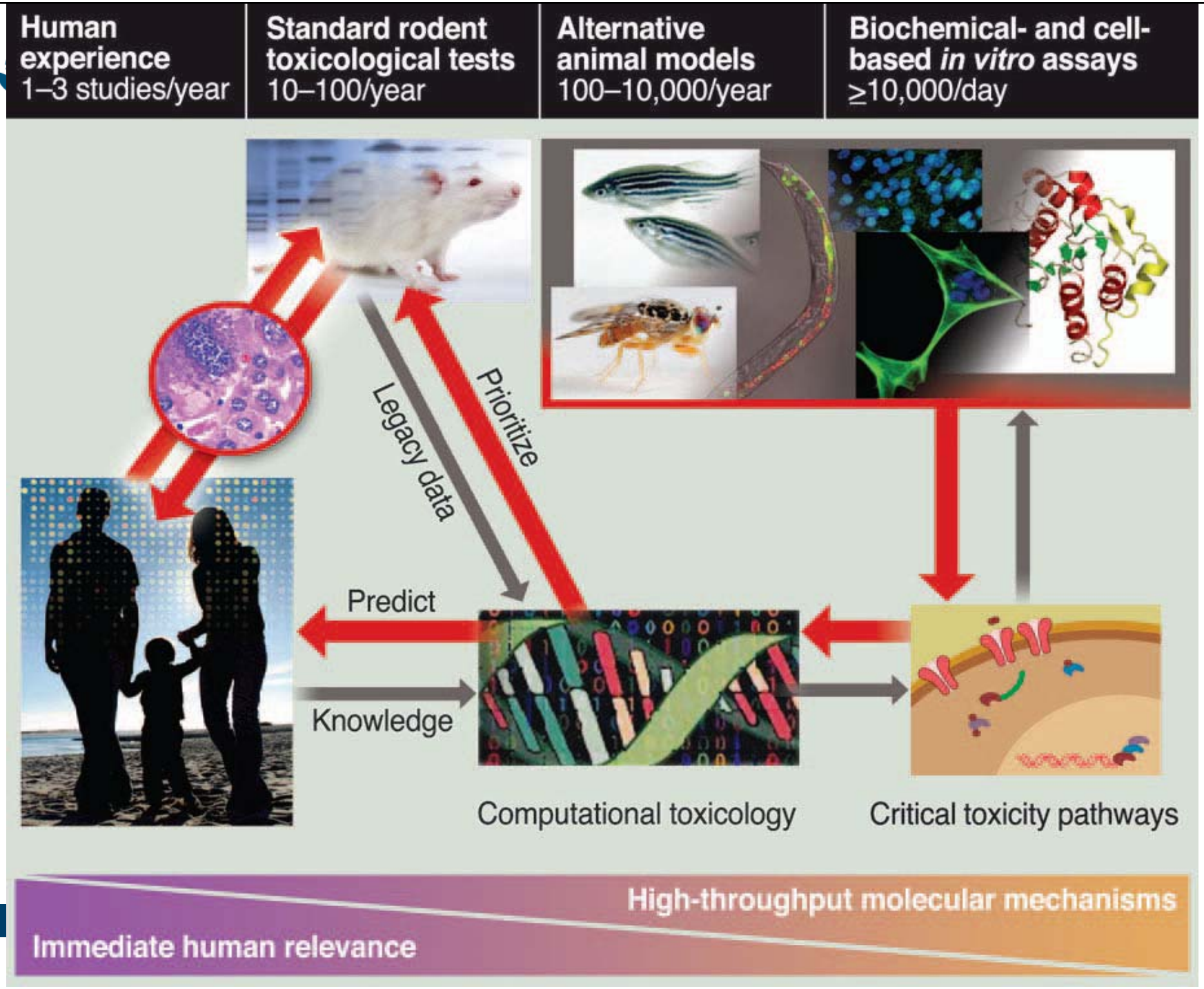
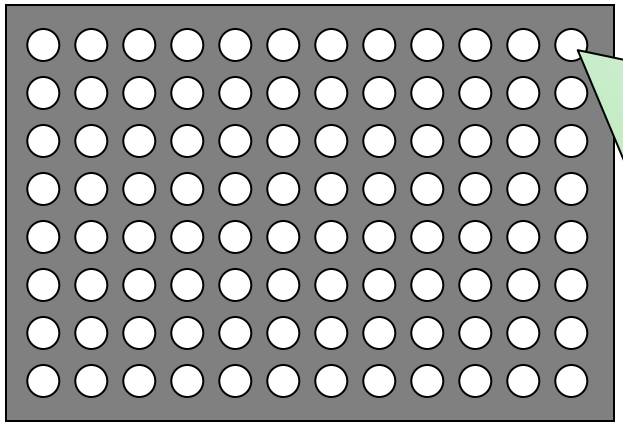
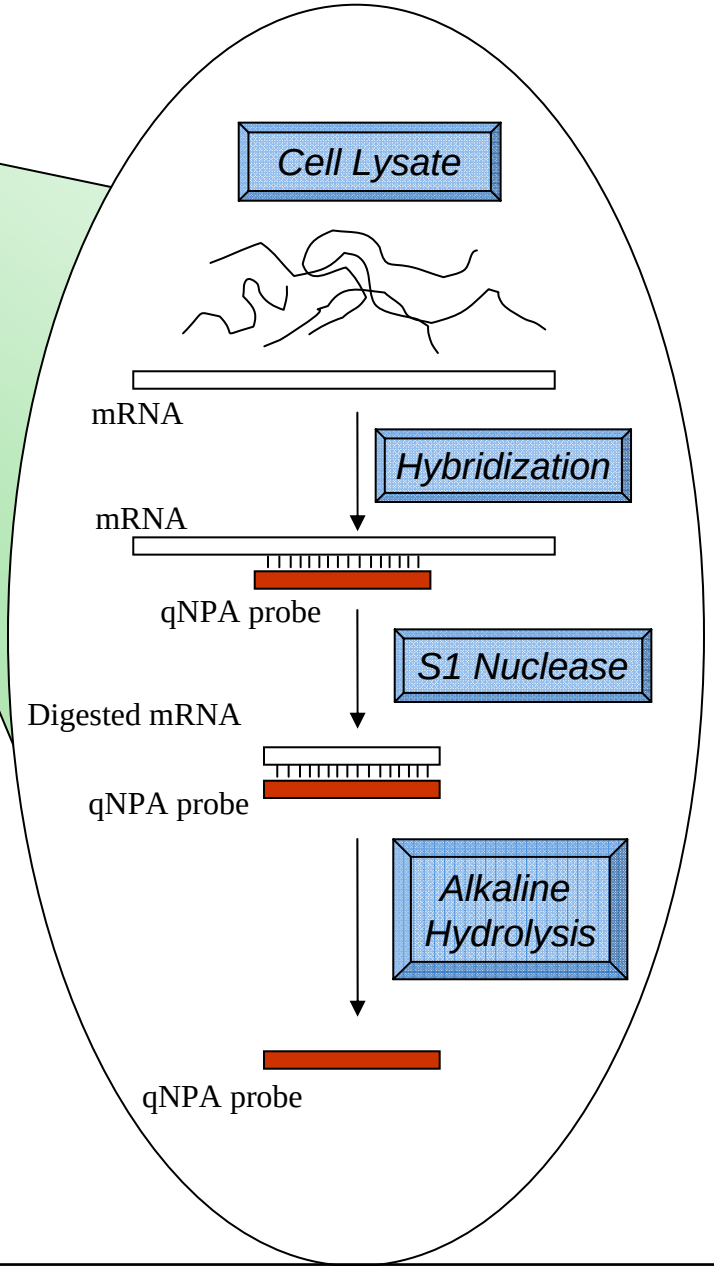
U.S. National Academy of Sciences, 2007


