Assessing the Exposure-Dose-Toxicity Relationship within the EPA's ToxCast Program

November 4, 2008

EPA Community of Practice

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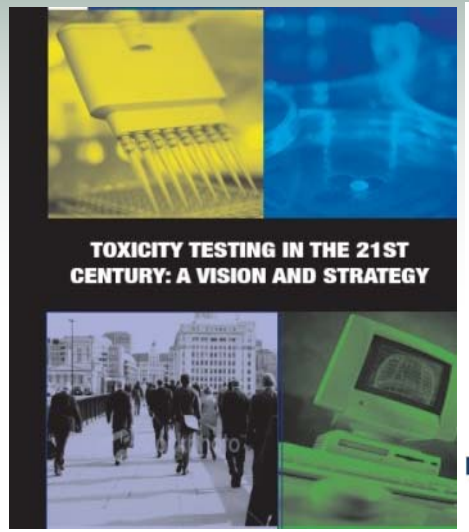
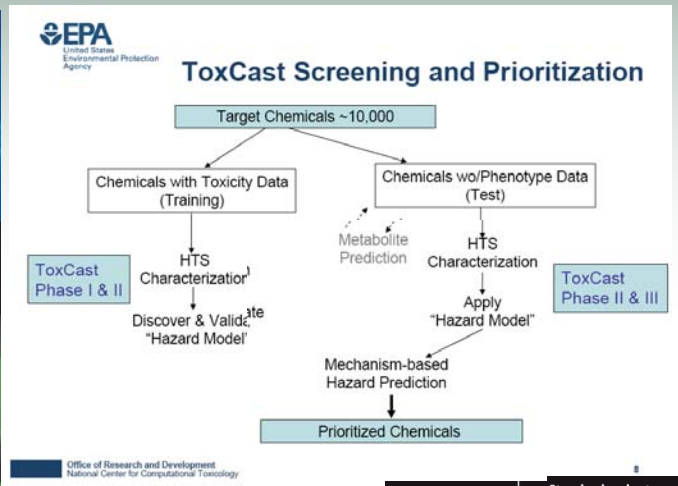
The Hamner Institutes for Health Sciences

There is a Broad-Based Movement in Toxicology Towards In Vitro Testing and Prediction of Hazard



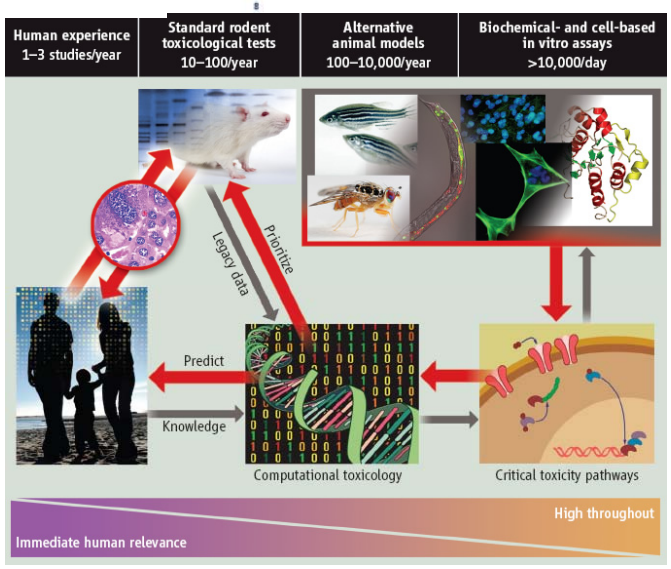


Roadmap Target #1: HTS Activity Matrix

SEQUENCE	TIMELINE		
	Short-term	Mid-term	Long-term
	('04) Catalogue assays	('06) Analyze metabolites from non-conforming	('09) Develop universal metabolizing system
	('05) Workshop to select tests	('07) Validation of assays to predict mechanistic endpoint	('10) Availability of agents & tissues for HTS to extramural scientists
	('05) Understand metabolism	('07) Develop systems biology method for analysis	('10) Validation of battery of tests for prediction
	('05) Test ±600 NTP carc., developmental & reprod. toxicity agents	('07) Develop database as part of CEBS	
		('07) Review after 36 months and periodically	('10) Review after 5 years and periodically

NIEHS The National Institute of Environmental Health Sciences The National Institutes of Health The Department of Health and Human Services

NTP The National Toxicology Program The Department of Health and Human Services



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

Current Focus of EPA and NTP Efforts is on Collecting HTS Screening Data

Table 1. Assays and Endpoints contained within Phase I signature development

Assay Type	Number of Assays	Number of Unique Endpoints	Assay Source	Comment	Source
Biochemical	+200	+200	Mostly human and rat	Enzyme inhibition, Ion channels, GPCRs, Cytochromes	NovaScreen Biosciences
Transcription Factor Profiling	2	+60	HepG2 cells (human liver)	Nuclear receptors and other transcription factors	Attagene
Nuclear receptor activation	+20	+20	Human and rodent	Reporter gene assay over 15 concentrations	NIH Chemical Genomics Center
Transcriptomics	1	+20,000	Primary hepatocytes-Kupffer cell co-cultures	Illumina microrrays	In Vitro ADMET Laboratories and Expression Analysis
Kinetic Cell Growth	1	Kinetic	A549 cells (human lung)	Real time recording of electrical impedance	ACEA Biosciences
Cytotoxicity and Bioactivation	1	6	Primary human liver, lung and kidney cells	Shared metabolism across cell types	In Vitro ADMET Laboratories
Complex cell culture	8	87	Primary human cells	Many cell signaling pathways	Bioseek
High content screening	1	11	HepG2 cells (human liver)	Fluorescence imaging of cells	Cellumen
Fish development	1	11	Zebrafish (Dana rerio)	Teratogenesis	Phylonix
TOTAL	>235	>20,395			

ToxCast Assays

Roadmap Target #1: High-Throughput Screening (HTS)

Target Date: Begin exploratory testing mid 2005

Activities:

ASSAYS:
- Catalogue assays in public domain
- Choice of Assays - pathways related to carcinogenicity, reproductive and developmental toxicity
- Metabolism - agents that have been tested
- Analyze metabolites
- Test each metabolite, if possible

AGENTS:
- 500+ that have been tested in bioassay
- HPV chemicals

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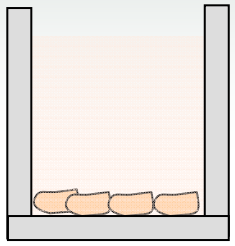
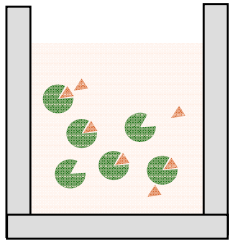
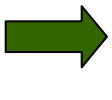
NTP Assays

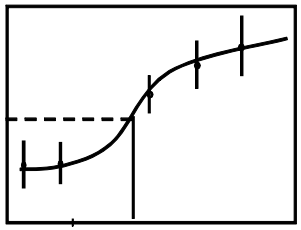
What is Missing from the Current High-Throughput Screening Approaches



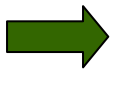


***In Vitro* High Throughput Screens**





EC_{50} or Single Point Activity Data



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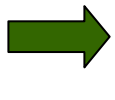
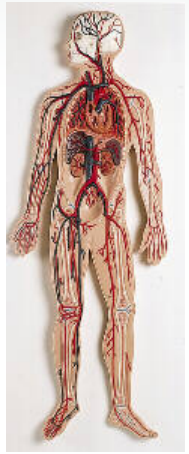
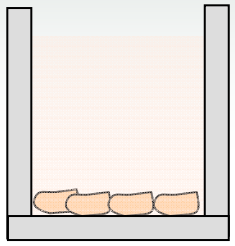
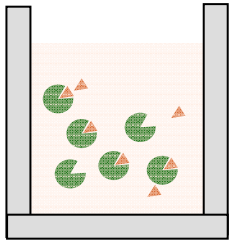




Human Toxicity

What is Missing from the Current High-Throughput Screening Approaches





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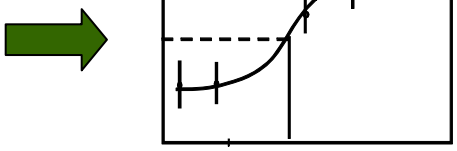
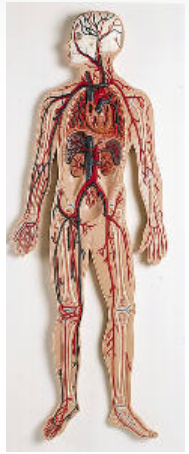


