An Integrated *In Vitro* and Computational Approach to Define the Exposure-Dose-Toxicity Relationships in High-Throughput Screens

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Society of Toxicology Platform

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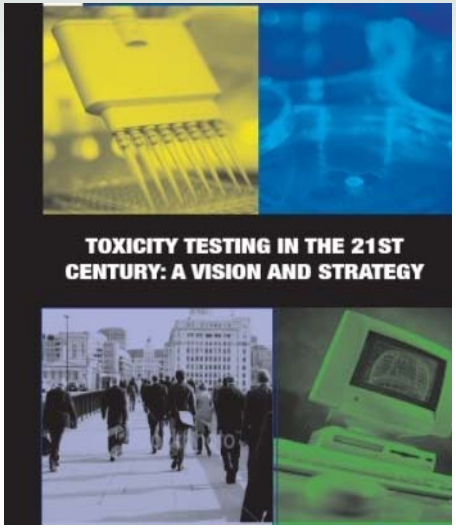
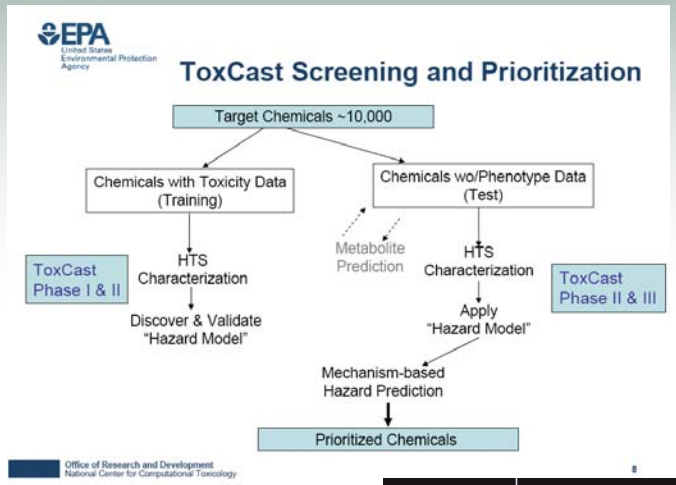
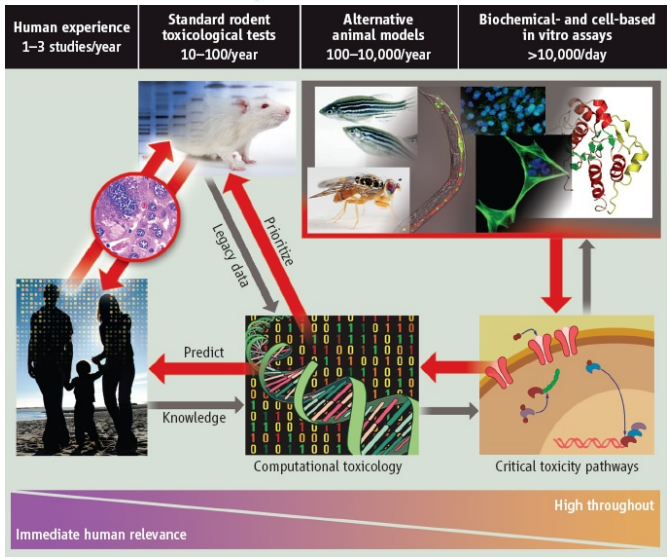
The Hamner Institutes for Health Sciences

Currently a Large Effort in Toxicology In Applying High-Throughput Screening for Toxicity Testing

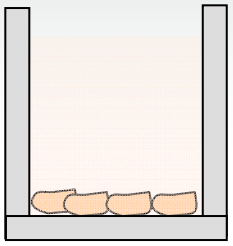


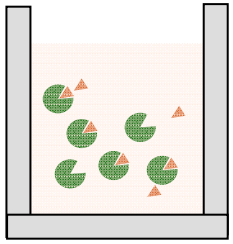




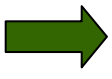
Collins *et al.*, Science 319:906, 2008

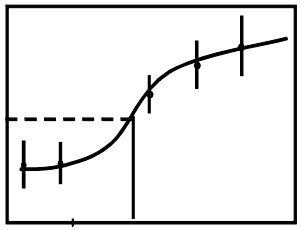
What Kind of Data are Produced from These Screens?



