Validation of the GastroPlus™ Software Tool and Applications

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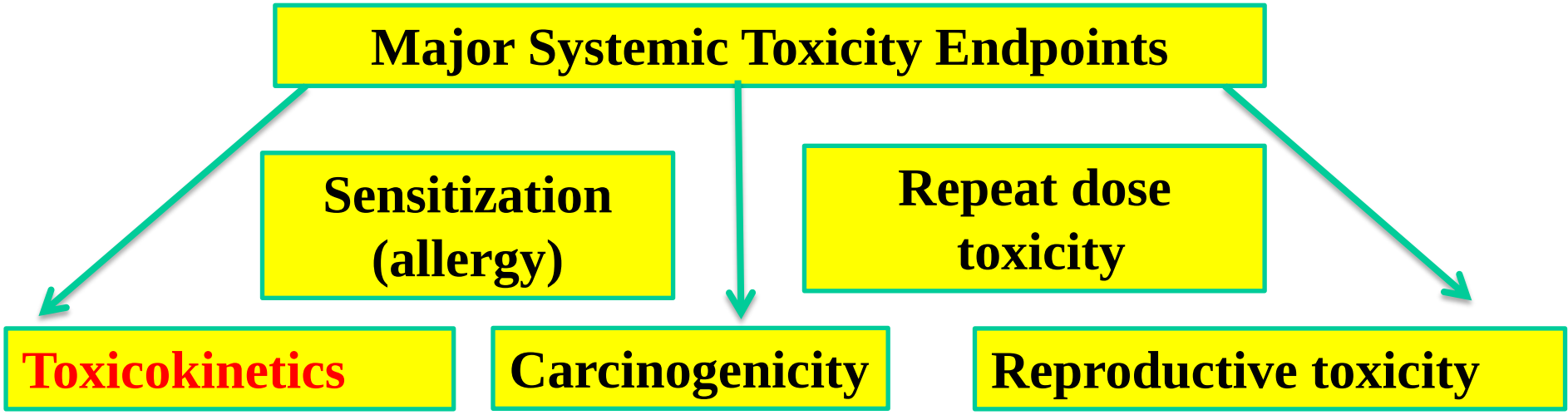
Simulations Plus, Inc.

Outline

- Introduction
- Validation Methods
- Validation Results
- Applications
- Conclusions

Introduction



Major five areas of systemic toxicity testing

Need for faster, cheaper, more predictive, and animal-free methods

Toxicokinetics in Product Development Process

Toxicokinetic activities

In silico Toxicokinetic modeling

Probe AME *in vivo* study (4 species)

In vitro Comparative metabolism study (EU)

ADME study (OECD 417)

In vivo Toxicokinetics Endpoint

Preliminary PBPK model (interspecies)

High-end PBPK models (interspecies & multiple routes)

In silico High throughput PBPK models (IVIVE)

In Silico Predictive Toxicokinetics

- **The Dow Toxicokinetics group conducts *in silico* Pharmacokinetic/Metabolism (ADME) assessments for a variety of product stewardship and regulatory needs**
  - *De novo* prediction of absorption (oral, inhalation, dermal)
  - Systemic exposures (blood levels)
  - Tissue distribution (bioaccumulation)

- **Primary tools are:**
  - ACD/Percepta (ACD/Labs) (Human Oral only)
  - Finite dose dermal penetration calculator (US CDC)
  - Dermwin (US EPA EpiSuite) (Human dermal only)
  - GastroPlus™ Software Suite (Simulations Plus)

HTS Toxicokinetic Model Requirements

Modeling software criteria:

- Support for multiple exposure routes and regimens
  - Oral, Inhalation, Dermal (critical for relevant Risk Assessments)
  - Acute, steady-state
- Incorporates critical QSARs for:
  - Absorption rates and amounts
  - Metabolic clearance
  - Plasma protein binding
  - Tissue distribution
- Based on Compartmental PK or PBPK designs
- Provides model predictions of parent compound and metabolite(s)
- Supports various species and lifestages
- Minimal to no coding required
  - Best option for regulatory buy-in
- Batch modeling feature

