The “Straw Man” System For Replacing Uncertainty Factors With Empirical Distributions For Traditional Systemic Toxicants—examples And Use For Value Of Information Analysis Of In Vitro Measurements

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"Tox21," the "Silver Book" and the "Straw Man" System

- "Tox21"—Start to deal with untested chemicals using high-throughput in vitro assays
- "Silver Book" (Science and Decisions)—
  - Redefine noncancer RfDs to be a "Risk Specific Dose" (no more than X incidence of harm with Z confidence)
  - Replace single-point "uncertainty factors" with empirical distributions representing prior experience
- "Straw Man"—Previously published Monte Carlo-based uncertainty propagation system using empirical distributions and a candidate risk specific dose criterion (< 1/100,000 risk with 95% confidence)

SG1

Steps in the Straw Man Approach

1. Estimate an **animal ED_{50}** by fitting dose-response models to the original toxicological data. (Otherwise, project from LOAEL)
2. Apply a **subchronic-to-chronic uncertainty factor distribution (UF_S)** to transform the subchronic dose that affects 50% of the lab animal populations to a chronic dose that would be expected affect 50% of the animals.
3. Apply the **animal-to-human uncertainty factor distribution (UF_A)** to convert the estimated animal ED50's to human ED50's.
4. Apply a **database uncertainty factor distribution (D)** to represent deficiencies in the toxicological datasets (different depending on whether repro or chronic toxicity studies are missing).
5. Apply an **interindividual uncertainty factor distribution** to account for uncertainties in the extent of susceptibility differences across humans, derived from human studies of analogous chemicals to produce separate distributions of human pharmacokinetic (**GSD_{PK}**) and pharmacodynamic (**GSD_{PD}**) variability. Use the combined GSD_{PK} and GSD_{PD} to assess risks as a function of dose, using the probit model.
6. Combine input from Steps 1 though 5 into a Monte Carlo simulation to evaluate a distribution of **Doses** corresponding to a given level of risk (**$P_{response}$**).

Slide 3

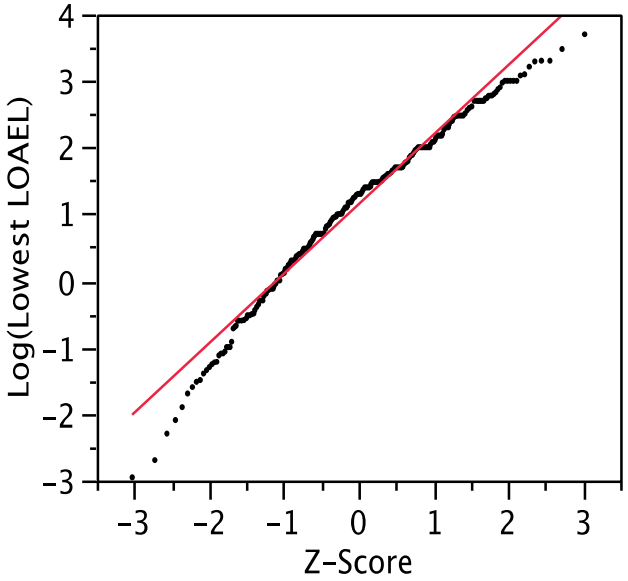
SG1 Meghan and I both thought that these two slides would be helpful for an audience not familiar with the details of Straw Man.
GrecoS, 4/26/2011

Initial Exploration of a Value of Information Framework to Asses Contributions of Nexgen In Vitro Data Toward Reducing Uncertainty in Toxic Potency—3 Steps

- First, define a “prior” distribution for an uncertain quantity of interest—that is, how uncertain should we be about a particular risk-related number (e.g. LOAEL) before any Nexgen information is considered?
- Second, assess the accuracy with which specific Nexgen results can predict the uncertain quantity of interest.
- Third, assessing the residual uncertainty that remains after the Nexgen results have been used to “update” the “prior” uncertainty distribution.

Lowest LOAEL Values for Chemical—An Initial Candidate for Juxtaposition with Nexgen Data

Lognormal Probability Plot of the Distribution of the Lowest Available LOAEL's in for 502 Chemicals in the Toxcast Database (LOAEL Data Graciously Provided by Matthew Martin, EPA National Center for Computational Toxicology)