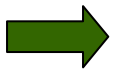


***In Vitro* High Throughput Screens**





EC_{50} or Single Point Activity Data



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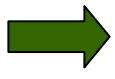
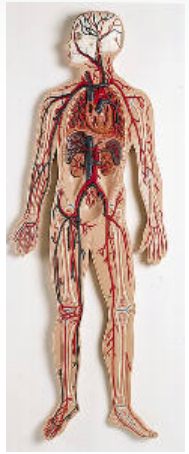
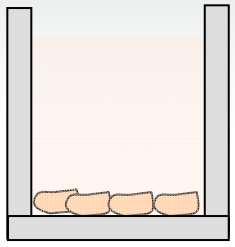
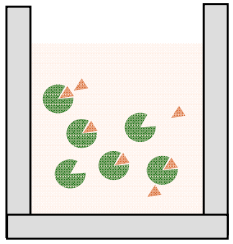




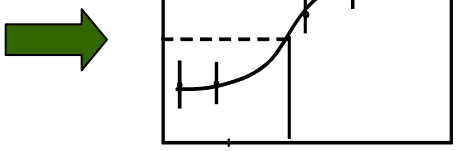
Human Toxicity

What is Missing from the Current High-Throughput Screening Approaches



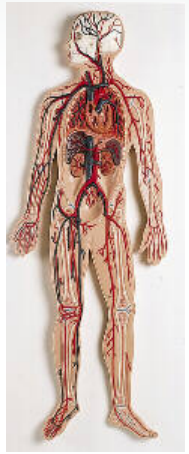


***In Vitro* High Throughput Screens**



EC_{50} or Single Point Activity Data

Dose/Exposure Context

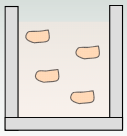


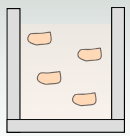
Human Toxicity

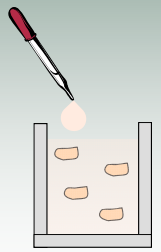
Question

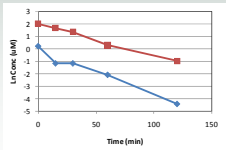
What do the EC/IC_{50} values measured using high-throughput screening mean in terms of human dosimetry and exposure?

Experimental Assays for Characterizing Steady-State Pharmacokinetics









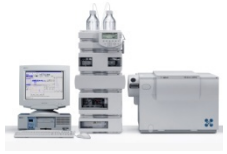
Human Hepatocytes (10 donor pool)

Add Chemical (1 and 10 uM)

Remove Aliquots at 15, 30, 60, 120 min

Analytical Chemistry

Hepatic Clearance



Plasma Protein Binding

Human Plasma (6 donor pool)

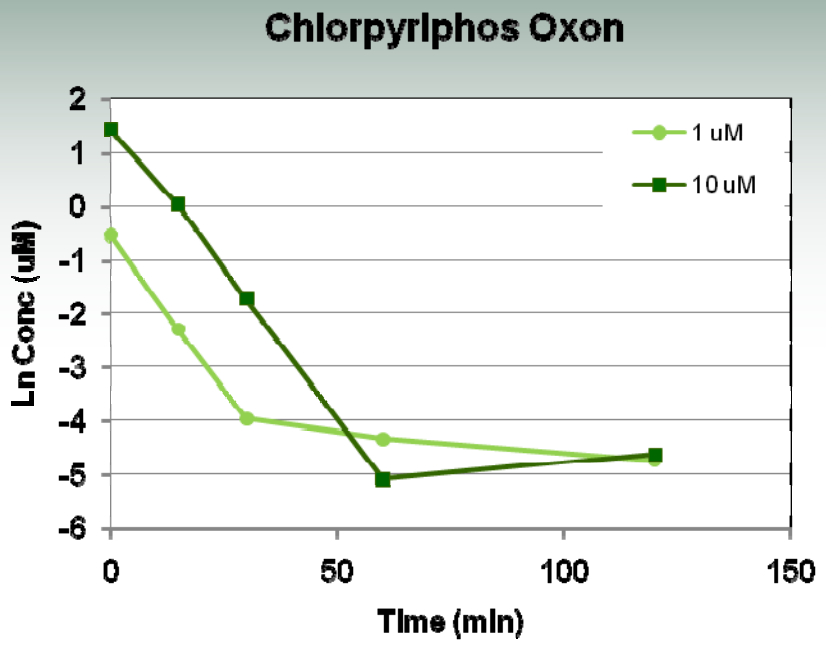
Add Chemical (1 and 10 uM)

