



THE
HAMNER INSTITUTES FOR HEALTH SCIENCES
WHERE GREAT MINDS & MEDICINE MEET

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

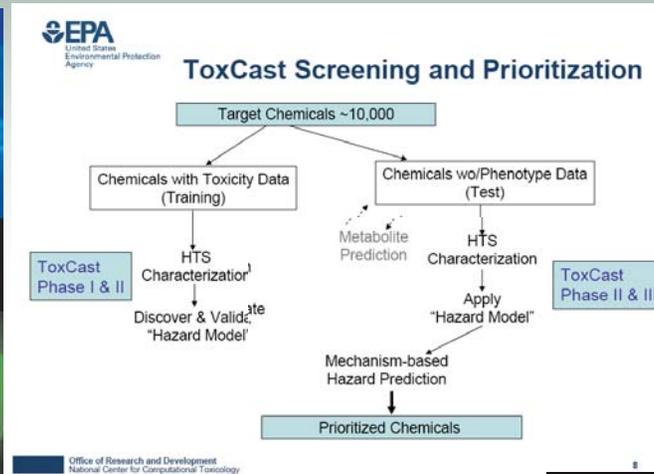
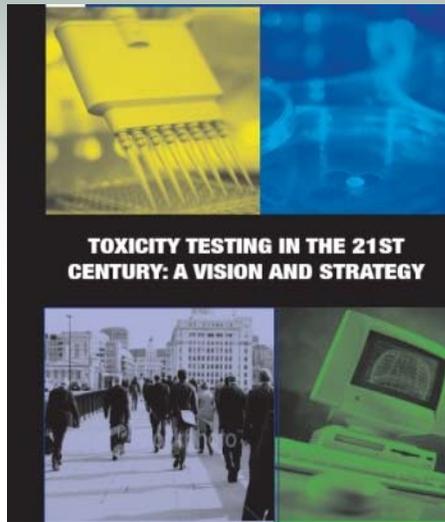
November 4, 2008

EPA Community of Practice

Russell Thomas

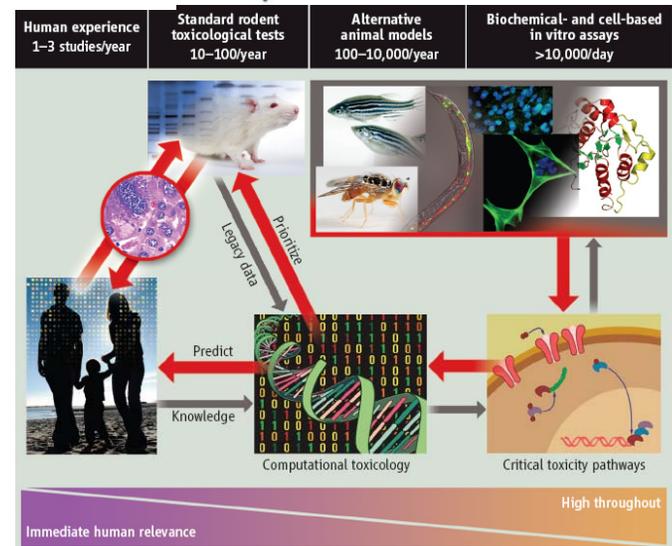
The Hamner Institutes for Health Sciences

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 | |



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 microarrays | 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

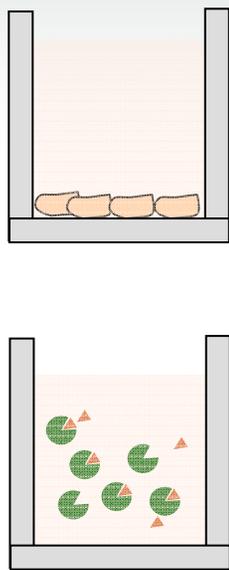
Activities:

- 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

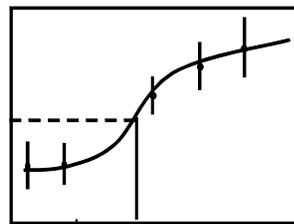


NTP Assays

What is Missing from the Current High-Throughput Screening Approaches



***In Vitro* High Throughput Screens**

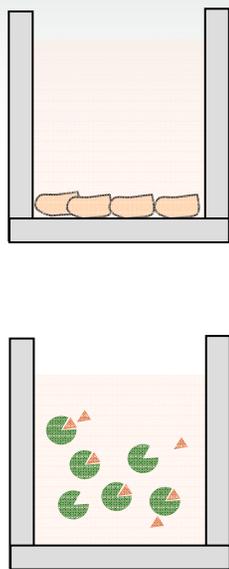


EC₅₀ or Single Point Activity Data

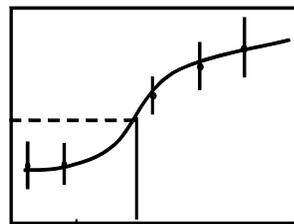


Human Toxicity

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

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**

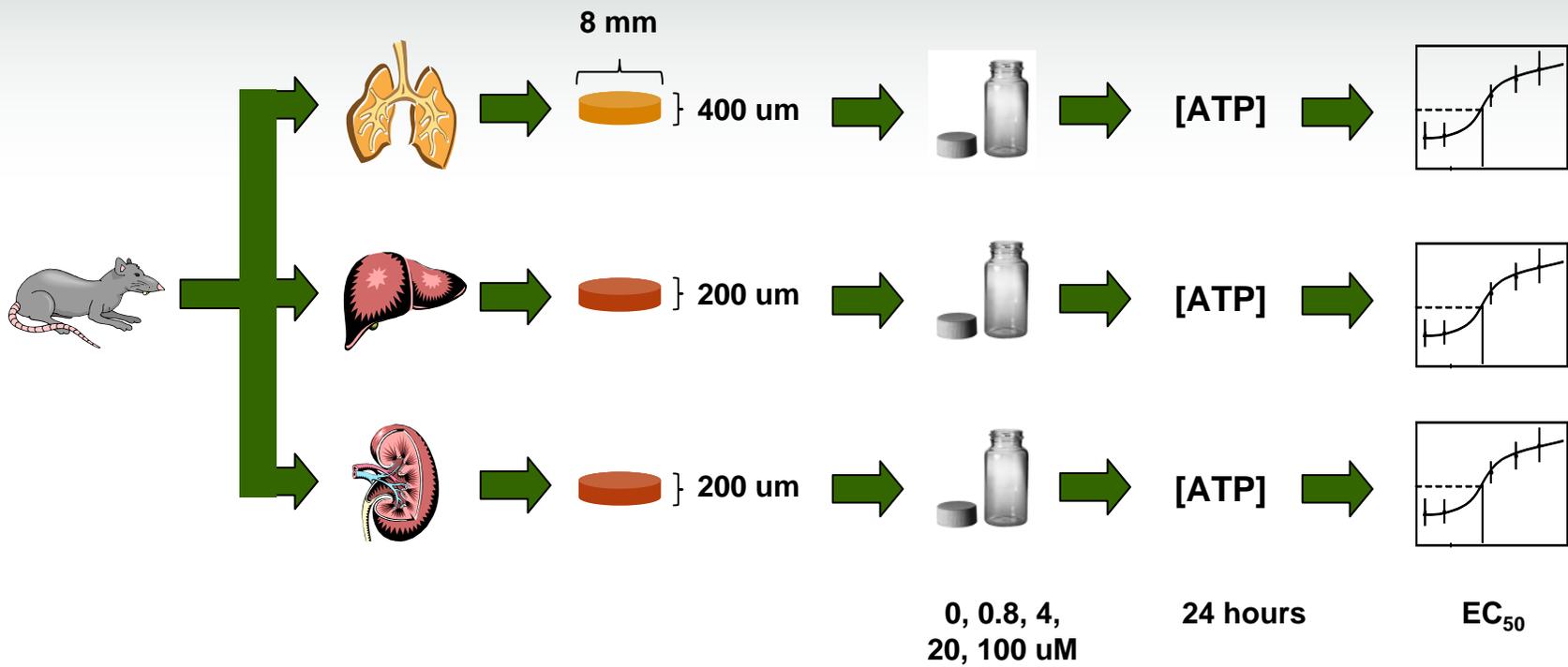
Outline

- 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

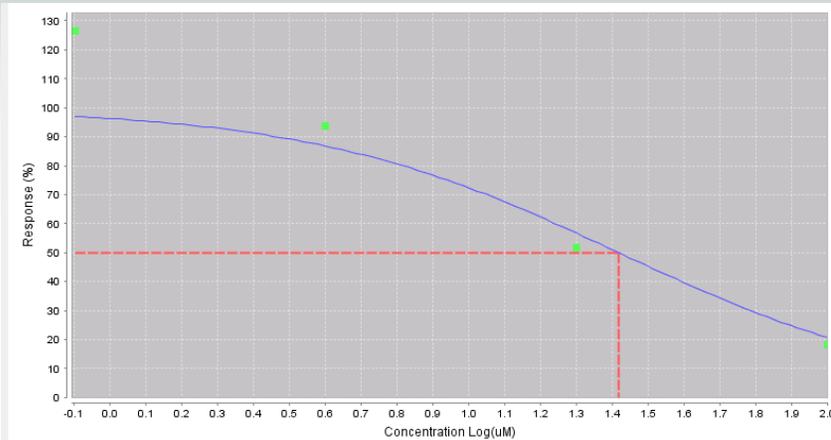
Can organ slice cultures be used to predict target organ toxicity in a whole animal?