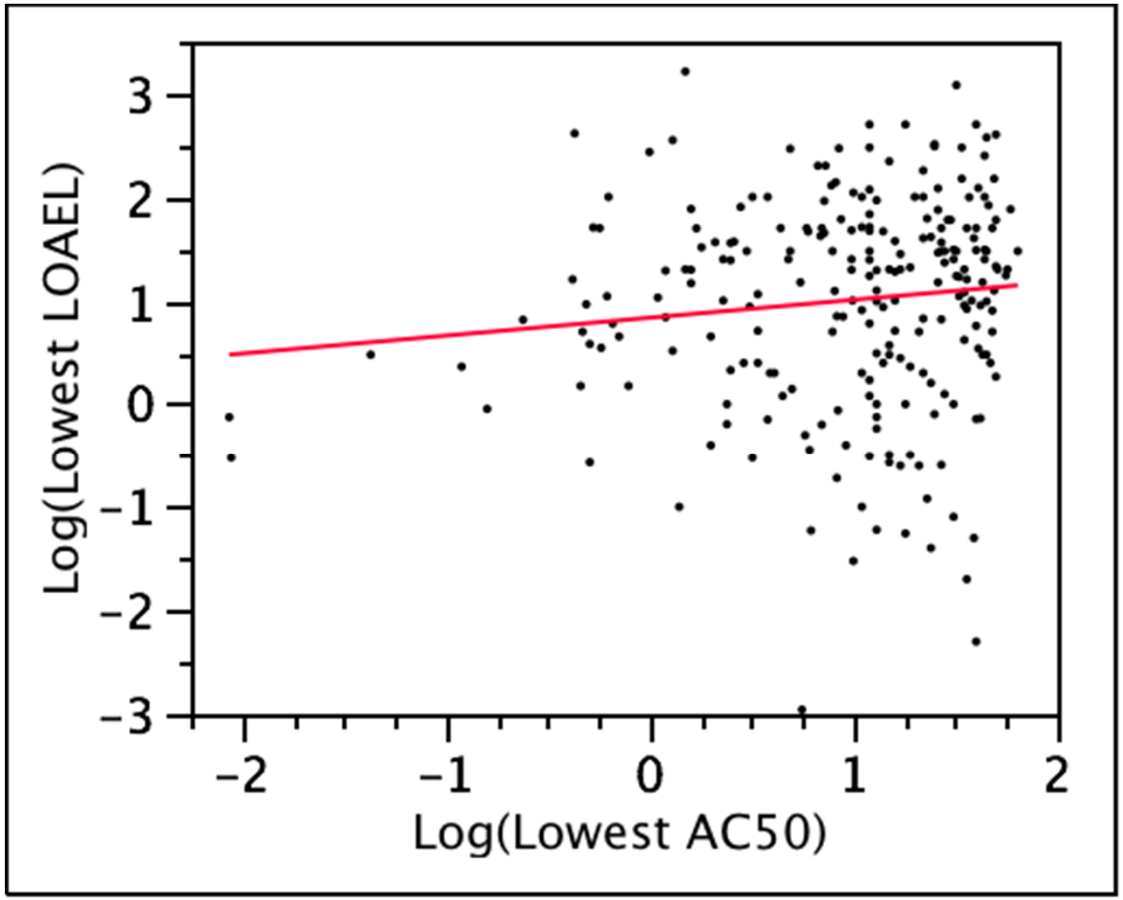


Percentile Distribution and Summary Statistics for 502 Lowest LOAEL Values (mg/kg-day)

1st	5th	50th	95th	99th	Geometric Mean	Geometric Std. Dev. (GSD)
0.015	0.20	20	500	1.8E+03	14	11.3

95th/5th percentiles	2.4E+03

Relationship Between Lowest In Vitro AC50's (µM) and Lowest LOAELs (mg/kg-day) for 235 Chemicals with Both Kinds of Data



Regression Statistics

Summary of Fit					
RSquare	0.0127				
RSquare Adj	0.0086				
Root Mean Square Error	1.011				
Mean of Response	1.002				
Observations	245				
Parameter Estimates					
Term	Antilog (Estimate)	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	6.82	0.834	0.115	7.26	<.0001
Log(Lowest AC50)	1.49	0.173	0.098	1.77	0.0782

Reduction in the “Prior” Uncertainty in Lowest LOAELs from the Relationship with Lowest In Vitro AC50’s