Human experience
1–3 studies/year
Standard rodent toxicological tests
10–100/year
Alternative animal models
100–10,000/year
Biochemical- and cell-based *in vitro* assays
≥10,000/day
Legacy data
Prioritize
Predict
Knowledge
Computational toxicology
Critical toxicity pathways
High-throughput molecular mechanisms
Immediate human relevance

HTG TECHNOLOGY





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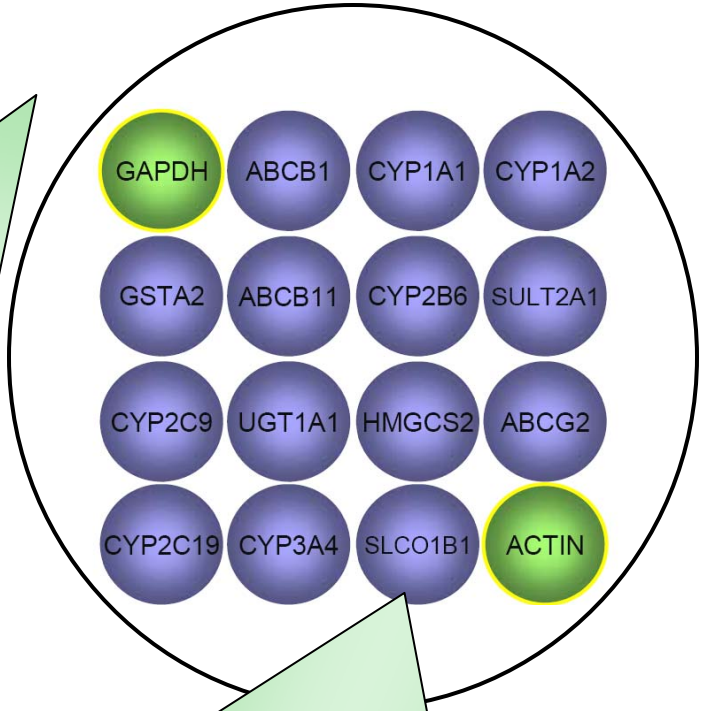
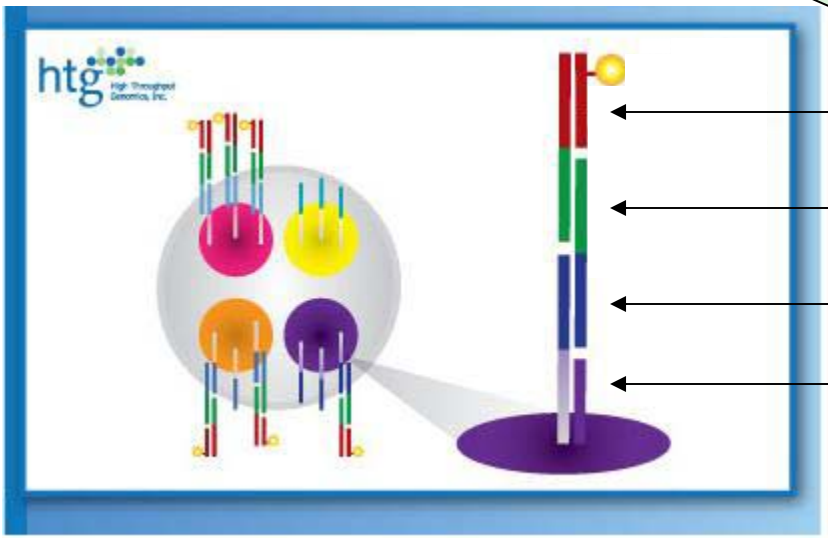
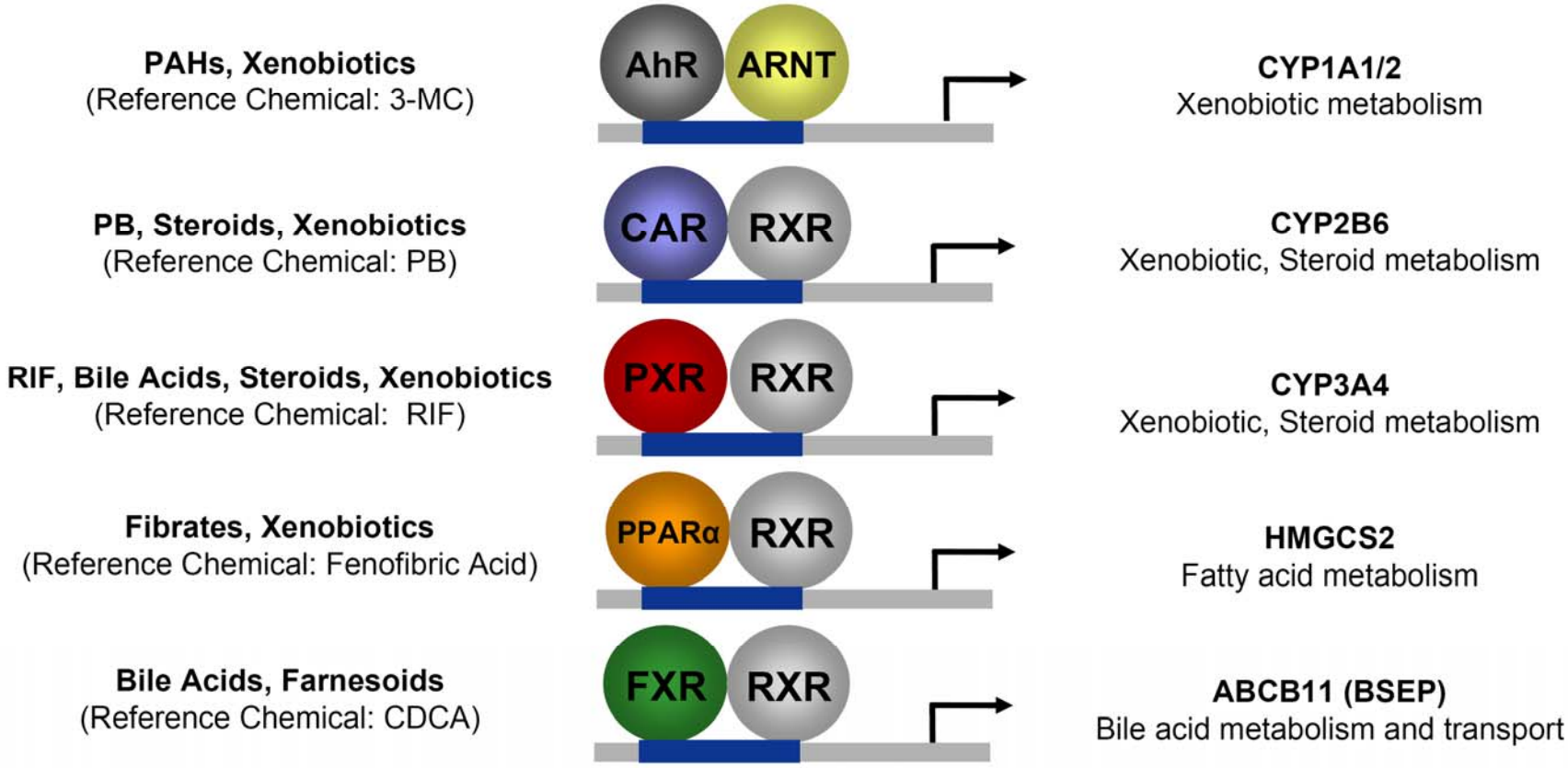