Experimental Design



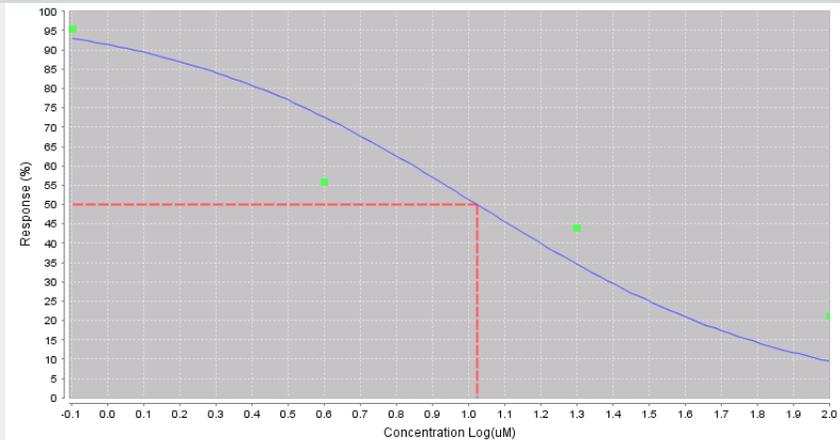
140 of the Phase I
ToxCast Chemicals

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

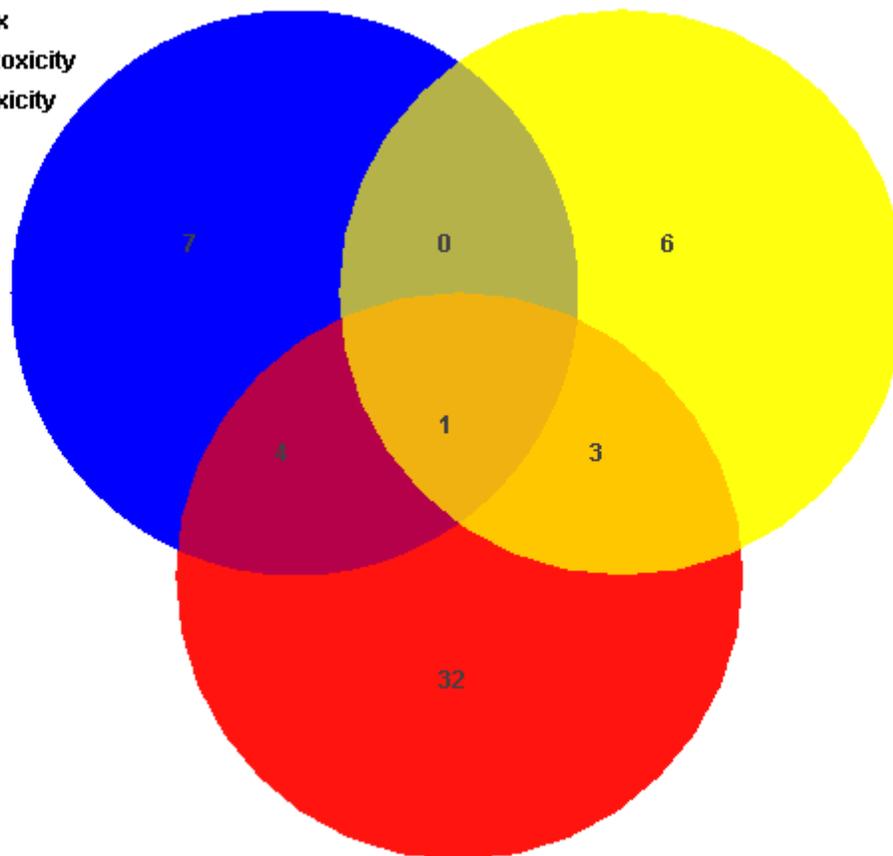


| | 119791-41-2 | Value |
|-----------------------|-------------|------------------|
| assay_id | | 550 |
| log_ac50 (uM) | | 1.024 |
| ac50 (Log uM) | | 10.5689996948242 |
| stdErr_log_ic50 | | 0.1767 |
| 95confidence_log_ic50 | | 0.4618 to 1.587 |
| 95confidence_ic50 | | 2.896 to 38.60 |
| degree_of_freedom | | 3 |
| r2 | | 0.8228 |
| abs_sum_of_square | | 509.8 |
| SYx | | 13.04 |
| number_of_points | | 4 |

Liver Toxicity of Emamectin

Preliminary Results Summary

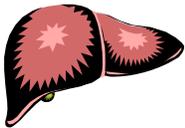
- A: Liver_Slice_Cytotox**
- B: Kidney_Slice_Cytotoxicity**
- C: Lung_Slice_Cytotoxicity**



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 μ M (Range: 1.3 – 99 μ M)

Predicting Target Organ Toxicity

| | 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 |
|-------------------------------|------------------|---------------------------------|------------------------------|-----------------------|------------------------|----------------------------------|-----------------------------------|--------------------|-------------------------|-----------------------|------------------------|----------------------|-------------------------------|---------------|--------------------|-----------------------------------|--------------------------------|-------------------------|------------------------|-------------------|-----------------|
| Relative Risk | | | | | | | | | | | | | | | | | | | | | |
| 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

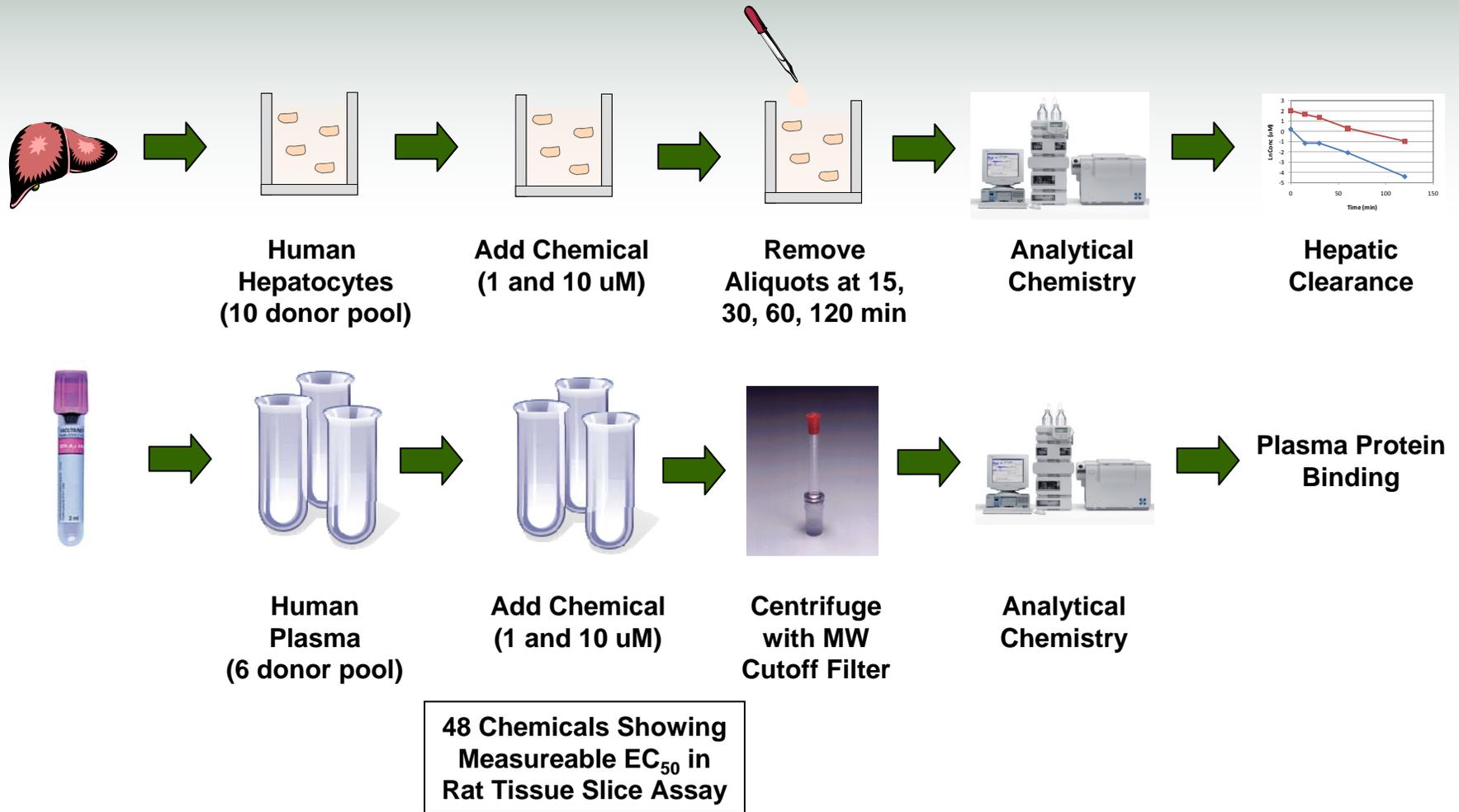
3. In Vitro to In Vivo Extrapolation and Reverse Dosimetry