Centrifuge with MW Cutoff Filter

Analytical Chemistry

48 Chemicals Showing Measureable EC_{50} in Rat Tissue Slice Assay

Example Chemicals for Hepatic Clearance

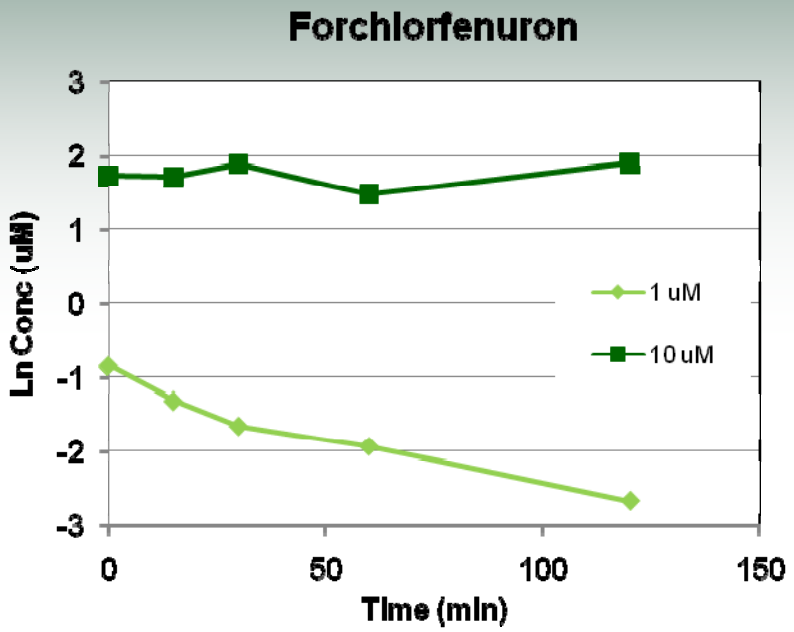


10 uM $T_{1/2}$ = 6.3 min

1 uM $T_{1/2}$ = 6.1 min

10 uM IC = 219 ul/min/10^6 cells

1 uM IC = 229 ul/min/10^6 cells



10 uM $T_{1/2}$ = Not determined

1 uM $T_{1/2}$ = 49.8 min

10 uM IC = Not determined

1 uM IC = 27.8 ul/min/10^6 cells

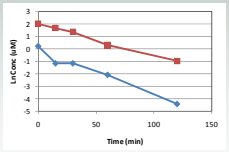
Clearance and Plasma Protein Binding Values