EC_{50} or Single Point Activity Data

Dose/Exposure Context



Human Toxicity

Outline

1. **Tissue Slice Studies**
2. **In Vitro Pharmacokinetic Assays**
3. **In Vitro to In Vivo Extrapolation and Reverse Dosimetry**
4. **ToxCast Visualization and Analysis Software**

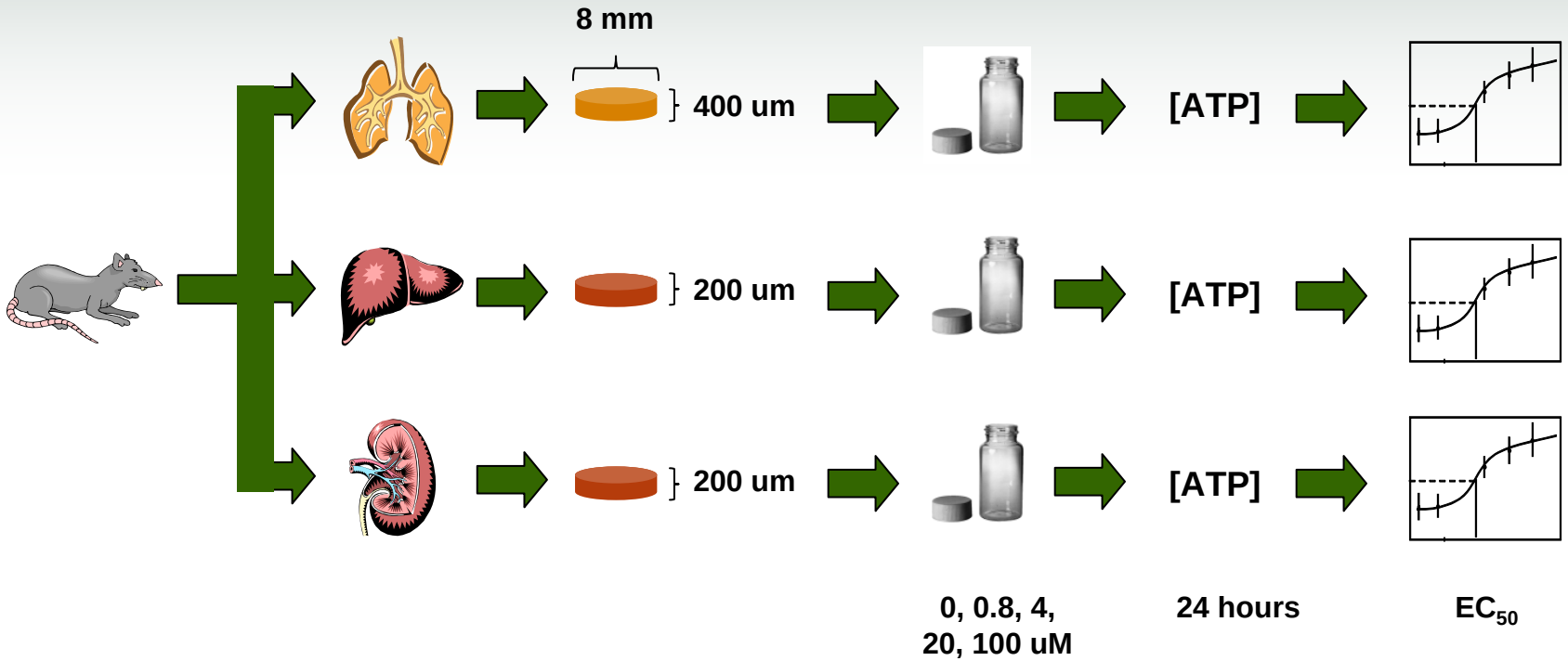
Outline

1. **Tissue Slice Studies**
2. **In Vitro Pharmacokinetic Assays**
3. **In Vitro to In Vivo Extrapolation and Reverse Dosimetry**
4. **ToxCast Visualization and Analysis Software**

Question

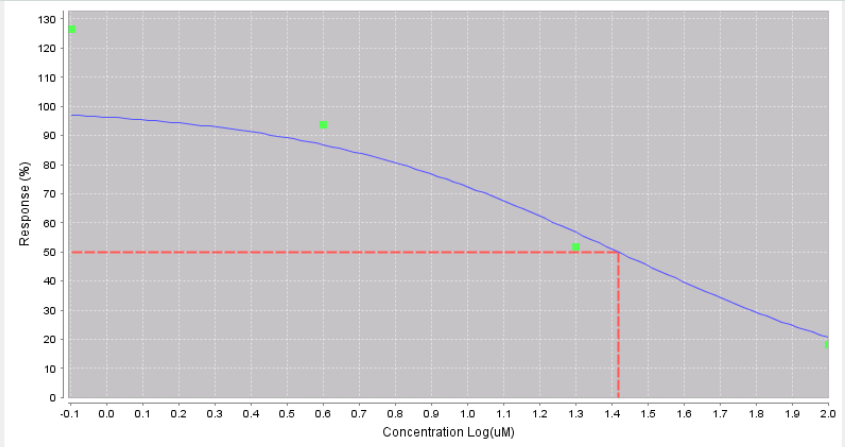
Can organ slice cultures be used to predict target organ toxicity in a whole animal?

Experimental Design



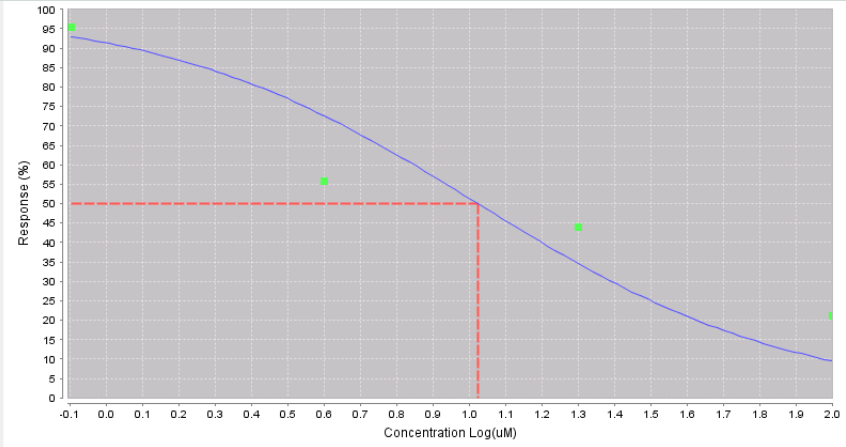
140 of the Phase I ToxCast Chemicals

Example Cytotoxicity Data



117718-60-2	Value
assay_id	549
log_ac50 (uM)	1.419
ac50 (Log uM)	26.2199993133545
stdErr_log_ic50	0.2412
95confidence_log_ic50	0.6511 to 2.186
95confidence_ic50	4.478 to 153.5
degree_of_freedom	3
r2	0.8605
abs_sum_of_square	938
SYx	17.68
number_of_points	4

