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

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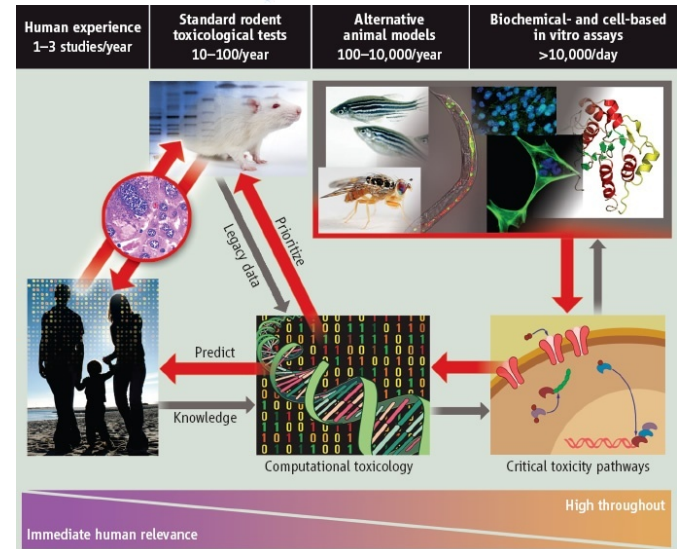
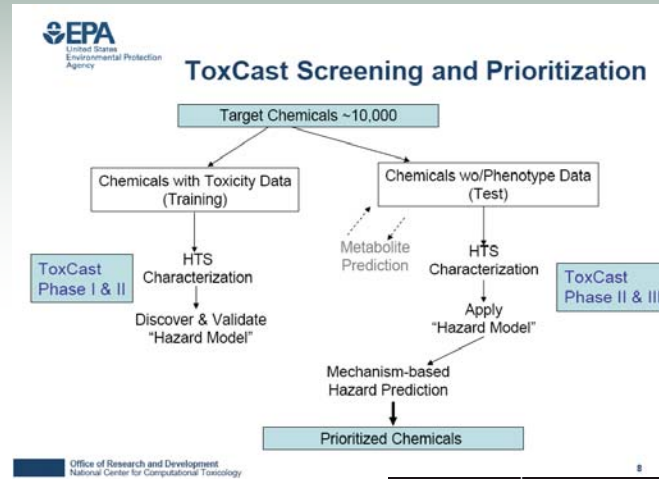
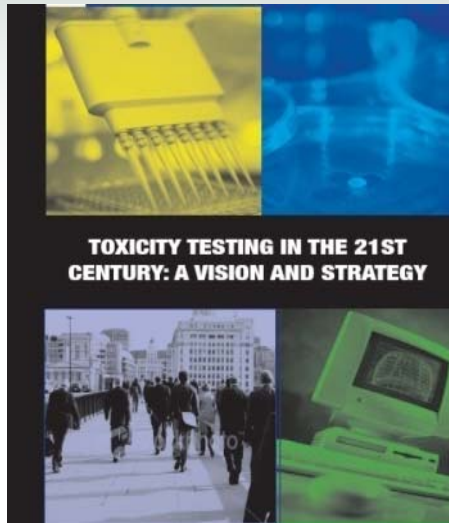
CellzDirect, Inc.

Daniel Rotroff and David Dix*

U.S. Environmental Protection Agency

*Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

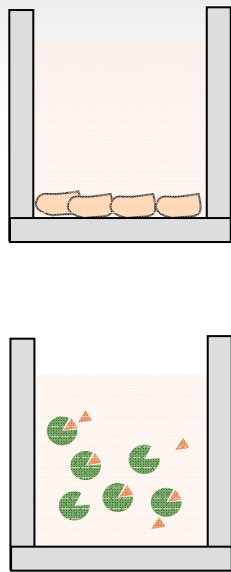
Currently a Large Effort in Toxicology In Applying High-Throughput Screening for Toxicity Testing



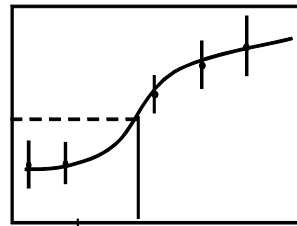
Collins et al., Science 319:906, 2008



What Kind of Data are Produced from These Screens?