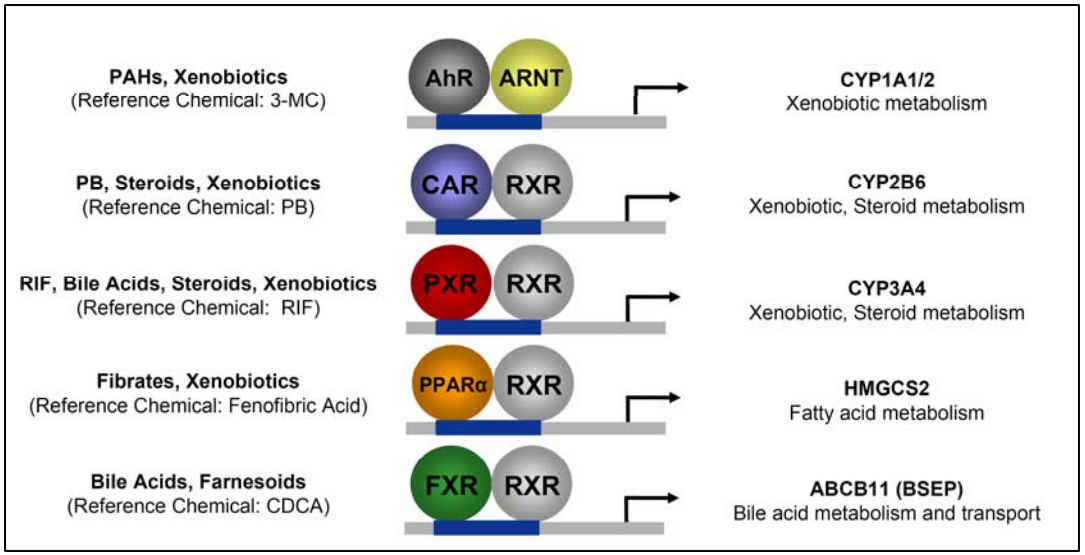
http://www.htgenomics.com/technology/arrayplate

Chemicals → NR → Genes



Experimental Design: Chemicals → NR → Genes

- ToxCast 320 Chemical Library
- Fresh Primary Human Hepatocytes
- 2 human donors
- 6 Reference Chemicals (Rif, PB, 3-MC, Fenofibric Acid, CDCA, CITCO)
- 5 receptors targets (AhR, CAR, PXR, PPARα, FXR)
- 2 endogenous control gene targets (GAPDH, Actin)
- 14 relevant gene targets
- 3 Time Points (6,24,48 hours)
- 5 Concentrations (.004, .04,0.4, 4, 40 µM)



Reference Chemical Decision Criteria

- High Efficacy
- Must have measurable EC50
- High Z-factor

Z-factor as defined by Zhang et al.

$$Z = 1 - \frac{(3\sigma_s + 3\sigma_c)}{|\mu_s - \mu_c|}$$

Zhang J, Chung T, Oldenburg K. *A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays*. Journal of Biomedical Screening. Vol. 4, Num.2. 1999.