Name	CAS	Hepatic Clearance (ul/min/10^6 cells) 1 uM	Hepatic Clearance (ul/min/10^6 cells) 10 uM	% Plasma Unbound 1 uM	% Plasma Unbound 10 uM	Renal Clearance (L/hr)[a] 1 uM	Renal Clearance (L/hr)[a] 10 uM
2,4-D	94-75-7	27.2	---[b]	4.82	4.00	0.32	0.27
Acetamiprid	135410-20-7	---	---	57.87	57.32	3.88	3.85
Acetochlor	34256-82-1	84.7	47.2	13.50	15.98	0.91	1.07
Atrazine	1912-24-9	9.2	---	10.04	12.37	0.67	0.83
Bentazone	25057-89-0	31.4	---	2.00	2.15	0.13	0.14
Bromacil	314-40-9	6.1	---	11.31	8.52	0.76	0.57
Buprofezin	69327-76-0	18.5	11.6	BD	0.04	---	0.00
Clothianidin	210880-92-5	10.7	10.2	52.85	50.59	3.55	3.39
Cyprodinil	121552-61-2	60.4	---	BD	0.21	---	0.01
Diazoxon	962-58-3	---	---	29.43	32.69	1.97	2.19
Dicrotophos	141-66-2	1.9	---	80.10	84.57	5.37	5.67
Fenamiphos	22224-92-6	68.9	30.3	3.00	4.14	0.20	0.28
Fenoxycarb	72490-01-8	23.1	12.7	0.51	0.33	0.03	0.02
Forchlorfenuron	68157-60-8	26.9	---	4.58	2.75	0.31	0.18
Isoxaben	82558-50-7	13.8	---	3.89	4.71	0.26	0.32
Isoxaflutole	141112-29-0	38.8	26.7	BD	1.66	---	0.11
Metribuzin	21087-64-9	10.4	4.3	59.54	47.87	4.00	3.21
Oxytetracycline dihydrate	6153-64-6	---	---	37.15	39.82	2.49	2.67
Propetamphos	31218-83-4	16.2	3.3	2.20	0.98	0.15	0.07
Thiazopyr	117718-60-2	41.5	41.3	1.07	1.14	0.07	0.08

[a]Renal clearance estimated as GFR*F_U

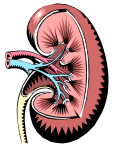
[b]Clearance not determined due to saturation kinetics.