***In Vitro* High Throughput Screens**

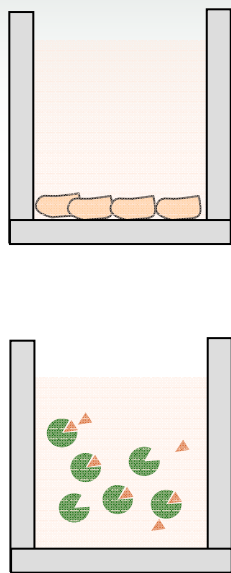


EC₅₀ or Single Point Activity Data

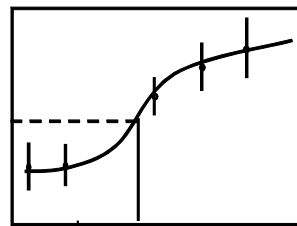


Human Toxicity

What is Missing from the Current High-Throughput Screening Approaches



***In Vitro* High Throughput Screens**



EC₅₀ or Single Point Activity Data



Dose/Exposure Context

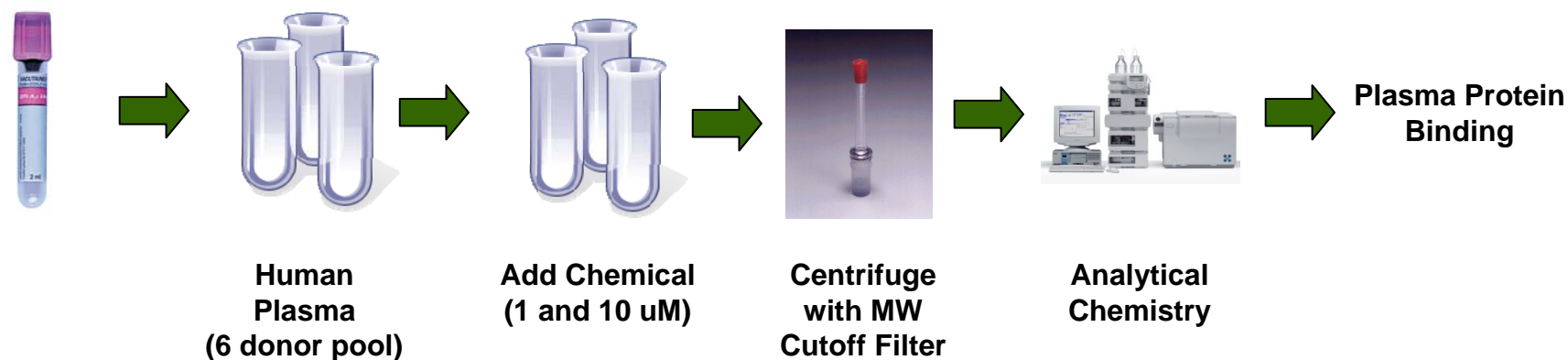
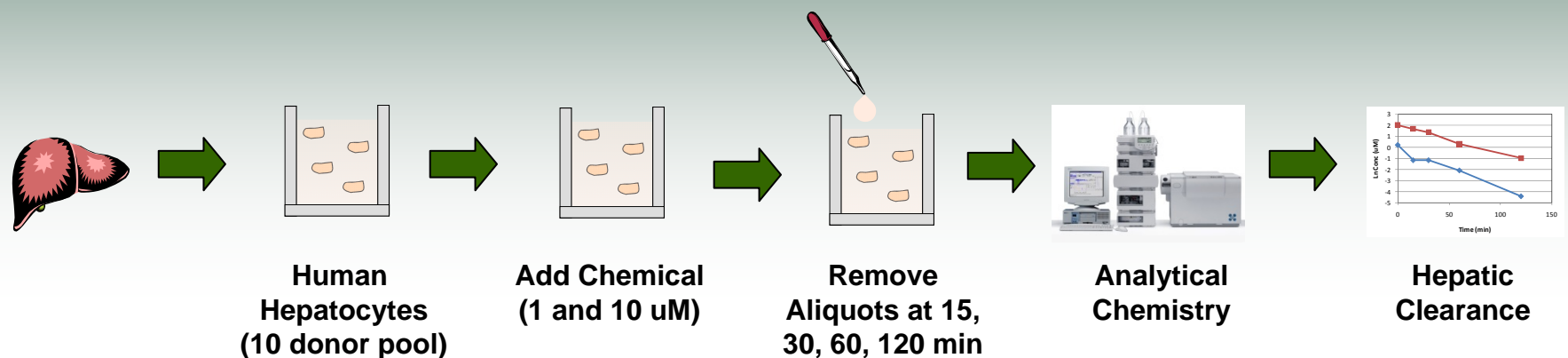