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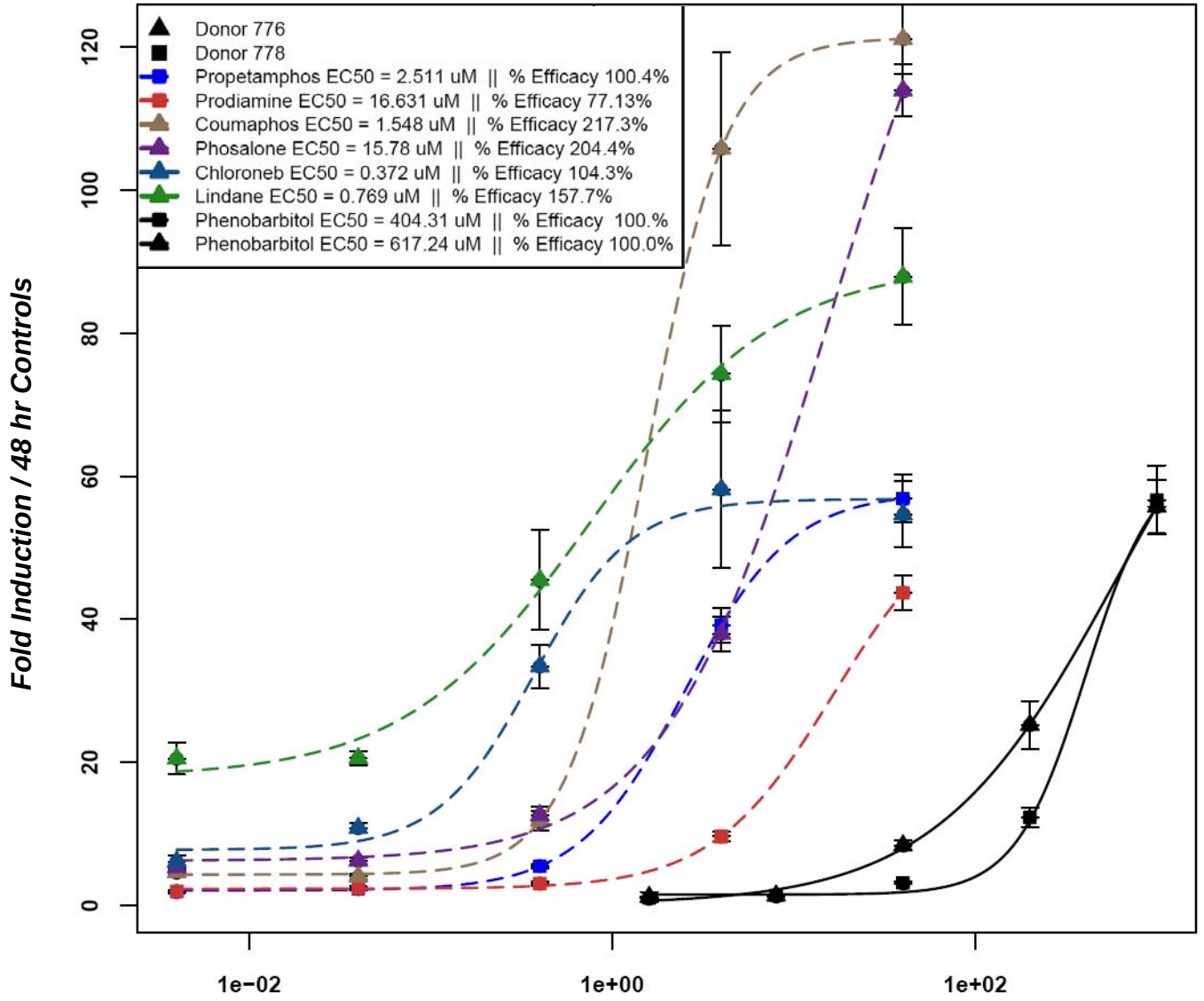
Representative ToxCast Chemicals for Potency and Efficacy for CYP2B6 at 48hrs



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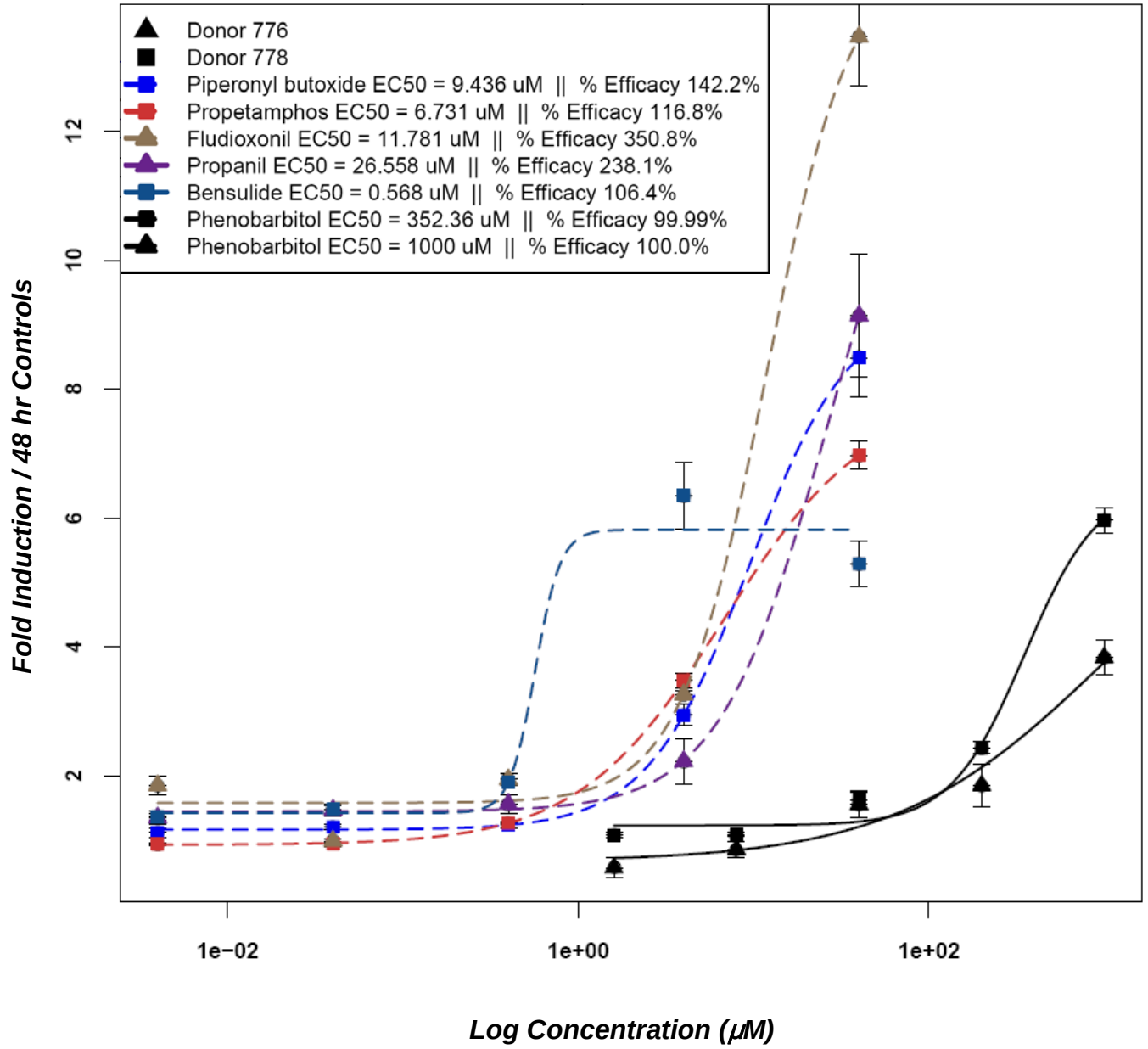
Representative ToxCast Chemicals for Potency and Efficacy for UGT1A1 at 48hrs