4. ToxCast Visualization and Analysis Software

Question

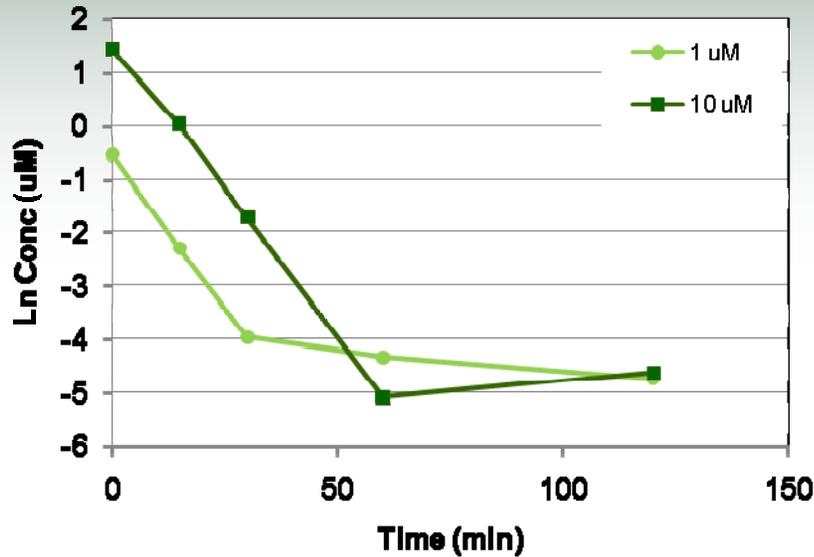
What in vitro assays are necessary to predict in vivo pharmacokinetic behavior?

Experimental Design



Example Chemicals for Hepatic Clearance

Chlorpyrifos Oxon



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

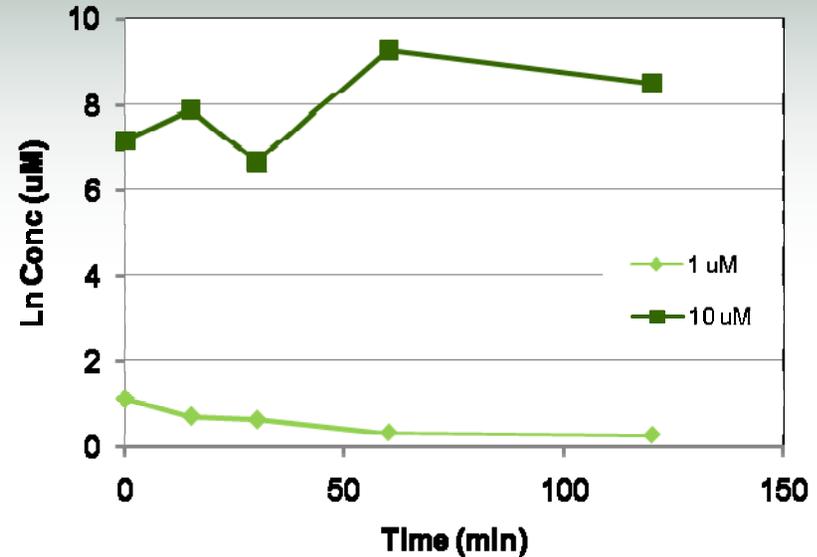
1 uM $T_{1/2}$ = 6.1 min



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

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

Atrazine



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

| Chemical | CAS No. | Clearance (ul/min/10 ⁶ cells) | | % Unbound | | Renal Clearance (L/hr) ^a | |
|-------------------|------------|--|------------------|-----------------|-----------------|-------------------------------------|-------------------|
| | | 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 |

^aRenal clearance estimated as $GFR \cdot F_U$

^bClearance not determined due to saturation kinetics.

^cPlasma protein binding not determined due to endogenous plasma esterase activity.

^dAssumed 10% unbound in plasma.