Human Toxicity

Question

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

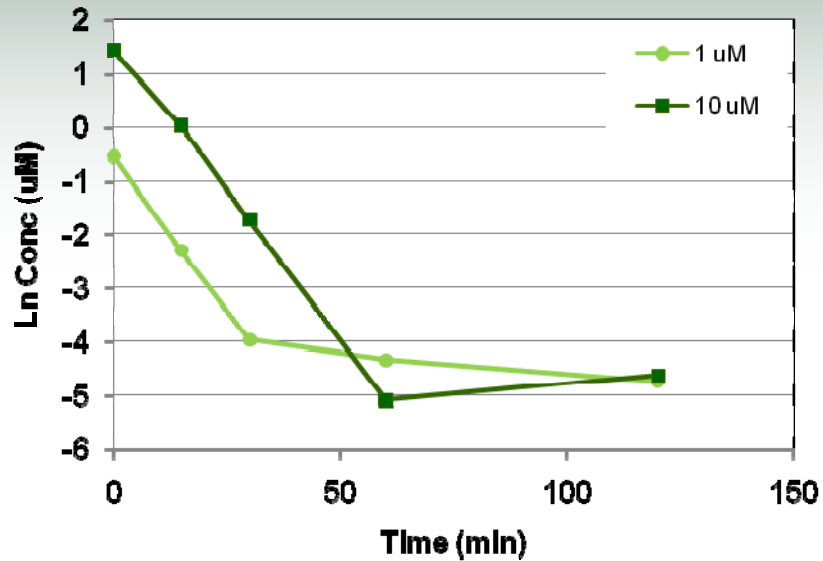
Experimental Assays for Characterizing Steady-State Pharmacokinetics



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

Example Chemicals for Hepatic Clearance

Chlorpyrifos Oxon



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

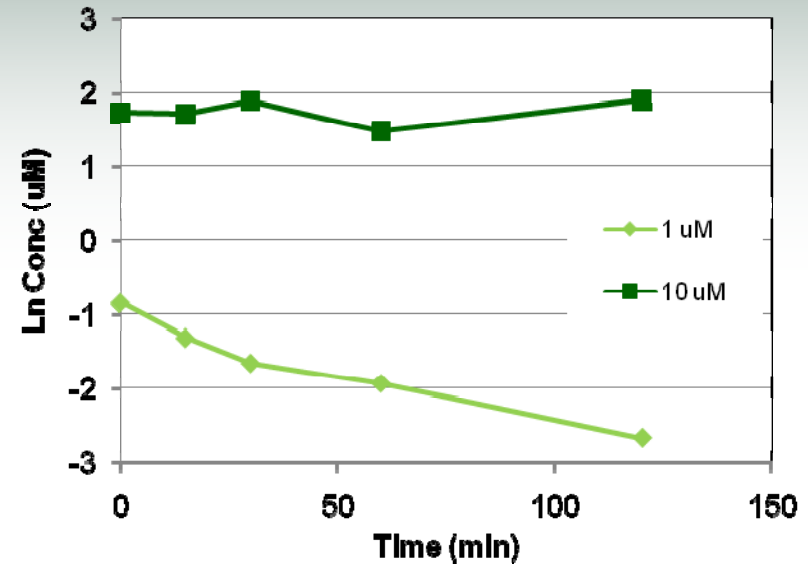
1 uM $T_{1/2}$ = 6.1 min



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

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

Forchlorfenuron



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)		% Plasma Unbound		Renal Clearance (L/hr) ^a	
		1 uM	10 uM	1 uM	10 uM	1 uM	10 uM
2,4-D	94-75-7	27.2	--- ^b	4.82	4.00	0.32	0.27
Acetamidrid	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
Bisphenol A	80-05-7	19.3	25.0	25.71	6.82	1.73	0.46
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
Nifedipine	21829-25-4	38.1	41.8	2.43	3.90		
Oxytetracycline dihydrate	6153-64-6	---	---	37.15	39.82	2.49	2.67
Parathion	56-38-2	6.65	---	---	---	---	---
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