Selected: GastroPlus™ from Simulations Plus

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Evaluation Methods

- **The accuracy of key physical-chemical properties of a variety of chemical classes used within GastroPlus for prediction of pharmacokinetics was evaluated against experimental data**
  - -pKa, LogP, Henry's Law Constant (HLC)
  - -GastroPlus predictions compared to other well-validated QSAR tools- Pipeline Pilot™, EPA EpiSuite

- **The accuracy of toxicokinetic parameters predictions from GastroPlus was evaluated for a variety of chemical classes with measured data from the oral, dermal and inhalation routes of exposure, either in animal species or human volunteers**

- **The correlation of predicted toxicokinetic values vs. literature data from oral, inhalation or dermal exposures was then determined:**
  - -Fraction absorbed (Fa%), Cmax, and AUC

- **Applications of GastroPlus for toxicology study design and high-throughput Exposure Assessments**

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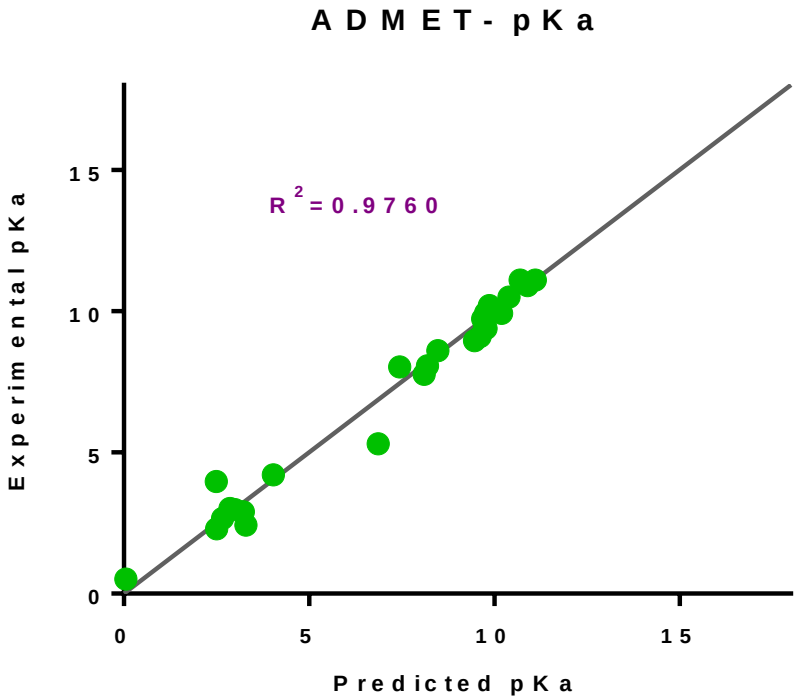
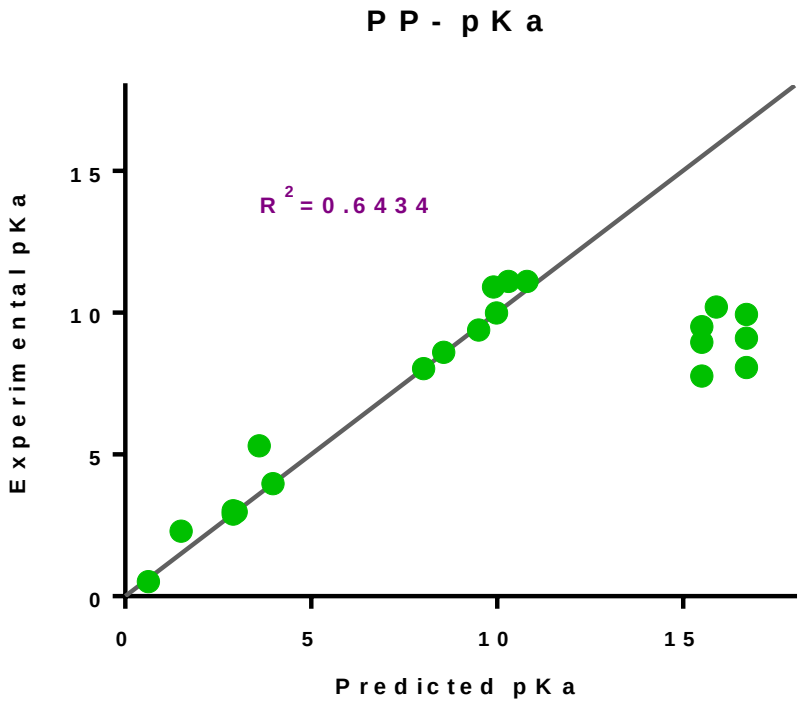
PhysChem Evaluation Results