Reverse Dosimetry Modeling for Interpreting *In Vitro* Assay Results



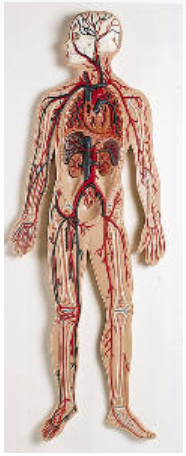
Hepatic Clearance

Plasma Protein Binding



Estimated Renal Clearance

Population-Based In Vitro to In Vivo Extrapolation Software

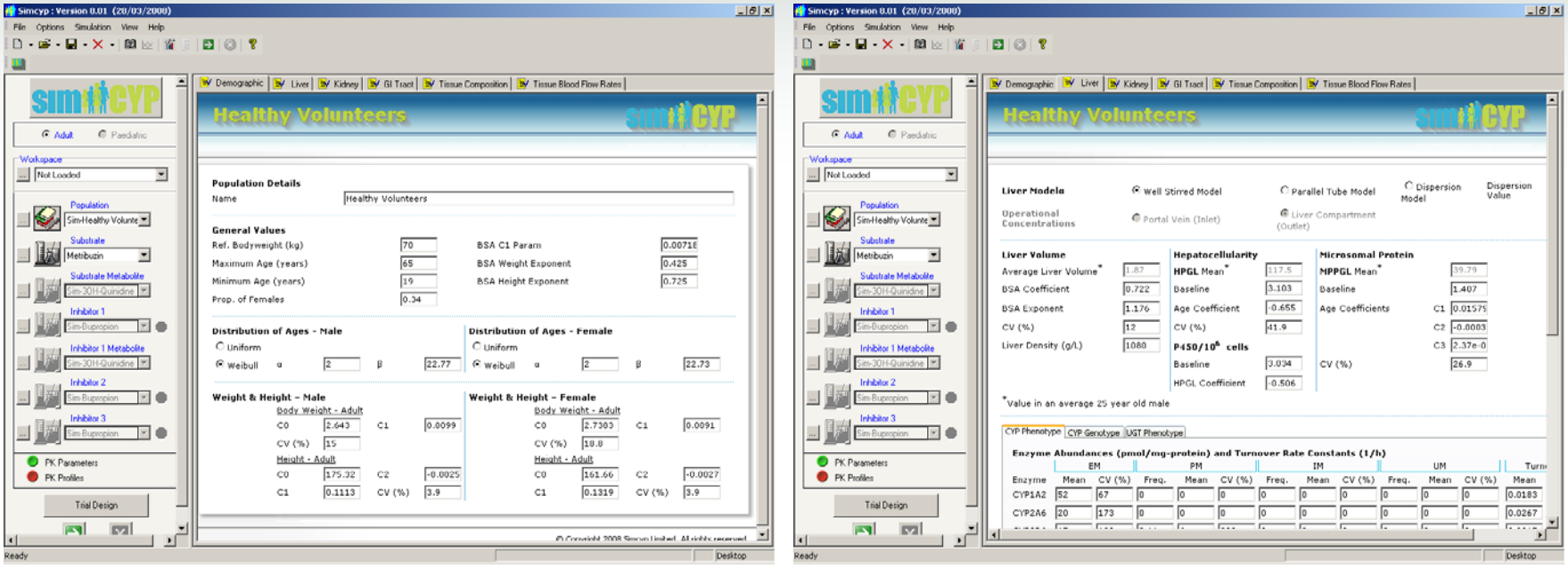


Plasma Concentration at Steady State

Tissue Dose **Exposure**

Reverse Dosimetry

Population-Based In Vitro to In Vivo Extrapolation Software



Population-Based Variability in PK Parameters

At Steady State the Kinetics are Linear

$$[Conc]_{SS} = \frac{DR * BW}{Cl_{Extrinsic}}$$

$Cl_{Extrinsic} = Cl_{Hepatic} + Cl_{Renal}$

$Cl_{Hepatic}$ ← F_U, BF_{Portal}, $M_{Hepatic}$

Cl_{Renal} ← F_U, GFR

***Conservatively assuming 100% GI absorption.**

Estimate Exposure Using Reverse Dosimetry

Statistics						
	CL (L/h)	CLpo (L/h)	Fg(Sub)	Fh(Sub)	Fa(Sub)	Css (mg/L)
Mean	3.15	3.85	1.00	0.96	0.90	0.93
Median	3.04	3.43	1.00	0.96	0.98	0.85
5th centile	1.62	1.83	1.00	0.93	0.55	0.38
95th centile	5.81	7.63	1.00	0.98	1.00	1.60
CV	0.40	0.50	0.00	0.02	0.16	0.44
Min Val	1.19	1.21	1.00	0.91	0.46	0.25
Max Val	7.84	11.78	1.00	0.99	1.00	2.41

$$\frac{1\ \text{mg/kg/day}}{[\text{Conc}]_{SS}} = \frac{\text{Est Oral Exposure at } EC_{50} \text{ Equivalent}}{EC_{50}}$$