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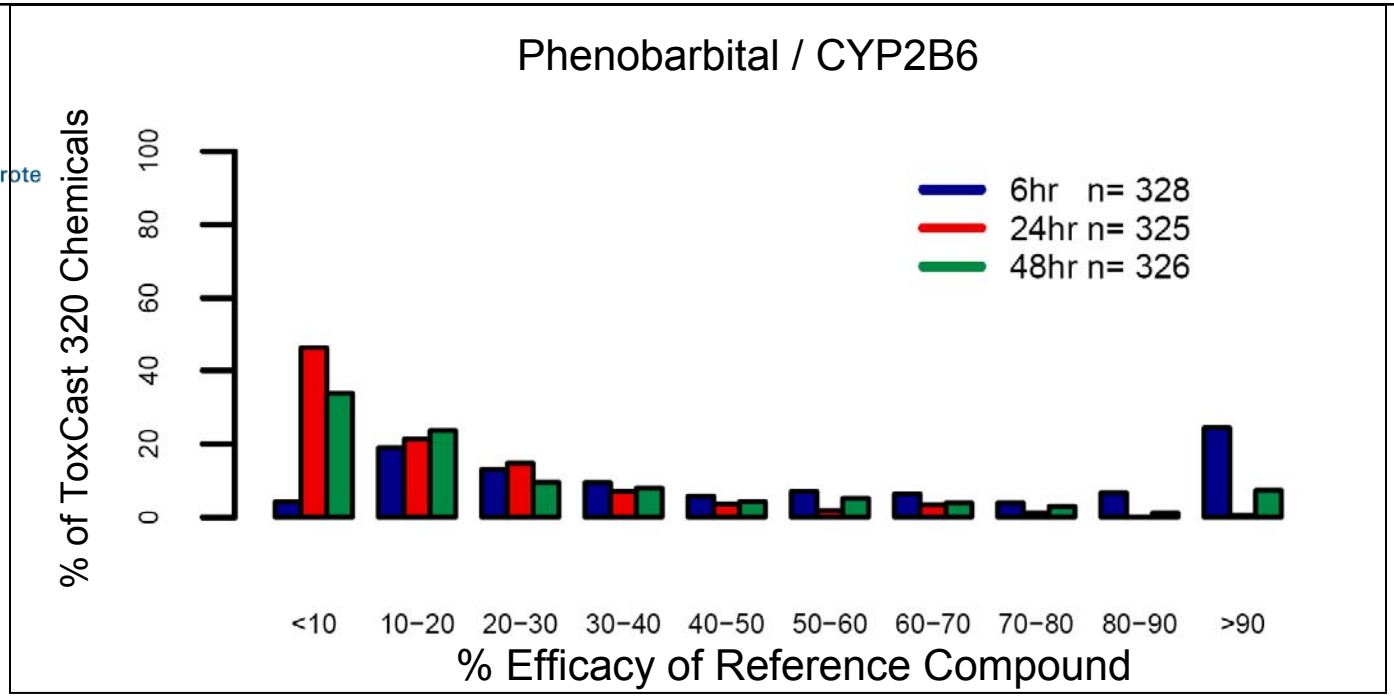