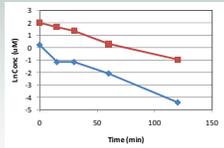
Outline

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

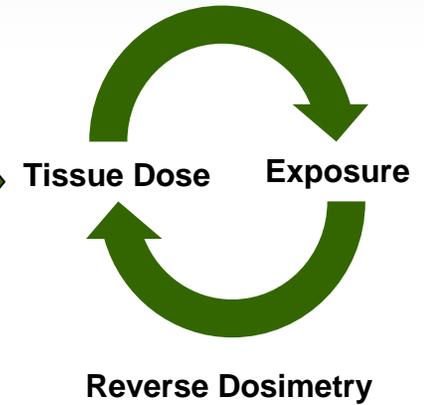


simCYP
real solutions from virtual populations

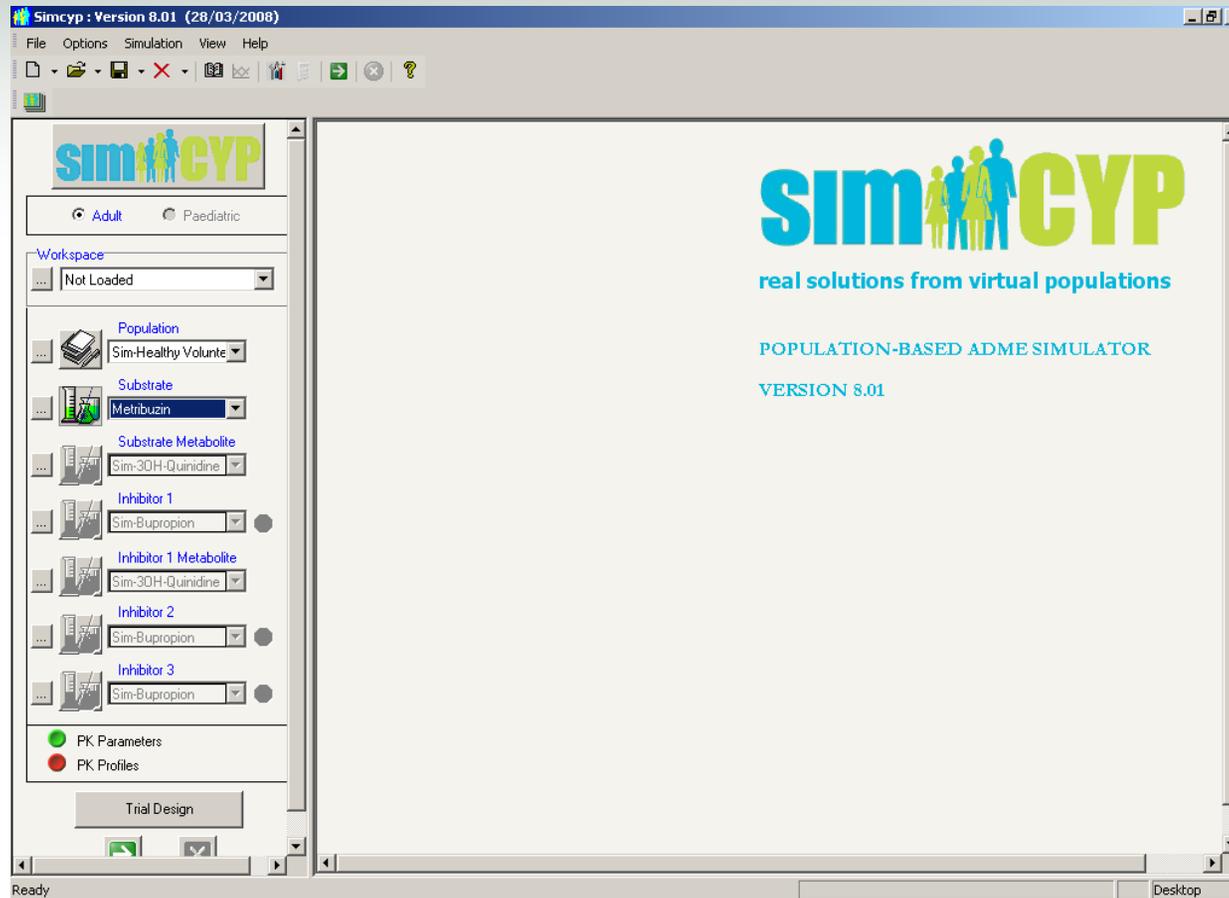
**Population-Based
In Vitro to In Vivo
Extrapolation
Software**



**Plasma
Concentration at
Steady State**



Population-Based In Vitro to In Vivo Extrapolation Software



Population-Based In Vitro to In Vivo Extrapolation Software

The screenshot displays the Simcyp software interface for configuring a population. The main window is titled 'Healthy Volunteers' and contains several sections for defining population parameters.

Population Details: Name: Healthy Volunteers

General Values:

| | | | |
|----------------------|------|---------------------|---------|
| Ref. Bodyweight (kg) | 70 | BSA C1 Param | 0.00716 |
| 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

Distribution of Ages - Female:

Uniform

Weibull α 2 β 22.73

Weight & Height - Male:

| | | | |
|----------------------------|--------|--------|---------|
| <u>Body Weight - Adult</u> | | | |
| C0 | 2.643 | C1 | 0.0099 |
| CV (%) | 15 | | |
| <u>Height - Adult</u> | | | |
| C0 | 175.32 | C2 | -0.0025 |
| C1 | 0.1113 | CV (%) | 3.9 |

Weight & Height - Female:

| | | | |
|----------------------------|--------|--------|---------|
| <u>Body Weight - Adult</u> | | | |
| C0 | 2.7383 | C1 | 0.0091 |
| CV (%) | 18.8 | | |
| <u>Height - Adult</u> | | | |
| C0 | 161.66 | C2 | -0.0027 |
| C1 | 0.1319 | CV (%) | 3.9 |

The interface also includes a left-hand sidebar for selecting population, substrate (Metribuzin), and inhibitors (Sim-Bupropion), and a bottom status bar showing 'Ready' and 'Desktop'.

Defining exposed population

Population-Based In Vitro to In Vivo Extrapolation Software

The screenshot displays the Simcyp software interface for 'Healthy Volunteers'. The main window is titled 'Healthy Volunteers' and contains several sections for parameter configuration:

- Liver Models:** Well Stirred Model (selected), Parallel Tube Model, Dispersion Model.
- Operational Concentrations:** Portal Vein (Inlet) (selected), 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), P450/10⁶ cells (Baseline: 3.034, 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).