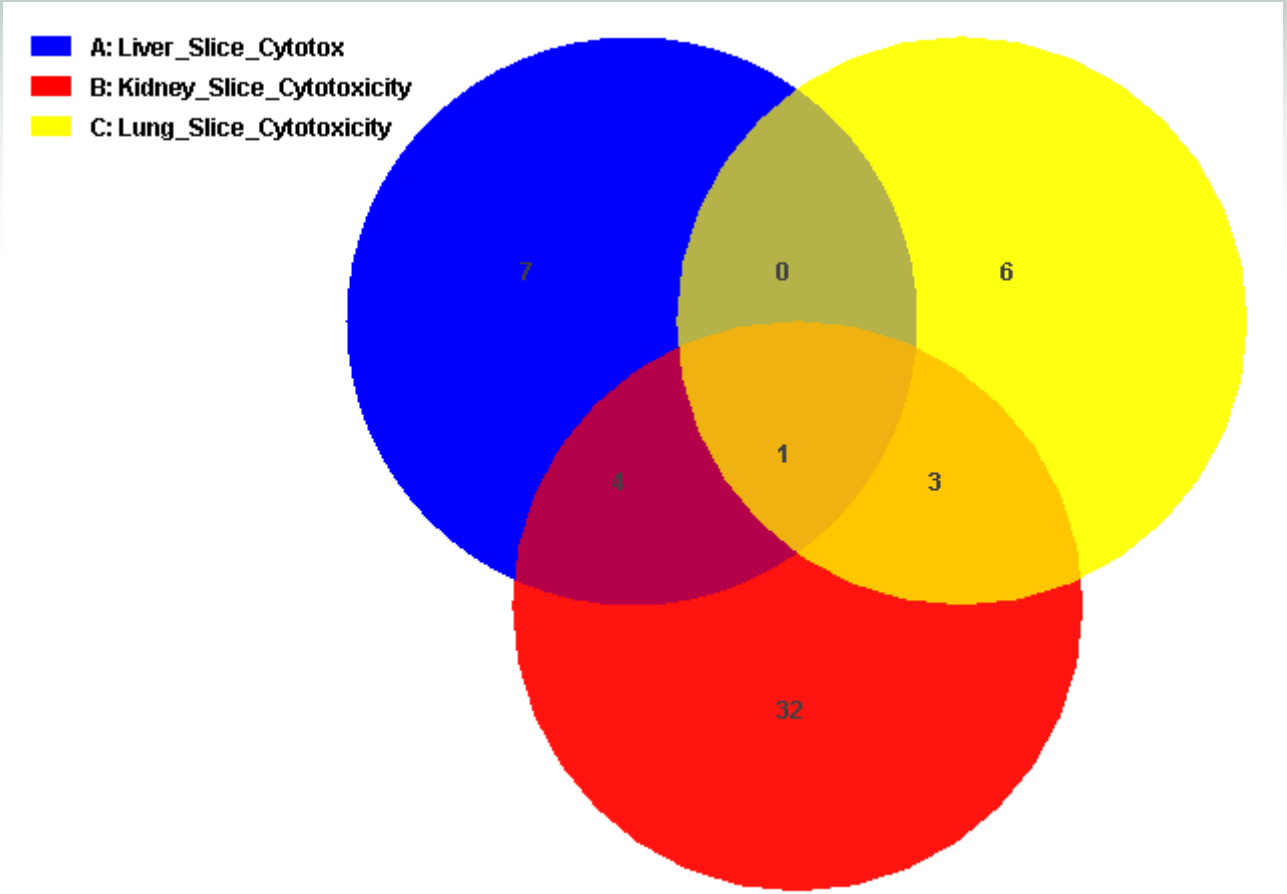
Kidney Toxicity of Thiazopyr



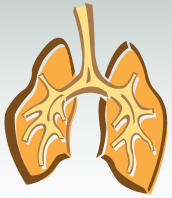
119791-41-2	Value
assay_id	550
log_ac50 (uM)	1.024
ac50 (Log uM)	10.5699996948242
stdErr_log_ic50	0.1767
95confidence_log_ic50	0.4618 to 1.587
95confidence_ic50	2.896 to 38.60
degree_of_freedom	3
r2	0.8228
abs_sum_of_square	509.8
SYx	13.04
number_of_points	4

Liver Toxicity of Emamectin

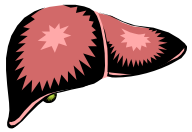
Preliminary Results Summary



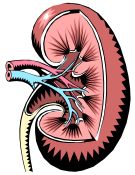
Preliminary Results Summary



- **Only 53 chemicals show measureable EC_{50}s in at least 1 tissue**



- **Kidney most sensitive organ (40 chemicals); lung next most sensitive (12 chemicals); and liver least sensitive (8 chemicals)**



- **8 chemicals showed toxicity in 2 organs and 1 chemical showed toxicity in all 3 organs**

- **Median EC_{50} was 31 uM (Range: 1.3 – 99 uM)**

Predicting Target Organ Toxicity

Relative Risk	Rat Liver Tumors	Rat Proliferative Liver Lesions	Rat Liver Apoptosis Necrosis	Rat Liver Hypertrophy	Rat Kidney Nephropathy	Rat Proliferative Kidney Lesions	Rat Proliferative Thyroid Lesions	Rat Thyroid Tumors	Rat Thyroid Hyperplasia	Rat Testicular Tumors	Rat Testicular Atrophy	Rat Spleen Pathology	Rat Cholinesterase Inhibition	Rat Tumorigen	Mouse Liver Tumors	Mouse Proliferative Liver Lesions	Mouse Liver Apoptosis Necrosis	Mouse Liver Hypertrophy	Mouse Kidney Pathology	Mouse Lung Tumors	Mouse Tumorigen
Rat Liver Slice Cytotoxicity	0.00	0.27	0.68	0.23	1.71	0.54	0.00	0.00	0.00	0.00	0.00	1.00	3.04	0.96	1.26	0.73	0.60	0.35	0.00	0.00	0.76
Rat Kidney Slice Cytotoxicity	0.59	1.35	2.88	0.85	1.43	1.09	1.82	1.52	1.62	1.45	1.48	0.76	0.28	1.34	1.04	1.37	1.98	1.84	1.72	0.32	0.99
Rat Lung Slice Cytotoxicity	0.00	1.07	1.06	1.74	0.97	4.40	0.00	0.00	0.00	4.13	0.00	0.65	0.00	0.22	0.00	0.00	0.86	1.33	0.56	2.51	0.24
Sensitivity																					
Rat Liver Slice Cytotoxicity	0.00	0.10	0.10	0.10	0.40	0.10	0.00	0.00	0.00	0.00	0.00	0.20	0.40	0.40	0.38	0.38	0.13	0.13	0.00	0.00	0.38
Rat Kidney Slice Cytotoxicity	0.06	0.31	0.23	0.29	0.34	0.17	0.26	0.17	0.14	0.06	0.11	0.17	0.09	0.46	0.33	0.50	0.27	0.37	0.33	0.03	0.43
Rat Lung Slice Cytotoxicity	0.00	0.29	0.14	0.43	0.29	0.43	0.00	0.00	0.00	0.14	0.00	0.14	0.00	0.14	0.00	0.00	0.17	0.33	0.17	0.17	0.17
Specificity																					
Rat Liver Slice Cytotoxicity	0.91	0.71	0.86	0.67	0.72	0.83	0.79	0.85	0.88	0.95	0.90	0.80	0.82	0.59	0.68	0.55	0.81	0.71	0.72	0.91	0.56
Rat Kidney Slice Cytotoxicity	0.91	0.75	0.91	0.68	0.73	0.84	0.84	0.88	0.91	0.96	0.92	0.79	0.75	0.61	0.68	0.58	0.85	0.76	0.77	0.90	0.56
Rat Lung Slice Cytotoxicity	0.91	0.73	0.86	0.70	0.71	0.85	0.80	0.85	0.88	0.96	0.90	0.80	0.79	0.57	0.65	0.53	0.81	0.73	0.74	0.93	0.55

Outline

1. Tissue Slice Studies

2. **In Vitro Pharmacokinetic Assays**

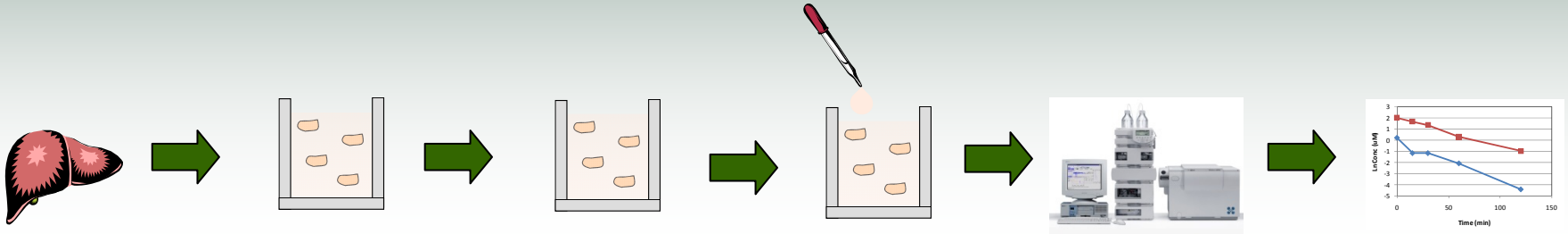
3. In Vitro to In Vivo Extrapolation and Reverse Dosimetry

4. ToxCast Visualization and Analysis Software

Question

What in vitro assays are necessary to predict in vivo pharmacokinetic behavior?