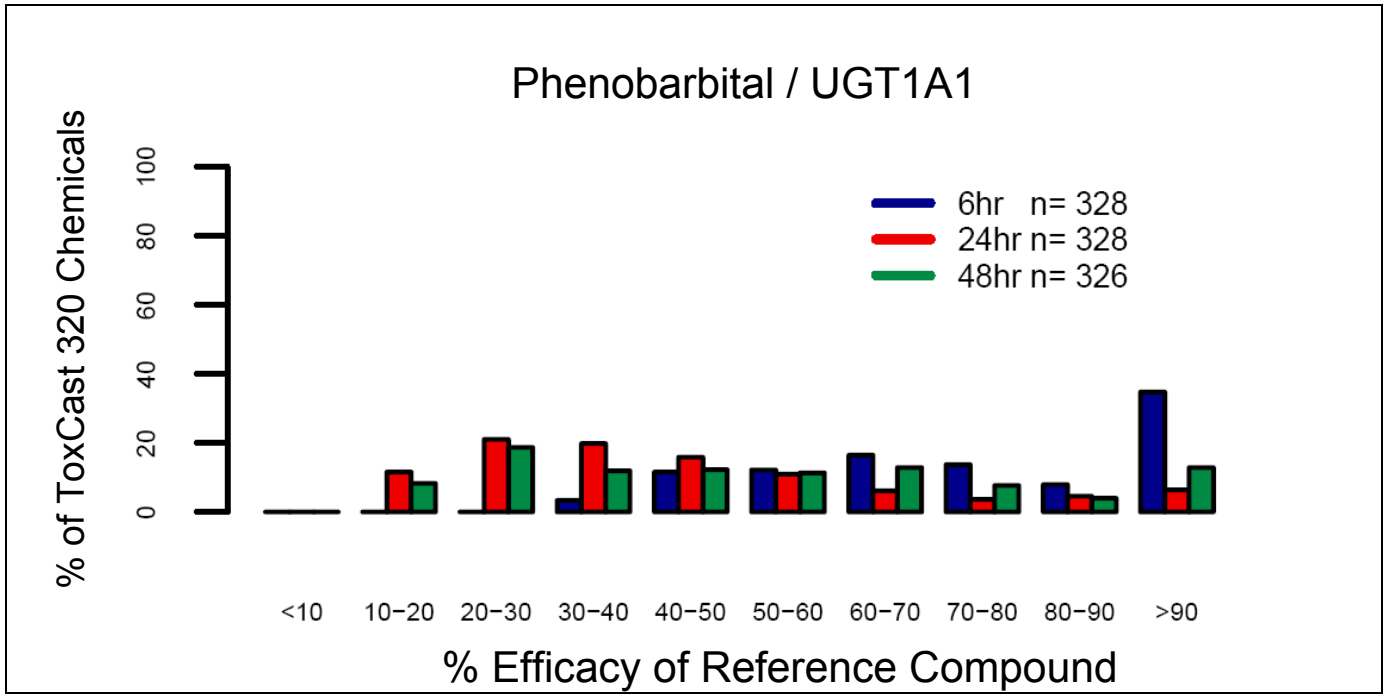

Criteria for *In Vitro-In Vivo* Comparison

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In Vitro Criteria

- Must be statistically significant
  $p < .01/14 = .000714$
- Must have an EC50 within the tested concentration range.
- Must not be cytotoxic at concentrations lower than the EC50.
- If cytotoxicity was observed at 48 hours, and compound met all criteria at 24 hours, then assume cytotoxicity confounded results at 48 hours

In Vivo Criteria

- Data came from DER entered into ToxRefDB
- Must have a lowest effect level (LEL)
- LEL assigned if statistically significant effect was observed, or if a clear dose response of effect was demonstrated.
- Described in detail:

Martin et al. *Profiling Chemicals Based on Chronic Toxicity Results from the U.S. EPA ToxRef Database.* Environ Health Perspect. Doi:10.1289/ehp.0800074.[Online 20 October 2008]

In Vitro – In Vivo

Analysis	Gene	Mouse Liver Apoptosis Necrosis	Rat Liver Apoptosis Necrosis	Rat Liver Hypertrophy	Rat Liver Tumors	Rat Proliferative Thyroid Lesions	Rat Thyroid Tumors
RR	CYP2B6	0.81	2.00	2.26	0.62	2.25	3.83
Sensitivity	CYP2B6	0.45	0.67	0.69	0.38	0.69	0.79
Specificity	CYP2B6	0.49	0.52	0.59	0.49	0.55	0.55
RR	ABCB11	2.20	0.79	1.39	1.49	1.23	1.51
Sensitivity	ABCB11	0.32	0.14	0.23	0.24	0.21	0.24
Specificity	ABCB11	0.86	0.82	0.85	0.83	0.83	0.84
RR	HMGCS2	1.27	1.10	1.00	1.65	1.19	1.35
Sensitivity	HMGCS2	0.61	0.57	0.55	0.67	0.59	0.62
Specificity	HMGCS2	0.47	0.46	0.45	0.47	0.46	0.47
RR	CYP1A1	1.12	1.51	1.44	2.01	1.04	1.36
Sensitivity	CYP1A1	0.27	0.33	0.32	0.40	0.26	0.31
Specificity	CYP1A1	0.76	0.76	0.79	0.77	0.75	0.76
RR	CYP3A4	1.10	0.87	1.41	0.87	1.06	1.52
Sensitivity	CYP3A4	0.35	0.30	0.41	0.30	0.34	0.43
Specificity	CYP3A4	0.68	0.67	0.71	0.67	0.67	0.69

Predicting Thyroid Tumorigenicity

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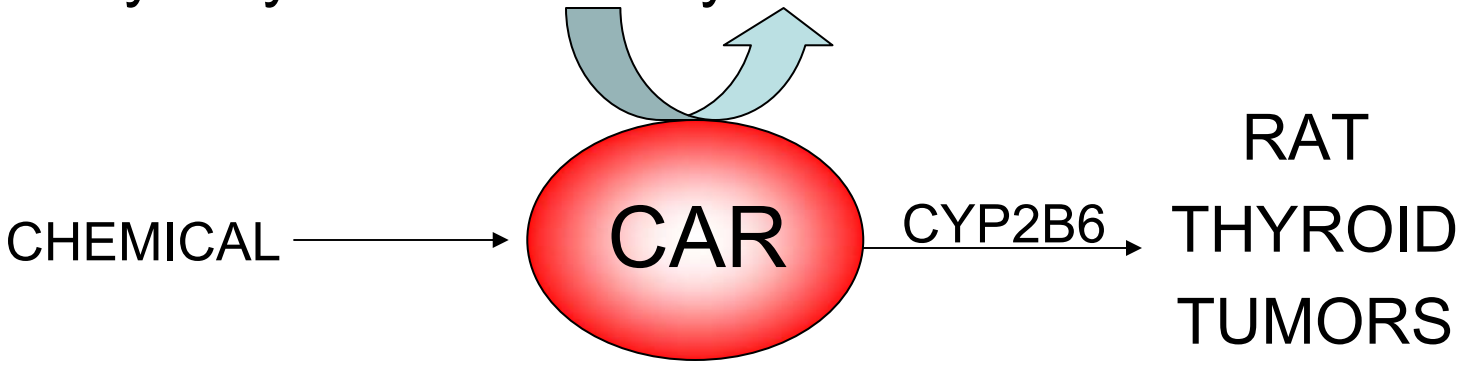
Analysis	Gene	Mouse Liver Apoptosis Necrosis	Rat Liver Apoptosis Necrosis	Rat Liver Hypertrophy	Rat Liver Tumors	Rat Proliferative Thyroid Lesions	Rat Thyroid Tumors
RR	CYP2B6	0.81	2.00	2.26	0.62	2.25	3.83
Sensitivity	CYP2B6	0.45	0.67	0.69	0.38	0.69	0.79
Specificity	CYP2B6	0.49	0.52	0.59	0.49	0.55	0.55