Experimental vs. Predicted pKa Values



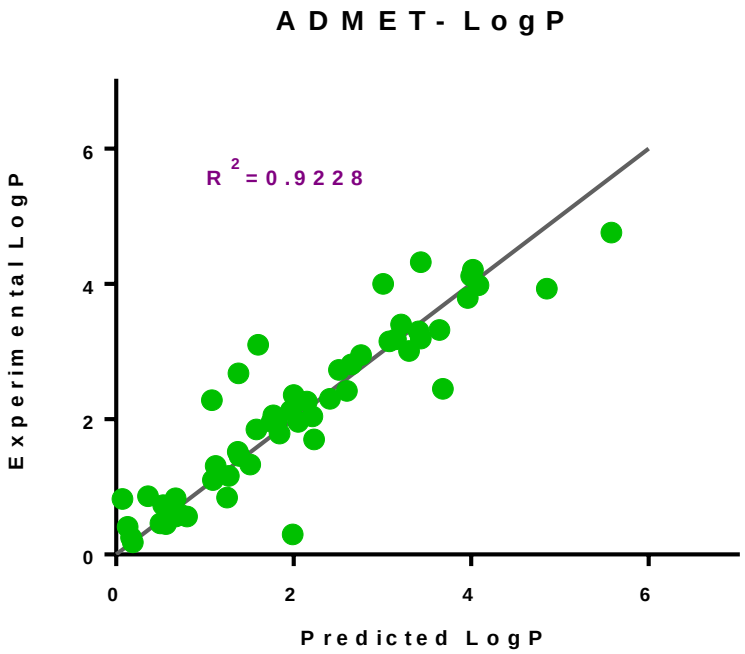


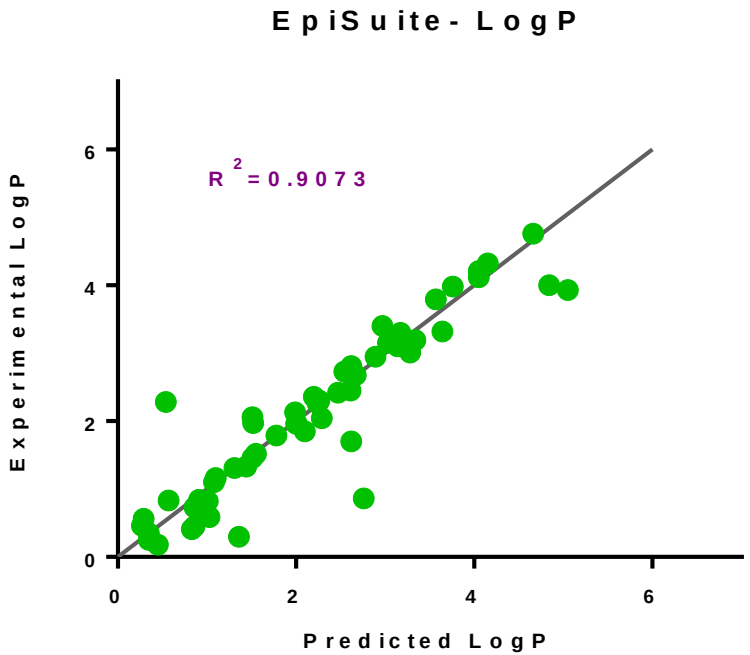
from ADMET Predictor model of GastroPlus™ (ADMET) or Pipeline Pilot™ (PP)

The predicted pKa values from ADMET correlated well with the literature data and were better than those predicted by PP.