Experimental Design



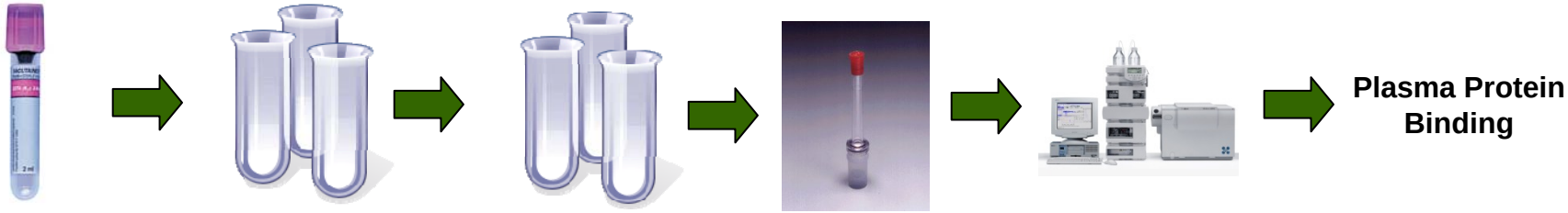
Human Hepatocytes (10 donor pool)

Add Chemical (1 and 10 uM)

Remove Aliquots at 15, 30, 60, 120 min

Analytical Chemistry

Hepatic Clearance



Human Plasma (6 donor pool)

Add Chemical (1 and 10 uM)

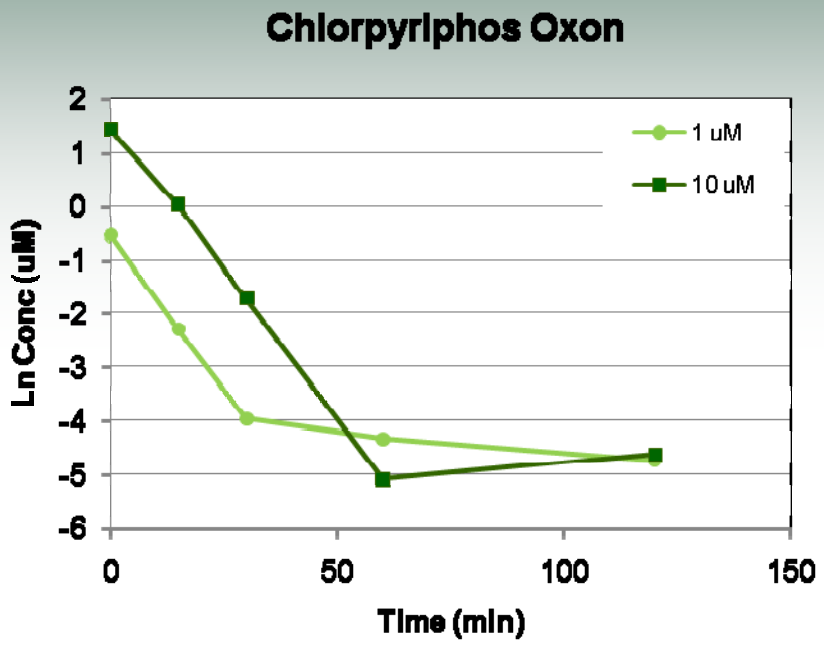
Centrifuge with MW Cutoff Filter

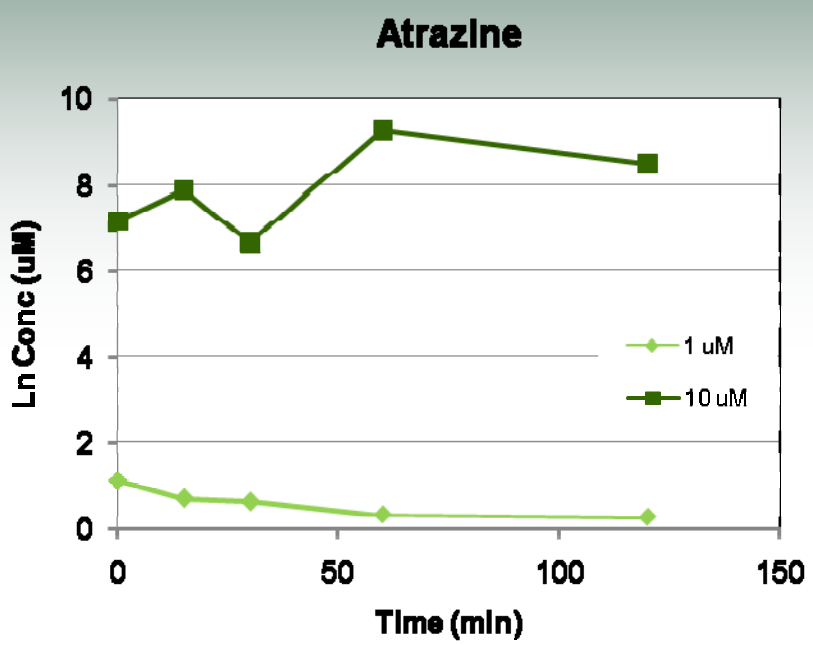
Analytical Chemistry

Plasma Protein Binding

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 $T_{1/2}$ = 61.1 min

10 uM IC = Not determined

1 uM IC = 22.7 ul/min/10^6 cells

Clearance and Plasma Protein Binding Values

		Clearance (ul/min/10^6 cells)		% Unbound		Renal Clearance (L/hr)[a]	
Chemical	CAS No.	1 uM	10 uM	1 uM	10 uM	1 uM	10 uM
Nifedipine	21829-25-4	70.9	50.7	2.4	3.9	0.16	0.26
Atrazine	1912-24-9	22.7	---[b]	15.1	8.7	1.02	0.59
Chlorpyrifos oxon	5598-15-2	228.5	219.4	ND[c]	ND[c]	0.68[d]	0.68[d]
Bromacil	314-40-9	---[b]	---[b]	2.9	3.5	0.19	0.24
Fenamiphos	22224-92-6	71.6	30.3	3.5	3.5	0.23	0.23
Forchlorfenuron	68157-60-8	27.8	---[b]	0.8	0.7	0.05	0.05
Metribuzin	21087-64-9	4.8	4.4	32.5 (?)	5.9	2.19 (?)	0.40

[a]Renal clearance estimated as GFR*F_U

[b]Clearance not determined due to saturation kinetics.

[d]Plasma protein binding not determined due to endogenous plasma esterase activity.

[c]Assumed 10% unbound in plasma.

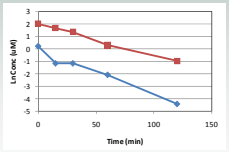
Outline

1. Tissue Slice Studies
2. In Vitro Pharmacokinetic Assays
3. **In Vitro to In Vivo Extrapolation and Reverse Dosimetry**
4. ToxCast Visualization and Analysis Software

Question

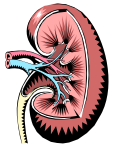
What do the EC/IC_{50} values measured using high-throughput screening mean in terms of human dosimetry and exposure?