Chemical Class	Chemical	In Vitro CYP2B6 EC50 (µM)	In Vitro CYP2B6 EMAX (F/C)	In Vitro UGT1A1 EC50 (µM)	In Vitro UGT1A1 EMAX (F/C)	Rat Thyroid Tumors LEL (mg/kg/day)	Human CYP2B6 Oral Equivalent (mg/kg)	Human UGT1A1 Oral Equivalent (mg/kg)
amide	Oryzalin	5.64	57.48	x	2.99	112	x	x
amide	Propyzamide	18.92	42.91	x	4.49	49	x	x
amide	Alachlor	8.98	11.33	5.11	3.05	126	x	x
amide	Boscalid	3.85	9.79	4.86	4.21	116	x	x
amide	Acetochlor	0.48	7.02	0.09	2.47	250	0.13	0.02
conazole	Triadimefon	22.19	57.70	34.87	4.02	114	7.61	11.97
conazole	Fenbuconazole	1.03	15.93	8.41	2.80	31	x	x
conazole	Imazalil	0.38	11.75	0.39	2.22	66	x	x
pyridine	Thiazopyr	0.91	55.20	0.20	2.38	44	0.26	0.06
pyridine	Fluazinam	0.16	5.89	0.44	2.04	4	x	x
pyridine	Fluroxypyr	0.02	4.17	0.01	0.97	500	x	x

- 23 True Positives; 7 False Negatives for CYP2B6 induction
- True Positives: 5 Amides, 3 Conazoles, 3 Pyridines

Conclusions:

- Suggestive that activation of the CAR nuclear receptor pathway may result in rat thyroid tumors.



Follow up:

- Would the rat thyroid tumor MOA be relevant to humans?
- Although biologically plausible, are current exposures relevant for human risk?

Acknowledgements

The Hamner

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Gene Target	Primary Receptor	Positive Control	Relavance
CYP2C9	CAR/PXR/Others	Phenobarbital	Involved in the metabolism of many non-steroidal anti-inflammatory drugs (NSAIDs) and sulfonylureas and the anticoagulant warfarin
CYP1A1	AhR	3-MC	Primarily involved in xenobiotic and drug metabolism and in the metabolic activation of aromatic hydrocarbons
CYP1A2	AhR/Others	3-MC	Involved in the metabolism of many drugs, higher basal expression in the liver than CYP1A1
CYP2C19	CAR/PXR/Others	Phenobarbital	P450 for metabolism of several groups of drugs including many proton pump inhibitors (omeprazole) and antiepileptics
CYP3A4	CAR/PXR	Rifamipicin	Involved in the metabolism of xenobiotics and endogenous substrates
CYP2B6	CAR/PXR	Phenobarbital	Principle CAR target gene involved in xenbiotic metabolism
GSTA2	CAR/PXR/Others	Rifamipicin	Phase II conjugating enzyme involved in glutathione conjugation/metabolism
SULT2A1	CAR/PXR/Others	Rifamipicin	Phase II conjugating enzyme involved in the sulfation of endogenous substrates and xenobiotics
UGT1A1	CAR/AhR/PXR/Others	Phenobarbital	Phase II conjugating enzyme, an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, and hormones into water-soluble compounds
ABCB1	PXR/CAR/Others	Phenobarbital	ATP-dependent efflux pump with broad substrate specificity,and involved in the efflux of chemicals and endogenous substrates
HMGCS2	PPARα/Others	Fenofibric Acid	Cholesterol metabolism enzyme and sentinel gene for human PPAR alpha nuclear receptor
ABCB11 (BSEP)	FXR/Others	CDCA	Hepatic transporter involved in the efflux of bile acids from hepatocytes into the bile
ABCG2 (BCRP)	CAR/PXR/PPARα/Others	Phenobarbital	Xenobiotic transporter which may play a major role in multi-drug resistance
SLCO1B1 (OATP-C)	PXR/Others	Rifamipicin	Solute carrier organic anion transporter family, mediates the Na(+)-independent transport of organic anions

Transcription factor	Dimerization partner	Examples of ligands	Genes Regulated
AHR	ARNT	Dioxins, non-*ortho* PCBs, some PAHs, bilirubin, omeprazole, etc.	**CYP1A,** CYP1B GST, UGT, NQO
CAR	RXR	Phenobarbital (PB), TCPOBOP, chlorinated pesticides, *ortho*-PCBs, androstanol/androstenol (inhibits)	**CYP2B**, CYP3A, CYP2C, GST, ABC transporters
PXR	RXR	Rifampicin, PB, *ortho*-PCBs, hyperforin pesticides, dexamethasone, pregnenalone, corticosterone, bile acids (lithocholic acid)	**CYP3A**, CYP2B, CYP2C, CYP7A (repression) GST, ABC transporters
PPAR	RXR	Fibrate drugs, phthalate esters, linoleic acid, arachidonic acid	CYP4A, CYP7A (repression), CYP8B, LX, HMGCS2
LXR	RXR	Cholesterol; (24 S)- hydroxycholesterol	CYP7A, ABC transporters, LXR
FXR	RXR	Bile acids, chenodeoxycholic acid	Represses CYP7A, CYP8B, CYP27A; Induces **BSEP**, BCRP