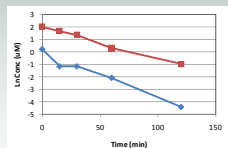
^aRenal clearance estimated as $GFR \cdot F_u$

^bClearance not determined due to saturation kinetics.

Current Validation Results

Name	CAS	PBPK or PK Model Css (mg/L)	In Vitro Predicted Css (mg/L)
Nifedipine	21829-25-4	0.06	0.22
Bisphenol A	80-05-7	<0.03	0.09
Parathion	56-38-2	0.049	0.043

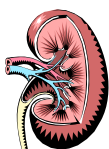
Reverse Dosimetry Modeling for Interpreting *In Vitro* Assay Results



Hepatic Clearance



Plasma Protein Binding



Estimated Renal Clearance

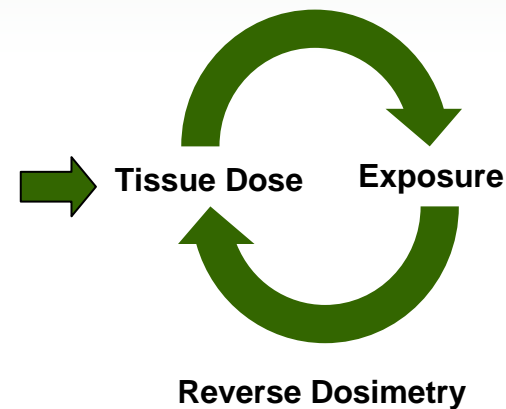


simCYP
real solutions from virtual populations

Population-Based
In Vitro to In Vivo
Extrapolation
Software



Plasma
Concentration at
Steady State



Population-Based In Vitro to In Vivo Extrapolation Software

The image displays two screenshots of the SimCYP software interface, version 0.01 (20/03/2000). Both screenshots are for a simulation titled 'Healthy Volunteers'.

Left Screenshot: Population Details

General Values

Ref. Bodyweight (kg)	70	BSA C1 Param	0.0071E
Maximum Age (years)	65	BSA Weight Exponent	0.425
Minimum Age (years)	19	BSA Height Exponent	0.725
Prop. of Females	0.34		

Distribution of Ages - Male

Uniform Weibull

α	2	β	22.77
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Weight & Height - Male

Body Weight - Adult			
C0	2.643	C1	0.0099
CV (%)	15		
Height - Adult			
C0	175.32	C2	-0.0025
C1	0.1113	CV (%)	3.9

Right Screenshot: Liver Models

Liver Models

Well Stirred Model Parallel Tube Model Dispersion Model

Operational Concentrations

Portal Vein (Inlet) Liver Compartment (Outlet)

Liver Volume

Average Liver Volume*	1.87
BSA Coefficient	0.722
BSA Exponent	1.176
CV (%)	12
Liver Density (g/L)	1080

Hepatocellularity

HPGL Mean*	117.5
Baseline	3.103
Age Coefficient	-0.655
CV (%)	41.9
HPGL Coefficient	-0.506

Microsomal Protein

MPPGL Mean*	39.79
Baseline	1.407
Age Coefficients	C1 -0.01575
	C2 -0.0003
	C3 2.37e-0
CV (%)	26.9

Enzyme Abundances (pmol/mg-protein) and Turnover Rate Constants (1/h)

Enzyme	EM			PM			IM			UM			Mean
	Mean	CV (%)	Freq.	Mean	CV (%)	Freq.	Mean	CV (%)	Freq.	Mean	CV (%)	Freq.	
CYP1A2	52	67	0	0	0	0	0	0	0	0	0	0	0.0183
CYP2A6	20	173	0	0	0	0	0	0	0	0	0	0	0.0267