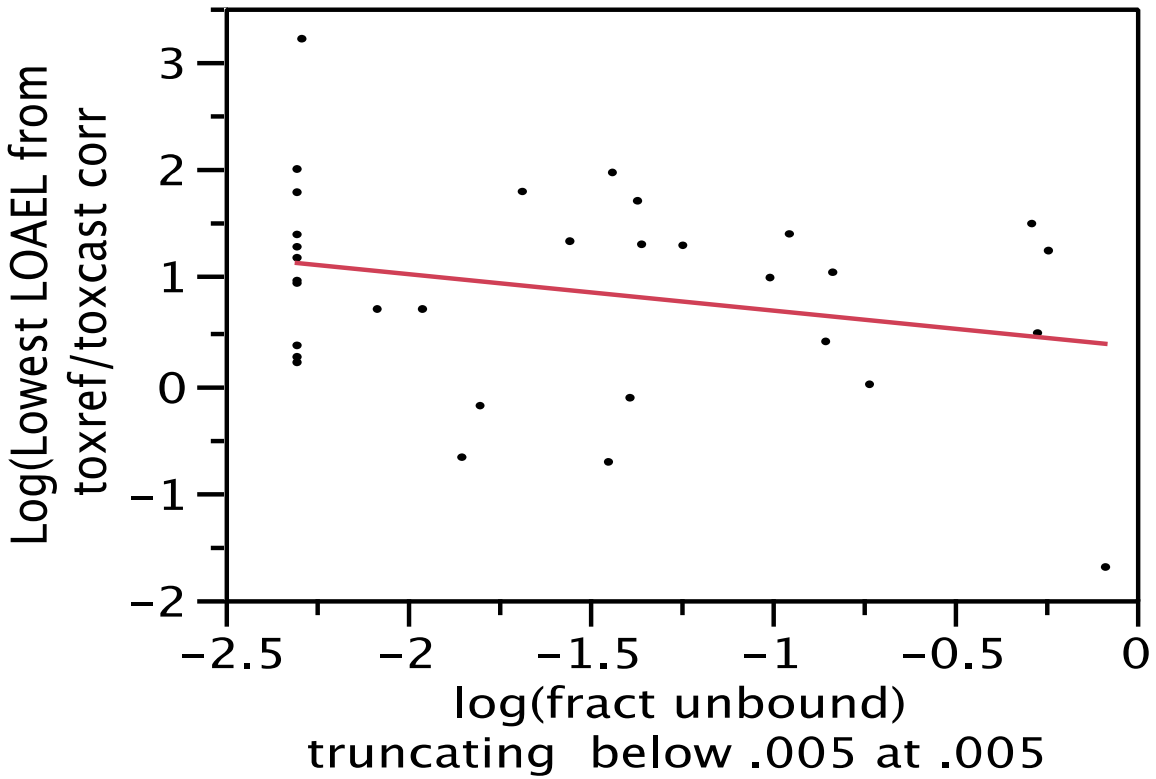
	1st	5th	50th	95th	99th	Geometric Std. Dev. (GSD)	Ratio 95th/5th
"Prior" Lowest LOAEL Fract Geom Mean	0.0016	0.011	1.6	30	58	10.35	2696
"Posterior" LOAEL Residual Fract Geom Mean	0.0012	0.012	1.5	29	74	10.20	2342

Bottom Line: About 1% of the “Prior” Log Variance in LOAELs is “Explained” by the Relationship with Log AC50s

Do In Vitro Predictors of In Vivo Pharmacokinetics Help?

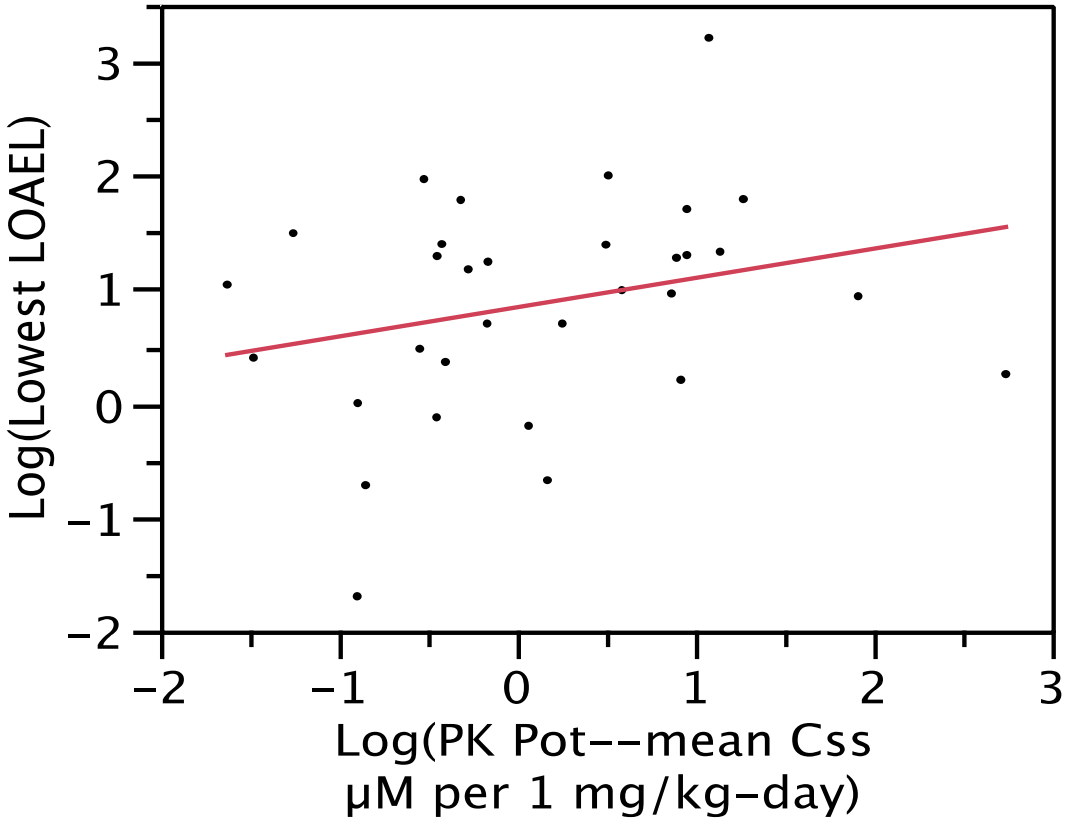
- Fraction Unbound
- In Vitro Hepatocyte Metabolism Rate
- Overall Expected Steady State Unbound Concentration Per 1 mg/kg-day External Dose