*Value in an average 25 year old male

Below these sections, there are tabs for 'CYP Phenotype', 'CYP Genotype', and 'UGT Phenotype'. The 'CYP Phenotype' tab is active, showing a table of enzyme abundances and turnover rate constants.

| Enzyme | EM | | | PM | | | IM | | | UM | | | Turnover |
|--------|------|--------|-------|------|--------|-------|------|--------|-------|------|--------|------|----------|
| | Mean | CV (%) | Freq. | Mean | CV (%) | Freq. | Mean | CV (%) | Freq. | Mean | CV (%) | Mean | |
| 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 |

Variability in population

Population-Based In Vitro to In Vivo Extrapolation Software

The screenshot shows the Simcyp software interface with the following settings:

- Substrate:** PhysChem and Blood Binding, Absorption, Distribution, Elimination, Interaction, Transport (all checked).
- Whole Organ Metabolic Clearance:**
 - Liver: HLM, Hep. CL_{int} : 4.4, CV (%): 30, $f_{u_{inc}}$: 1.
 - Intestine: HIM, CL_{int} : 0, CV (%): 30, $f_{u_{inc}}$: 1.
 - Kidney: HKM, CL_{int} : 0, CV (%): 30, $f_{u_{inc}}$: 1.
- Enzyme Kinetics:**
 - CYPs:** CYP2C9 Allelics, UGTs.
 - Recombinant, HLM, Use Allelic variants for CYP2C9.

| Metabolite | Pathway | Enzyme | CL_{int} | V_{max} | Km(Ks) | f _{mic} | rCYP system | ISEF | α | β |
|------------|---------|--------|------------|-----------|--------|------------------|-------------|------|----------|---------|
| 4-OH | | CYP3A4 | 0 | 2.69E+003 | 660 | 0.99 | User | 1 | 1 | 1 |
| alpha-OH | | CYP3A4 | 0 | 151 | 295 | 0.99 | User | 1 | 1 | 1 |
- Additional Glucuronidation Clearance:**
 - HLM: CL_{int} : 0, CV (%): 30, $f_{u_{mic}}$ ($f_{u_{inc}}$): 1.
 - HIM: CL_{int} : 0, CV (%): 30, $f_{u_{mic}}$ ($f_{u_{inc}}$): 1.
 - HKM: CL_{int} : 0, CV (%): 30, $f_{u_{mic}}$ ($f_{u_{inc}}$): 1.

Defining plasma protein binding and metabolic clearance

Population-Based In Vitro to In Vivo Extrapolation Software

The screenshot displays the Simcyp software interface for trial design. The main window is titled "Trial Design" and "Population Design". The "Virtual Population" tab is selected. The "Trials" section includes the following parameters:

| Parameter | Value |
|-------------------------------|-------|
| No. of trials | 10 |
| No. of subjects in each trial | 10 |
| Size | 100 |
| Minimum age (years) | 19 |
| Maximum age (years) | 65 |
| Proportion of females | 0.34 |
| Duration of study (days) | 7 |
| Fluid intake with dose (mL) | 250 |
| CV (%) | 30 |

The "Substrate" and "Inhibitor 1" sections are also visible. The "Substrate" section is set to "Oral" and "Single" dose. The "Dose (mg)" field is circled and labeled "Input Dose". The "Inhibitor 1" section is also set to "Oral" and "Single" dose.

Workspace:

- Population: Sim-Healthy Volunteers
- Substrate: Metribuzin
- Substrate Metabolite: Sim-3OH-Quinidine
- Inhibitor 1: Sim-Bupropion
- Inhibitor 1 Metabolite: Sim-3OH-Quinidine
- Inhibitor 2: Sim-Bupropion
- Inhibitor 3: Sim-Bupropion

PK Parameters: PK Parameters, PK Profiles

Trial Design:

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

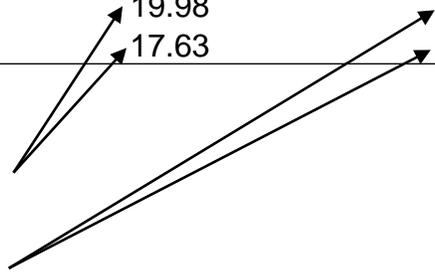
Estimate exposure using reverse dosimetry

Results From Reverse Dosimetry Analysis

| Chemical | CAS No. | C _{ss} (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 EC₅₀ Values