Population-Based Variability in PK Parameters

At Steady State the Kinetics are Linear

$$[\text{Conc}]_{\text{ss}} = \frac{\text{DR} * \text{BW}}{\text{Cl}_{\text{Extrinsic}}}$$

$\text{Cl}_{\text{Extrinsic}}$

$\text{Cl}_{\text{Hepatic}} \qquad \text{Cl}_{\text{Renal}}$

$\uparrow \qquad \qquad \qquad \uparrow$

$F_U, \text{BF}_{\text{Portal}}, M_{\text{Hepatic}} \qquad F_U, \text{GFR}$

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

Estimate Exposure Using Reverse Dosimetry

Statistics						
	CL (L/h)	CL _{po} (L/h)	F _g (Sub)	F _h (Sub)	F _a (Sub)	C _{ss} (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



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

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 Rodent LEL (mg/kg/day)	Ratio Oral Equiv:LEL
Acetamiprid	135410-20-7	BSK_BE3C_uPAR	1.481	0.384	0.256	17.5	0.022
Acetochlor	34256-82-1	ATG_NRF2_ARE_CIS	0.587	6.862	3.625	1.1	6.24
Atrazine	1912-24-9	BSK_KF3CT_IP10	1.481	1.215	0.584	9.5	0.13
Bromacil	314-40-9	BSK_BE3C_IP10	1.481	0.888	0.435	179	0.005
Buprofezin	69327-76-0	ACEA_LOC2	0.141	0.001	0.001	8.7	0.0001
Cyprodinil	121552-61-2	ATG_PPRE_CIS	1.186	0.121	0.062	73.6	0.0016
Fenamiphos	22224-92-6	ATG_PXRE_CIS	0.391	1.026	0.465	0.098	10.47
Fenoxycarb	72490-01-8	ATG_PPRE_CIS	0.391	0.041	0.021	24.7	0.0016
Forchlorfenuron	68157-60-8	BSK_BE3C_uPAR	1.481	1.277	0.588	7	0.18
Isoxaben	82558-50-7	ATG_PXRE_CIS	0.129	0.092	0.050	61.8	0.0015
Metribuzin	21087-64-9	BSK_hDFCGF_MMP1	1.481	6.577	3.755	13.8	0.48
Isoxaflutole	141112-29-0	BSK_hDFCGF_EGFR	1.481	1.209	0.549	20	0.06
Thiazopyr	117718-60-2	ATG_NRF2_ARE_CIS	0.129	0.083	0.038	44.2	0.002
Dicrotophos	141-66-2	BSK_hDFCGF_PA11	1.481	2.632	1.529	0.02	131.6
Clothianidin	210880-92-5	BSK_hDFCGF_EGFR	1.481	7.580	4.336	82	0.092
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	0.017
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	0.63	0.04
2,4-D	94-75-7	BSK_BE3C_IL1a	1.481	1.389	0.641	62.5	0.02

Similar LEL Values

Different Oral Equivalents

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

Reasons for Discrepancy Between Human Oral Equivalents and Rodent LELs

- Relevance of the *in vitro* assay to the *in vivo* endpoint
 - Relationship of molecular endpoints to integrated tissue response
 - Species differences between *in vitro* assays and *in vivo* studies
- Cross-species pharmacokinetic differences
- Assumptions in *in vitro* to *in vivo* extrapolation and pharmacokinetic modeling
 - 100% gastrointestinal absorption
 - Renal excretion
 - Others

Relevance of the *In Vitro* Assay to the *In Vivo* Endpoint

Chemical	ToxCast Endpoint	Relevant EC50 or LEL (uM)	Est Oral Equivalent (mg/kg/day)	Lower 95th Confidence Bound	Relevant LOAEL or LEL (mg/kg/day)	Ratio Oral Eqiv:LEL	
Acetochlor	Phospho H2AX	25.4	296	157	250	1.18	Rat liver prolifer
	p53	17.5	199	105		0.80	
Atrazine	ATG_ERa_TRANS	100	8.94	5.98	3.65	2.45	Estrous cycle
	ATG_ERE_CIS	100					

****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 EC₅₀ 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.
- Challenges remain for interpreting the relevance of the *in vitro* assay results to *in vivo* endpoints, cross-species differences in assay design and pharmacokinetics, and relevance to human health and exposure.

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