Experimental Design



Hepatic Clearance

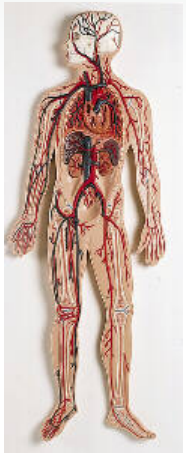
Plasma Protein Binding



Estimated Renal Clearance

SIMCYP
real solutions from virtual populations

Population-Based In Vitro to In Vivo Extrapolation Software

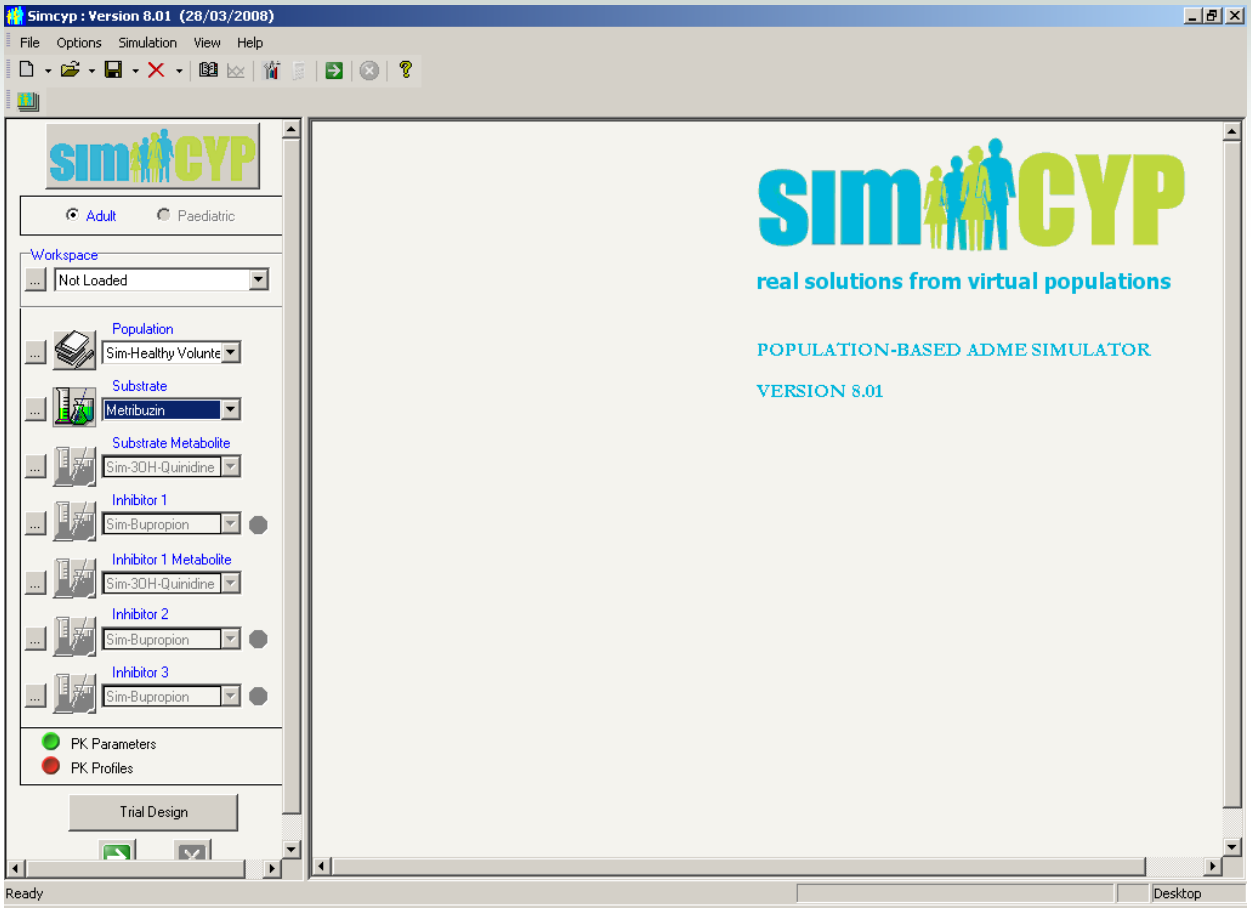


Plasma Concentration at Steady State

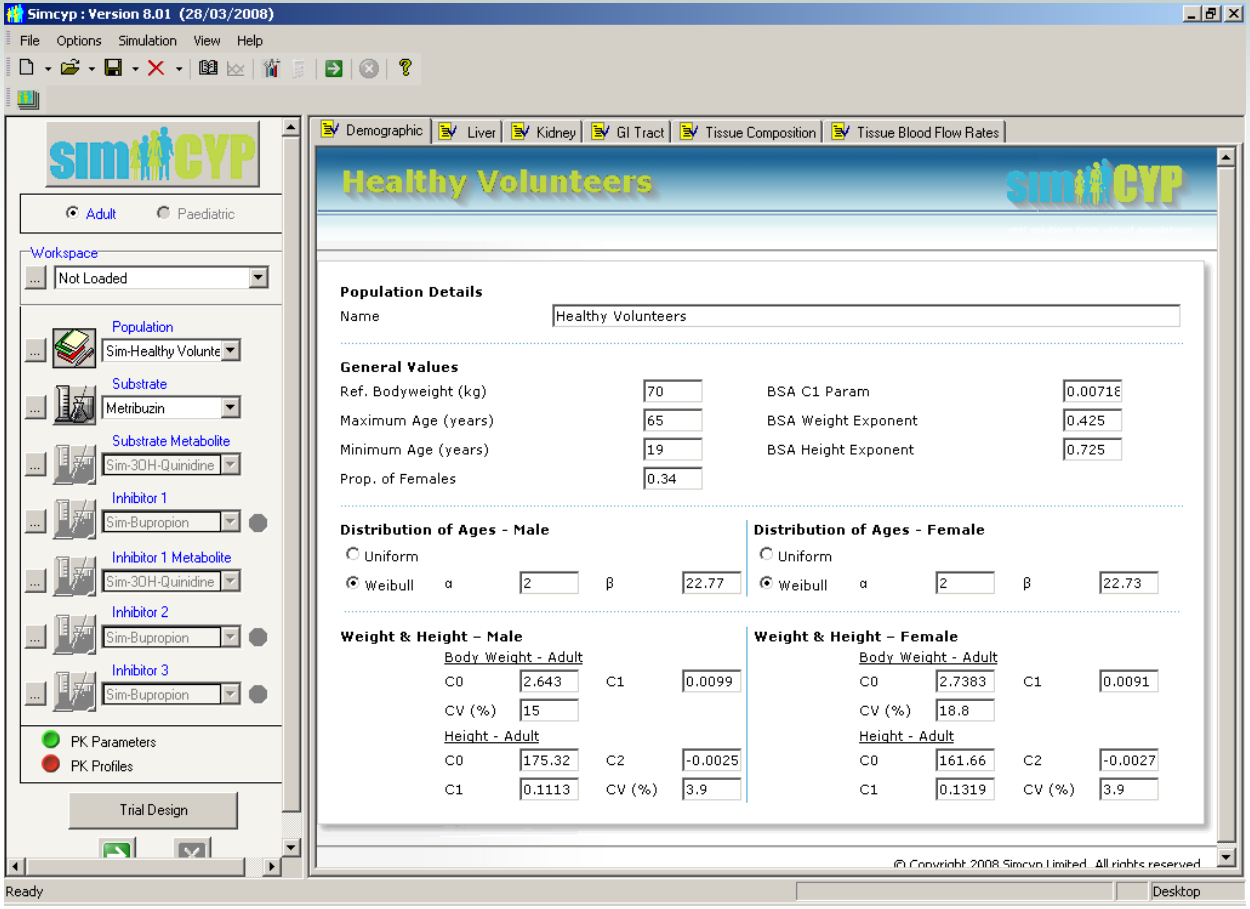
Tissue Dose **Exposure**

Reverse Dosimetry

Population-Based In Vitro to In Vivo Extrapolation Software

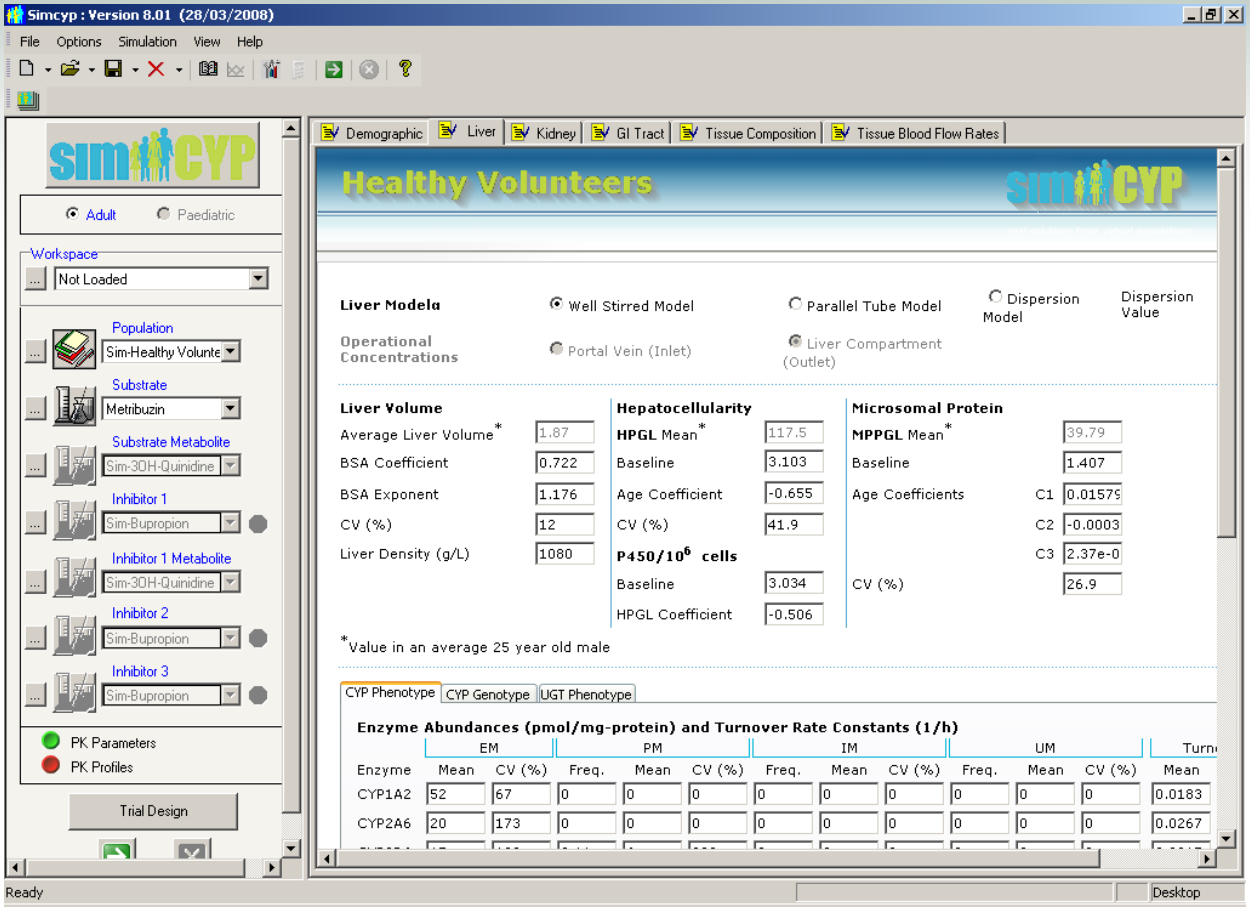


Population-Based In Vitro to In Vivo Extrapolation Software



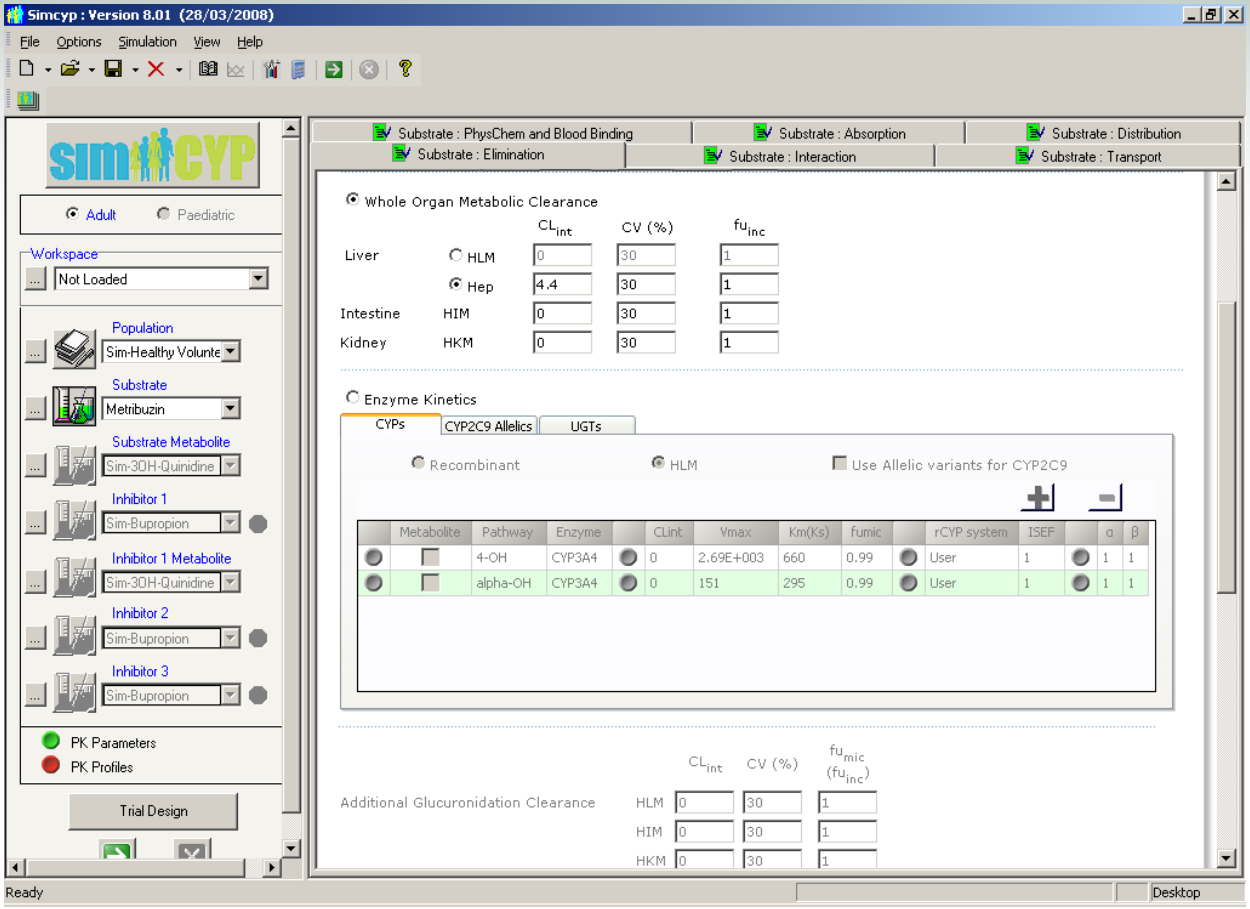
Defining exposed population

Population-Based In Vitro to In Vivo Extrapolation Software



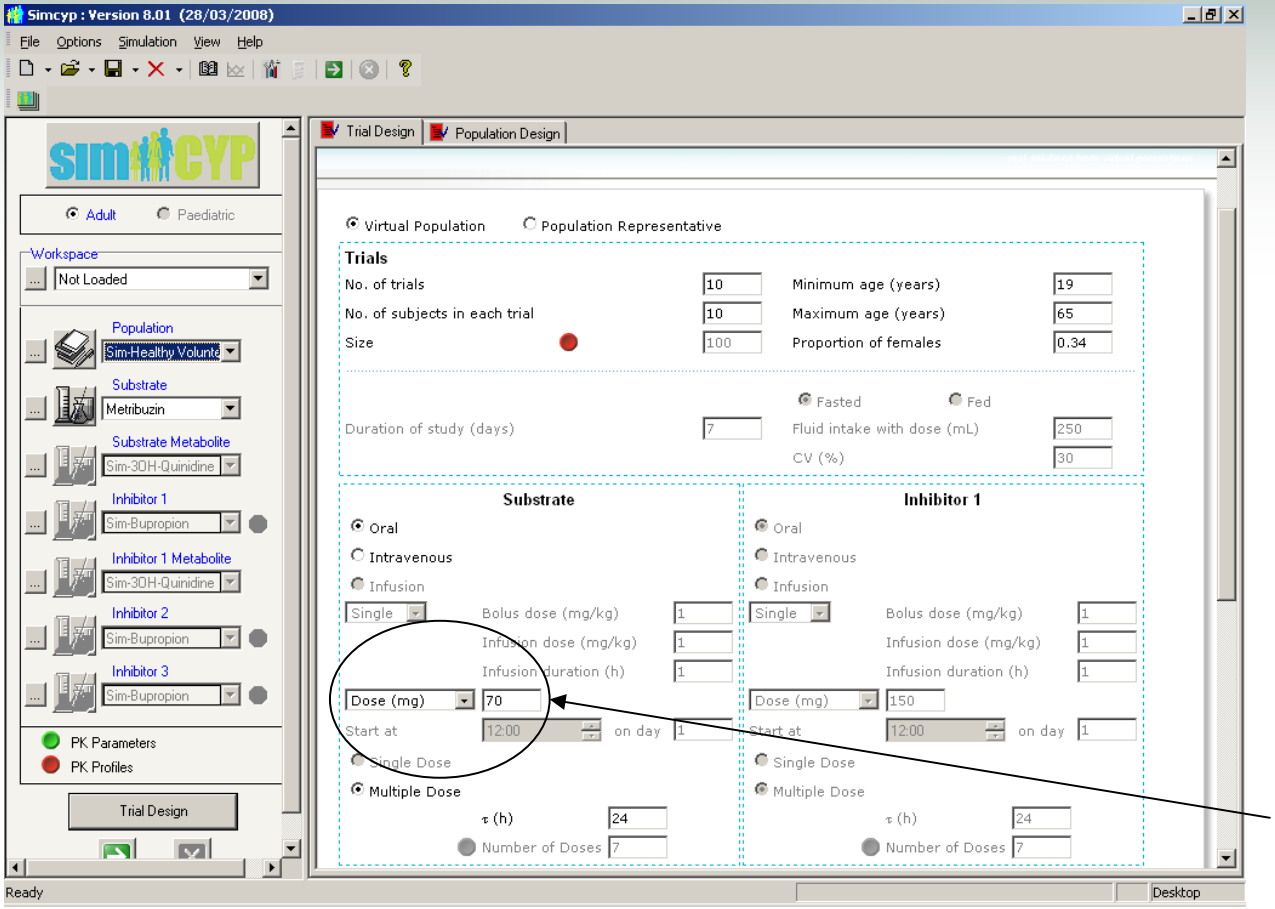