Results From Reverse Dosimetry Analysis

Chemical	CAS No.	ToxCast Endpoint	Minimum EC50 or LEL (uM)	Est Oral Equivalent (mg/kg/day)	Lower 95th Confidence Bound	ToxRef LEL (mg/kg/day)	EPA Chronic Dietary RfD-General Population (mg/kg/day)
Acetamiprid	135410-20-7	BSK_BE3C_uPAR	1.481	0.384	0.256	17.5	0.07
Acetochlor	34256-82-1	ATG_NRF2_ARE_CIS	0.587	6.862	3.625	1.1	0.2
Atrazine	1912-24-9	BSK_KF3CT_IP10	1.481	1.215	0.584	9.5	0.018
Bromacil	314-40-9	BSK_BE3C_IP10	1.481	0.888	0.435	179	---
Buprofezin	69327-76-0	ACEA_LOC2	0.141	0.001	0.001	8.7	0.0033
Cyprodinil	121552-61-2	ATG_PPRE_CIS	1.186	0.121	0.062	73.6	0.03
Fenamiphos	22224-92-6	ATG_PXRE_CIS	0.391	1.026	0.465	0.098	0.0001
Fenoxycarb	72490-01-8	ATG_PPRE_CIS	0.391	0.041	0.021	24.7	---
Forchlorfenuron	68157-60-8	BSK_BE3C_uPAR	1.481	1.277	0.588	7	0.07
Isoxaben	82558-50-7	ATG_PXRE_CIS	0.129	0.092	0.050	61.8	0.0500*
Metribuzin	21087-64-9	BSK_hDFCGF_MMP1	1.481	6.577	3.755	13.8	0.013
Isoxaflutole	141112-29-0	BSK_hDFCGF_EGFR	1.481	1.209	0.549	20	
Thiazopyr	117718-60-2	ATG_NRF2_ARE_CIS	0.129	0.083	0.038	44.2	
Dicrotophos	141-66-2	BSK_hDFCGF_PAI1	1.481	2.632	1.529	0.02	
Clothianidin	210880-92-5	BSK_hDFCGF_EGFR	1.481	7.580	4.336	82	
Diazoxon	962-58-3	BSK_KF3CT_IP10	1.481	0.266	0.175		
Bentazone	25057-89-0	ACEA_LOC2	1.230	0.680	0.310	40	
Oxytetracycline dihydrate	6153-64-6	BSK_BE3C_IL1a	1.481	0.567	0.374		
Propetamphos	31218-83-4	NVS_ADME_hCYP2C19	0.098	0.026	0.012	0.63200003	
2,4-D	94-75-7	BSK_BE3C_IL1a	1.481	1.389	0.641	62.5	

Similar EC_{50} Values

Different Oral Equivalents

****ToxCast endpoint data and reverse dosimetry results are preliminary and subject to change**

Cross-Assay Oral Equivalents for Thiazopyr

Company	ToxCast Endpoint	EC50 or LEL (uM)	Est Oral Equivalent (mg/kg/day)	Lower 95th Confidence Bound
Attagene	ATG_PPRE_CIS	33.0	21.0	10.9
Attagene	ATG_PXRE_CIS	0.129	0.083	0.038
Attagene	ATG_NF_kB_CIS	33.0	21.0	10.9
Attagene	ATG_AP_1_CIS	100.0	63.7	33.0
Attagene	ATG_NRF2_ARE_CIS	0.13	0.08	0.04
Attagene	ATG_PPARg_TRANS	10.9	6.9	3.6
Bioseek	BSK_KF3CT_IP10	40.0	25.5	13.2
Bioseek	BSK_hDFCGF_VCAM1	40.0	25.5	13.2
Bioseek	BSK_SAg_CD38	13.3	8.6	4.4
Bioseek	BSK_BE3C_uPA	40.0	25.5	13.2
Bioseek	BSK_BE3C_uPAR	13.3	8.5	4.4
Bioseek	BSK_SAg_CD69	40.0	25.5	13.2
Bioseek	BSK_BE3C_IP10	13.3	8.5	4.4
Bioseek	BSK_hDFCGF_MMP1	13.3	8.5	4.4
Novascreen	NVS_ADME_rCYP2C6	3.33	2.14	0.97
Novascreen	NVS_ADME_hCYP2C19	1.52	0.98	0.44
	ACEA_LOC2	33.1	21.1	10.9

*Not active in 10/27 assays.
**ToxRef LEL is 44.2 mg/kg/day (Rat liver, kidney, and thyroid).

****ToxCast endpoint data and reverse dosimetry results are preliminary and subject to change**

Conclusions

- *In vitro* assays for hepatocyte clearance and plasma protein binding have been developed to provide critical pharmacokinetic information on a subset of ToxCast chemicals.

- Integration of *in vitro* pharmacokinetic assays with computational modeling allows estimation of oral exposures required to produce steady state *in vivo* concentrations equivalent to EC_{50} values in HTS assays.

- Comparisons of equivalent oral exposures to RfD values allows the estimation of margins-of-exposure and provides additional context for prioritization.

Acknowledgements

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