LOAEL vs Fraction Unbound



Relationship is in the expected direction—more unbound yields lower LOAEL, but only about 4% of the lognormal variance in LOAEL is explained.

LOAEL vs Expected Steady State Internal Concentration (Based on In Vitro Metabolism and Renal Excretion)



Slope is in the opposite direction to that expected (higher LOAELs for greater Expected steady state concentrations per unit external dose)

Exploring Reasons for the Poor Prediction of LOAELs from AC50s

- Survey of Most Extreme Off-Diagonal Cases With
- Breakdown by Chemical Structure Categories (future work)

9 Chemicals with the Greatest Underpredictions of Toxic Potency from the Lowest In Vitro AC50s

Chemical	Residual [Obs - Pred Log(LOAEL)]	Use/Mode of Action
Cyfluthrin	-3.92	Synthetic pyrethroid derivative that is used as an insecticide
Cyanazine	-3.41	S-triazine herbicide classified as a Restricted Use Pesticide (RUP) because of its teratogenicity
Dicrotophos	-2.80	Organophosphate acetylcholinesterase inhibitor used as an insecticide
Fenthion	-2.53	Organothiophosphate insecticide, avicide, and acaricide
Disulfoton	-2.47	Organophosphate insecticide and acaricide
Mevinphos	-2.41	Organophosphate insecticide
Dichlorvos	-2.31	Organophosphate insecticide
Diazinon	-2.25	Organophosphate insecticide
Fipronil	-2.20	Broad spectrum slow acting insecticide that disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels

Conclusion #1

Prediction of LOAELs would be improved by including assays related to intercellular signaling—particularly inhibition of cholinergic and other neural signaling.

9 Chemicals with the Greatest Overpredictions of Toxic Potency from the Lowest In Vitro AC50s

Chemical	Residual [Obs - Pred Log(LOAEL)]	Use/Mode of Action	Toxcast Assay
Fenhexamid	1.59	Imidazolinone herbicide--Inhibits acetolactate synthase, which is essential in the production of specific amino acids	PXRE_CIS
Imazapic	1.59	Hydrazide plant growth inhibitor with suspected mutagenic/carcinogenic activity	PXRE_CIS
Maleic hydrazide	1.60	Broad spectrum fungicide	PXRE_CIS
Fluoxastrobin	1.65	Imidazolinone herbicide—Inhibits the synthesis of specific amino acids (valine, leucine & isoleucine)	PXRE_CIS
Imazaquin	1.68	Broadleaf herbicide	PXRE_CIS
Flumetsulam	1.69	Insecticide that functions by accelerating the moulting process—insect hormone analog	PXRE_CIS
Methoxyfenozide	1.84	Anti-worm medicine--inhibits oxidative phosphorylation in the mitochondria of cestodes	PXRE_CIS
Niclosamide-olamine	1.98	Antibiotic that binds to the 30S subunit of microbial ribosomes, inhibing protein synthesis	NRF2_ARE_CIS
Oxytetracycline	2.35	Benzamide fungicide that acts by inhibiting mitosis	PPARg_TRANS

Conclusion #2

Predictions of general toxicity (LOAELs) from toxcast potencies Would be improved by eliminating or downweighting results from “PXRE_CIS“ (a specific transcription factor) and possibly a few other specific assays.