Variability in population

Population-Based In Vitro to In Vivo Extrapolation Software



Defining plasma protein binding and metabolic clearance

Population-Based In Vitro to In Vivo Extrapolation Software



Input Dose

Estimate exposure using reverse dosimetry

Population-Based In Vitro to In Vivo Extrapolation Software

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
Skewness	1.41	1.52	n/a	-1.10	-1.45	0.87
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
Fold	6.57	9.73	1.00	1.09	2.17	9.73
Std Dev	1.25	1.93	0.00	0.02	0.15	0.41

Output Conc at Steady State

Estimate exposure using reverse dosimetry

Results From Reverse Dosimetry Analysis

Chemical	CAS No.	Css (mg/L)*	Minimum EC_{50} (uM)	Est Oral Exposure at EC_{50} Equivalent (mg/kg/day)	Lower 95th Confidence Bound	Upper 95th Confidence Bound
Atrazine	1912-24-9	0.074	1.98	5.77	2.79	13.35
Chlorpyrifos oxon	5598-15-2	0.012	24.06	670.71	335.35	1609.71
Bromacil	314-40-9	10.13	97.28	2.51	1.70	4.18
Fenamiphos	22224-92-6	0.24	76.57	96.78	47.40	211.17
Forchlorfenuron	68157-60-8	50.87	19.98	0.10	0.07	0.16
Metribuzin	21087-64-9	0.85	17.63	4.44	2.45	9.44

Similar EC_{50} Values

Much Different Oral Equivalents

Outline

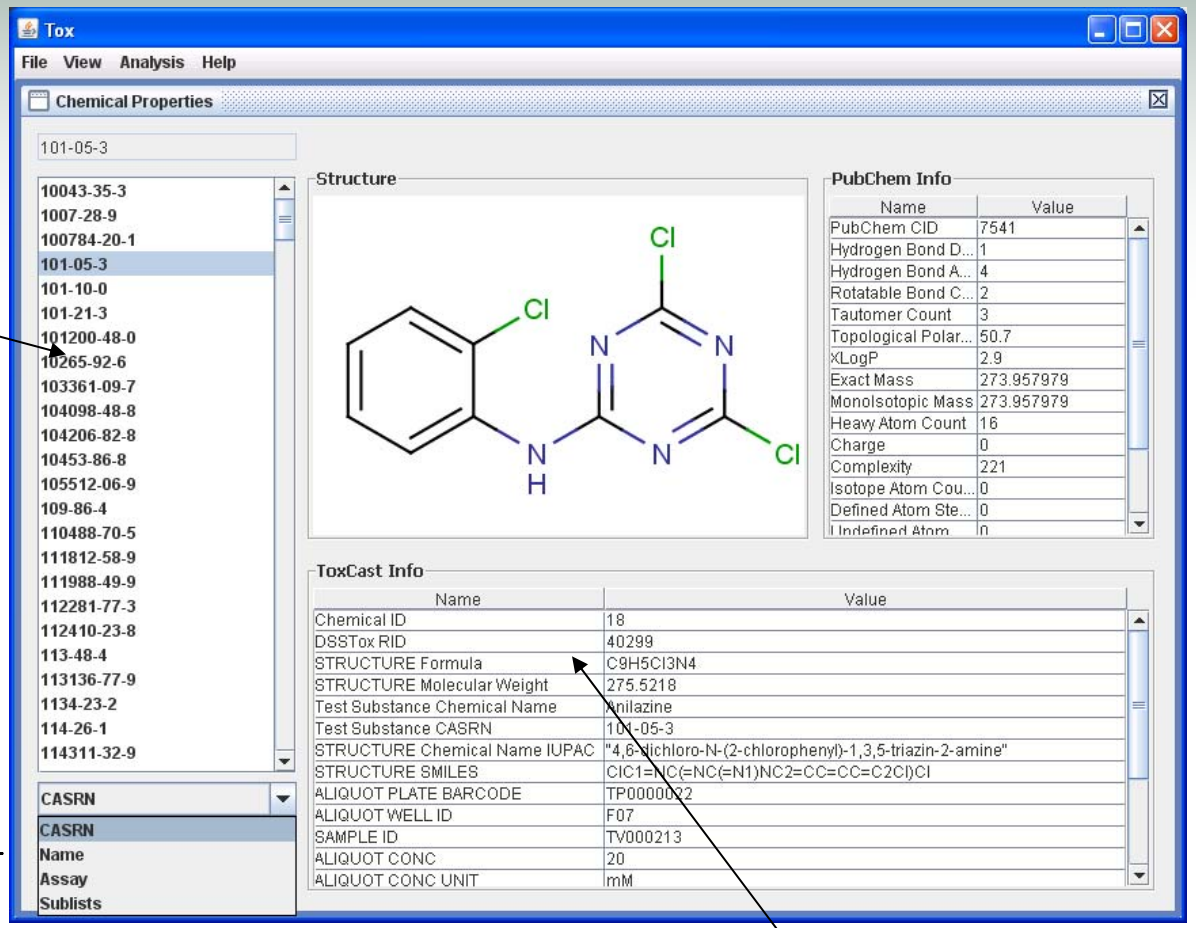
1. Tissue Slice Studies
2. In Vitro Pharmacokinetic Assays
3. In Vitro to In Vivo Extrapolation and Reverse Dosimetry
4. **ToxCast Visualization and Analysis Software**

ToxCast Visualization and Analysis Software

Constructed database and Java-based interface for analyzing and searching ToxCast-related data