CAS No.	Chemical	In Vitro CYP2B6 EC50 (µM)	In Vitro CYP2B6 EMAX (F/C)	In Vitro CYP2B6 Hit	In Vitro UGT1A1 Hit	Rat Thyroid Tumors LEL (mg/kg/day)	Chemical Class Tier 1	Chemical Class Tier 2
63-25-2	Carbaryl	18.75	71.01	1	0	350	carbamate	
43121-43-3	Triadimefon	22.19	57.70	1	1	114	conazole	triazole
19044-88-3	Oryzalin	5.64	57.48	1	0	112	amide	sulfonamide
29091-21-2	Prodiamine	16.63	56.88	1	1	151	dinitroaniline	
117718-60-2	Thiazopyr	0.91	55.20	1	1	44	pyridine	
40487-42-1	Pendimethalin	28.26	54.66	1	0	213	dinitroaniline	
82-68-8	Quintozene	5.32	43.37	1	0	150	aromatic	
23950-58-5	Propyzamide	18.92	42.91	1	0	49	amide	
74115-24-5	Clofentezine	4.84	41.63	1	1	17	tetrazine	
10453-86-8	Resmethrin	6.73	34.71	1	0	401	pyrethroid	pyrethroid ester
53112-28-0	Pyrimethanil	6.92	28.32	1	0	221	pyrimidine	
834-12-8	Ametryn	0.54	27.35	1	1	145	triazine	methylthiotriazine
114369-43-6	Fenbuconazole	1.03	15.93	1	1	31	conazole	triazole
148-79-8	Thiabendazole	5.61	13.67	1	0	30	benzimidazole	
87818-31-3	Cinmethylin	5.05	13.54	1	0	150	unclassified	herbicide
35554-44-0	Imazalil	0.38	11.75	1	1	66	conazole	imidazole
15972-60-8	Alachlor	8.98	11.33	1	1	126	amide	anilide
188425-85-6	Boscalid	3.85	9.79	1	1	116	amide	anilide
34256-82-1	Acetochlor	0.48	7.02	1	1	250	amide	anilide
79622-59-6	Fluazinam	0.16	5.89	1	1	4	pyridine	
361377-29-9	Fluoxastrobin	0.66	5.28	1	0	1083	antibiotic	strobilurin
69377-81-7	Fluroxypyr	0.02	4.17	1	0	500	pyridine	
8018-01-7	Mancozeb	0.33	2.62	1	1	31	dithiocarbamate	polymeric dithiocarbamate
113-48-4	MGK	NA	78.30	0	1	450	unclassified	insect repellent
111988-49-9	Thiacloprid	NA	24.29	0	1	3	nicotinoid	pyridylmethylamine
141112-29-0	Isoxaflutole	NA	13.15	0	0	500	cyclopropylisoxazole	
51338-27-3	Diclofop-methyl	NA	7.77	0	0	32	phenoxy	aryloxyphenoxypropionic
121-75-5	Malathion	NA	6.27	0	1	29	organophosphorus	organothiophosphate
133-07-3	Folpet	0.04	1.82	0	1	9	dicarboximide	phthalimide
104206-82-8	Mesotrione	0.00	0.88	0	0	189	benzoylcyclohexanedione	

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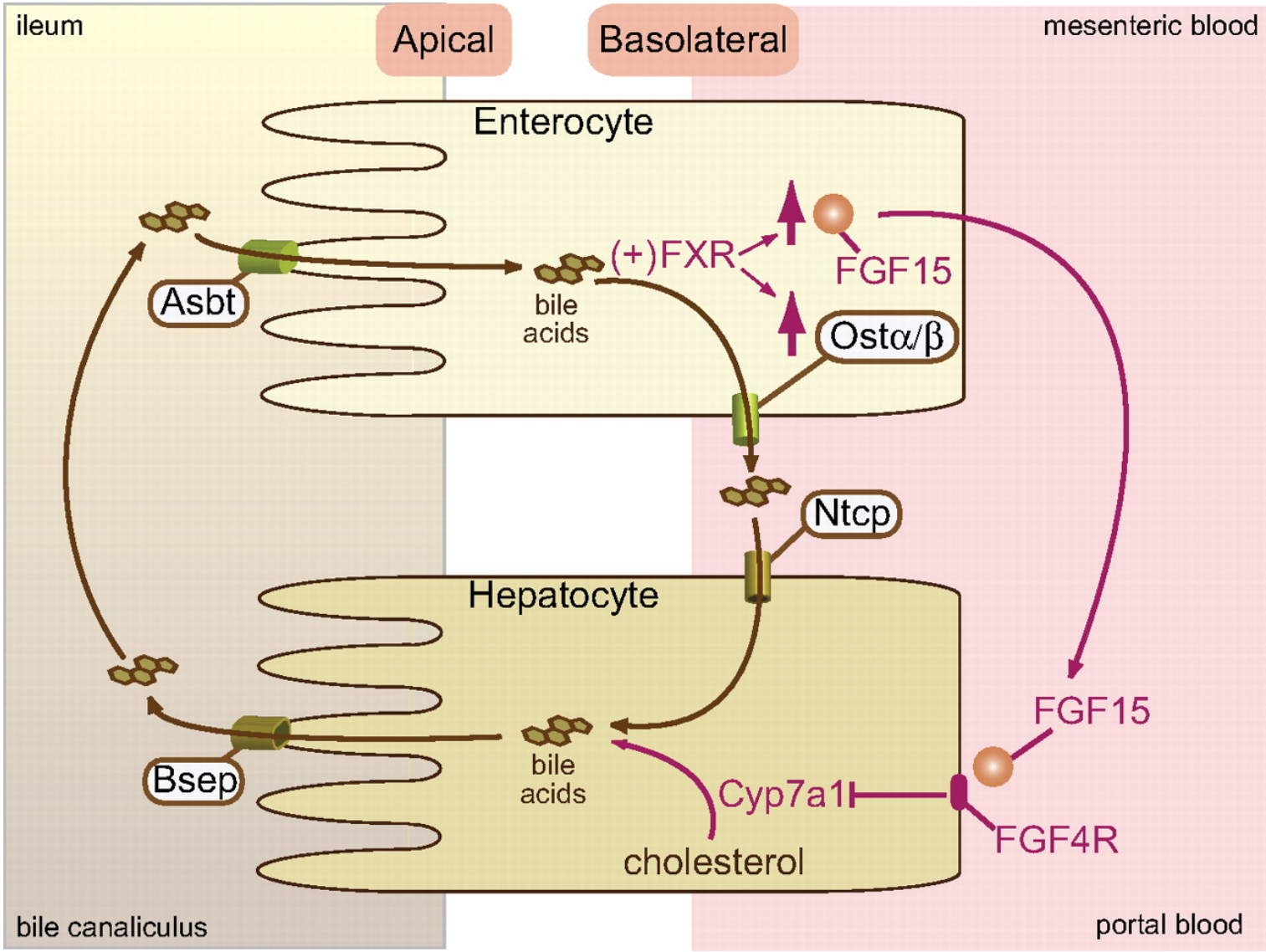
Regulation of bile acid synthesis by an ileal bile acid sensing system



Davis R. A., Attie A. D. PNAS 2008;105:4965-4966