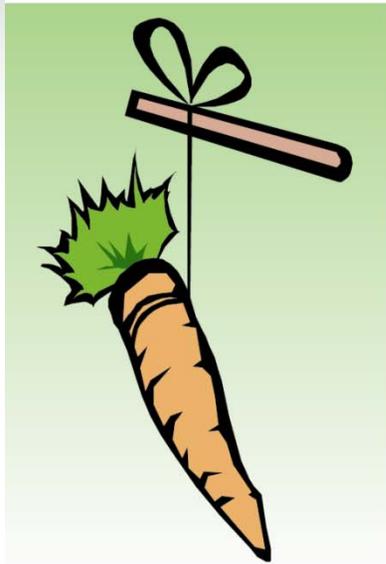
Much Different Oral Equivalents



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



TEASER

**ToxCast Exploration, Analysis,
and Search Resource**

The Hamner Institutes for Health Sciences
Bioinformatics Group
Version 1.0 Beta

**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

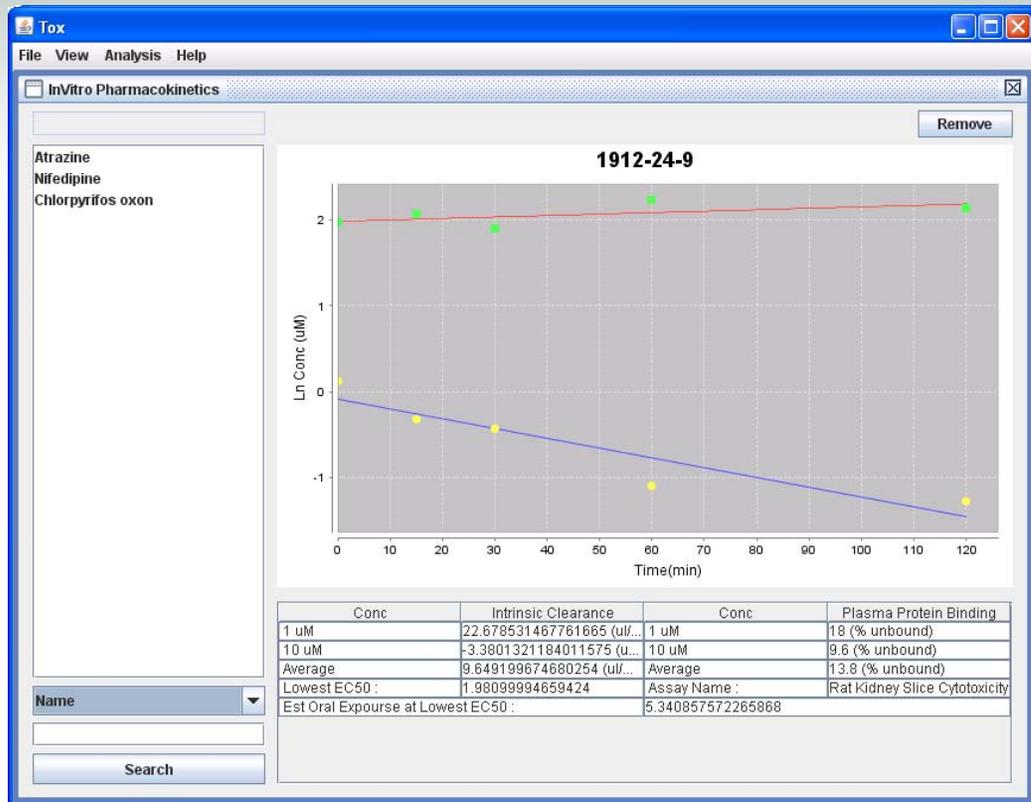
The screenshot displays the ToxCast software interface. On the left is a 'Navigation and Search Pane' with a list of chemical IDs, including '101-05-3' which is highlighted. Below this list are dropdown menus for 'CASRN' and 'Sublists'. The main area is divided into three panes: 'Structure' showing a chemical structure of 4,6-dichloro-N-(2-chlorophenyl)-1,3,5-triazin-2-amine; 'PubChem Info' with a table of chemical properties; and 'ToxCast Info' with a table of assay-related data.

| Name | Value |
|----------------------|------------|
| PubChem CID | 7541 |
| Hydrogen Bond D... | 1 |
| Hydrogen Bond A... | 4 |
| Rotatable Bond C... | 2 |
| Tautomer Count | 3 |
| Topological Polar... | 50.7 |
| xLogP | 2.9 |
| Exact Mass | 273.957979 |
| Monoisotopic Mass | 273.957979 |
| Heavy Atom Count | 16 |
| Charge | 0 |
| Complexity | 221 |
| Isotope Atom Cou... | 0 |
| Defined Atom Ste... | 0 |
| Undefined Atom | n |

| Name | Value |
|-------------------------------|---|
| Chemical ID | 18 |
| DBSTox RID | 40299 |
| STRUCTURE Formula | C9H5Cl3N4 |
| STRUCTURE Molecular Weight | 275.5218 |
| Test Substance Chemical Name | Anilazine |
| Test Substance CASRN | 101-05-3 |
| STRUCTURE Chemical Name IUPAC | "4,6-dichloro-N-(2-chlorophenyl)-1,3,5-triazin-2-amine" |
| STRUCTURE SMILES | C1C1=NC(=NC(=N1)NC2=CC=CC=C2Cl)Cl |
| ALiquot Plate Barcode | TP0000022 |
| ALiquot Well ID | F07 |
| SAMPLE ID | TV000213 |
| ALiquot Conc | 20 |
| ALiquot Conc Unit | mM |

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

The screenshot shows the ToxCast software interface. The main window is titled 'Relative Risk Ratios' and contains two side-by-side tables. The left table lists CASRN numbers and their corresponding relative risk ratios for Rat Liver Slic., Rat Kidney S., and Rat Lung Sli. The right table lists CASRN numbers, Chemical Names, and relative risk ratios for Rat Study E., Rat Liver Tu., Rat Prolifer., and Rat Liver Ap. Below these tables is a summary table with tabs for 'Relative Risk Ratios', 'Sensitivity', and 'Specificity'. The 'Relative Risk Ratios' tab is active, showing a table with columns for various assay types and their corresponding values.

| casrn | Rat Liver Slic... | Rat Kidney S... | Rat Lung Sli... |
|-------------|-------------------|-----------------|-----------------|
| 10043-35-3 | 0 | 0 | 0 |
| 1007-28-9 | 0 | 0 | 0 |
| 101200-48-0 | 0 | 0 | 0 |
| 10265-92-6 | 0 | 0 | 0 |
| 103361-09-7 | 0 | 0 | 0 |
| 105512-06-9 | 0 | 0 | 0 |
| 109-86-4 | 0 | 0 | 0 |
| 110488-70-5 | 0 | 0 | 0 |
| 113-48-4 | 1 | 1 | 0 |
| 113136-77-9 | 0 | 0 | 0 |
| 115-29-7 | 0 | 0 | 0 |
| 115-32-2 | 0 | 0 | 0 |
| 116-06-3 | 0 | 0 | 0 |
| 116714-46-6 | 0 | 0 | 0 |
| 117-81-7 | 0 | 1 | 0 |
| 117718-60-2 | 0 | 1 | 0 |
| 119446-68-3 | 0 | 0 | 0 |
| 119791-41-2 | 1 | 1 | 1 |
| 120-32-1 | 0 | 0 | 0 |
| 120068-37-3 | 1 | 0 | 0 |
| 121-75-5 | 0 | 0 | 0 |
| 121552-61-2 | 0 | 1 | 0 |
| 122-14-5 | 0 | 1 | 0 |