ToxCast Visualization and Analysis Software

Navigation and Search Pane

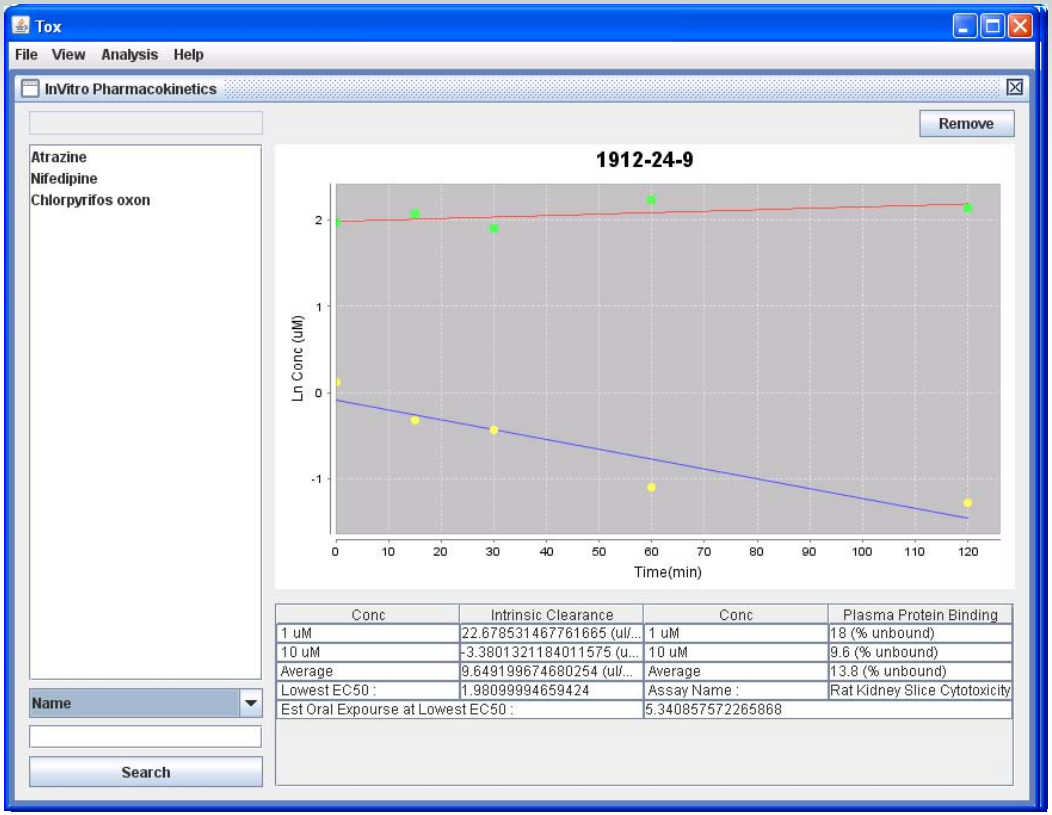


Organized Around:

1) Chemicals
2) Assays
3) Sublists

View Panes

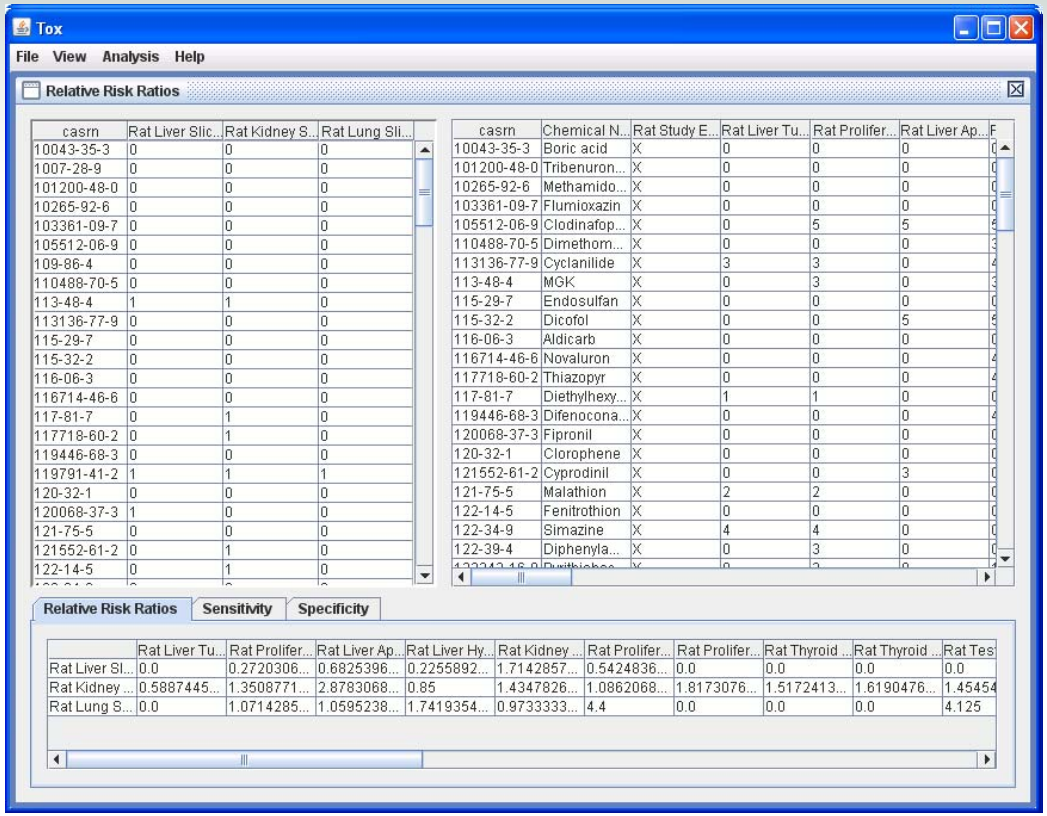
ToxCast Visualization and Analysis Software



5 Standard Views

1) Chemical Properties
2) Assay Properties
3) Sublists
4) Assay Results
5) In Vitro PK

ToxCast Visualization and Analysis Software



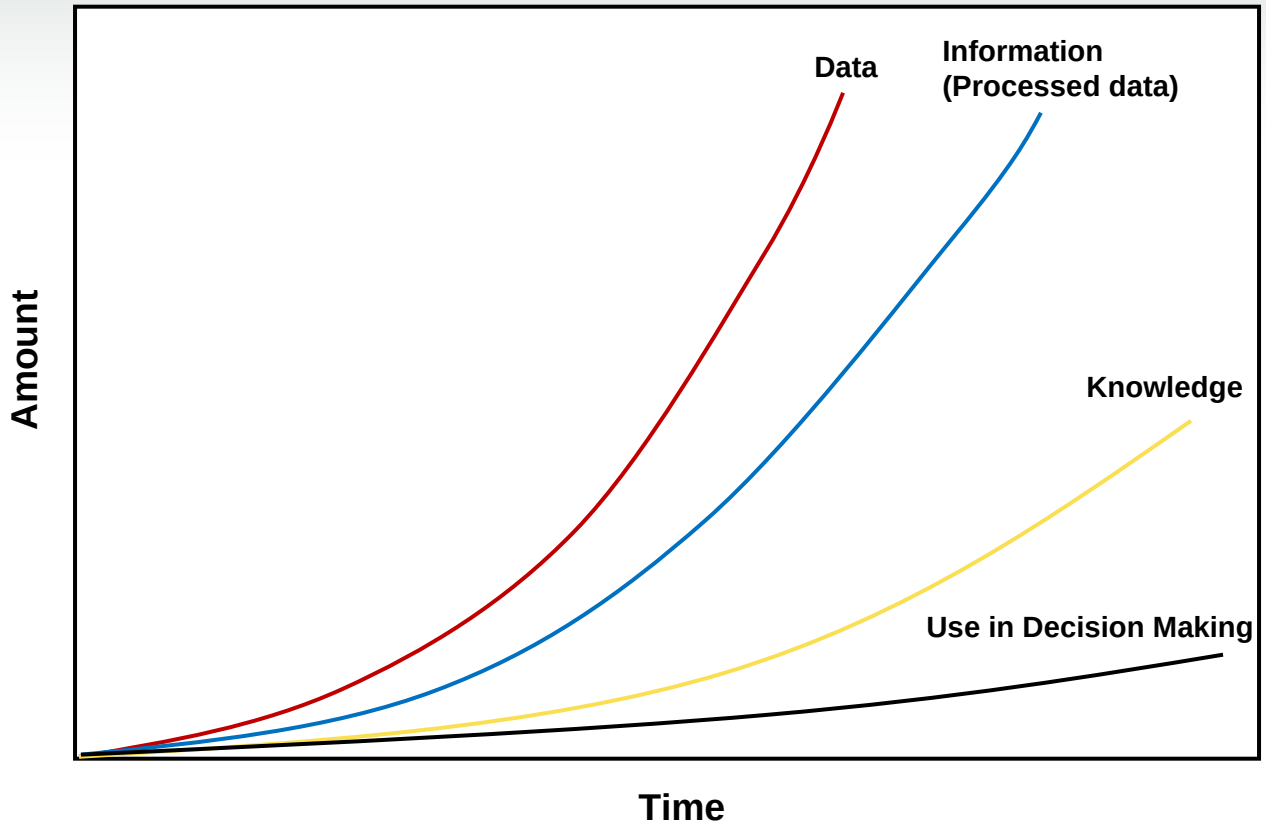
5 Analysis Tools

1) Sublist Creation
2) Assay Comparison
3) Correlation Matrix
4) Hierarchical Clustering
5) Relative Risk Calcs

Conclusions

- Although limited in scope, rat tissue slice cytotoxicity assays do not appear to reliably predict target organ toxicity.
- *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.

The Translation of In Vitro Concentrations to Equivalent Human Exposures Will Be Necessary for Regulatory Decision Making



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