| casrn | Chemical N... | Rat Study E... | Rat Liver Tu... | Rat Prolifer... | Rat Liver Ap... |
|-------------|----------------|----------------|-----------------|-----------------|-----------------|
| 10043-35-3 | Boric acid | X | 0 | 0 | 0 |
| 101200-48-0 | Tribenuron... | X | 0 | 0 | 0 |
| 10265-92-6 | Methamido... | X | 0 | 0 | 0 |
| 103361-09-7 | Flumioxazin | X | 0 | 0 | 0 |
| 105512-06-9 | Clodinafop... | X | 0 | 5 | 5 |
| 110488-70-5 | Dimethom... | X | 0 | 0 | 0 |
| 113136-77-9 | Cyclanilide | X | 3 | 3 | 0 |
| 113-48-4 | MGK | X | 0 | 3 | 0 |
| 115-29-7 | Endosulfan | X | 0 | 0 | 0 |
| 115-32-2 | Dicofol | X | 0 | 0 | 5 |
| 116-06-3 | Aldicarb | X | 0 | 0 | 0 |
| 116714-46-6 | Novaluron | X | 0 | 0 | 0 |
| 117718-60-2 | Thiazopyr | X | 0 | 0 | 0 |
| 117-81-7 | Diethylhexy... | X | 1 | 1 | 0 |
| 119446-68-3 | Difenocona... | X | 0 | 0 | 0 |
| 120068-37-3 | Fipronil | X | 0 | 0 | 0 |
| 120-32-1 | Chlorophene | X | 0 | 0 | 0 |
| 121552-61-2 | Cyprodinil | X | 0 | 0 | 3 |
| 121-75-5 | Malathion | X | 2 | 2 | 0 |
| 122-14-5 | Fenitrothion | X | 0 | 0 | 0 |
| 122-34-9 | Simazine | X | 4 | 4 | 0 |
| 122-39-4 | Diphenyla... | X | 0 | 3 | 0 |

| Relative Risk Ratios | Sensitivity | Specificity |
|-----------------------------|-----------------|-----------------|
| Rat Liver Tu... | Rat Prolifer... | Rat Liver Ap... |
| Rat Liver Si... 0.0 | 0.2720306... | 0.6825396... |
| Rat Kidney ... 0.5887445... | 1.3508771... | 2.8783068... |
| Rat Lung S... 0.0 | 1.0714285... | 1.0595238... |

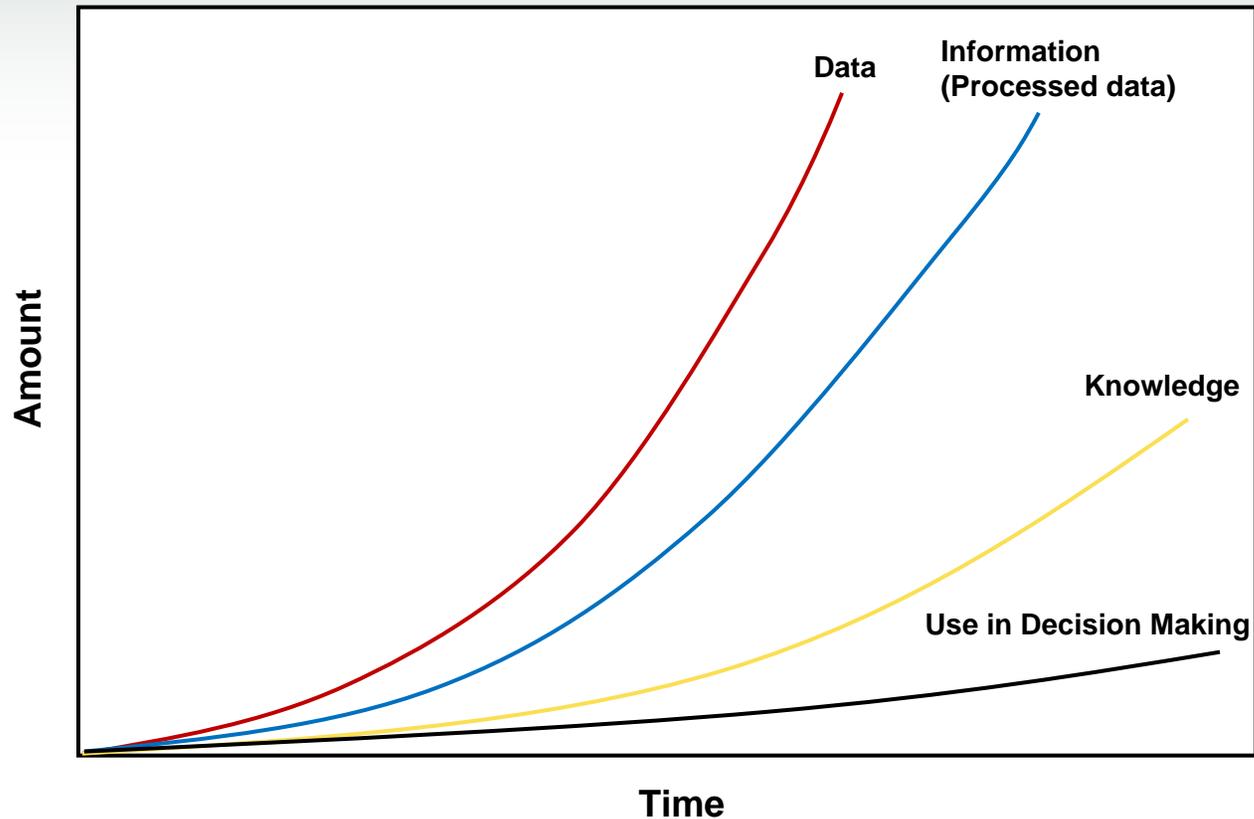
5 Analysis Tools

- 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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