PhysChem Evaluation Results

Experimental vs. Predicted LogP Values



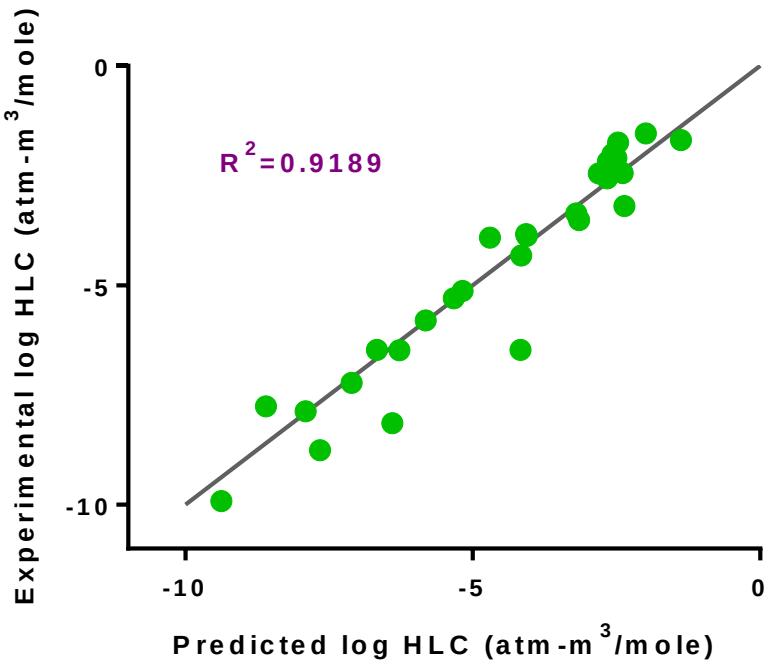


from ADMET Predictor model of GastroPlus™ (ADMET) or US EPA EpiSuite

The predicted LogP values from ADMET correlated well with the literature data and were comparable to those predicted by EpiSuite.

PhysChem Evaluation Results

Experimental vs. Predicted HLC Values *via* ADMET



The predicted values correlated well with the literature data.

Major PK Parameter Evaluation Results

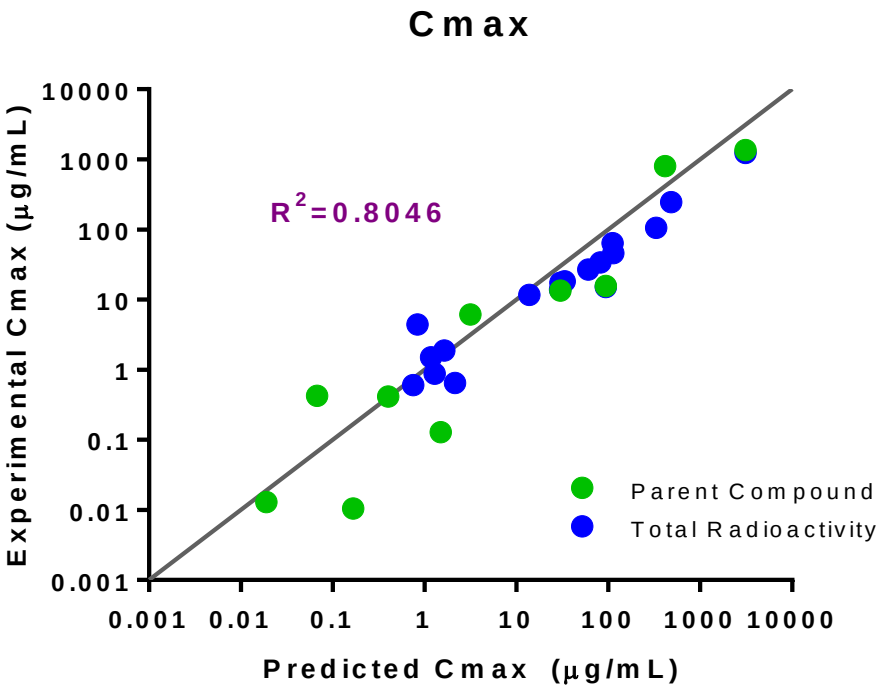
Cl_{int}		Fraction Unbound in Plasma	
Fold difference from empirical data	Percent of the total compounds *	Percent (%) difference from empirical data	Percent of the total compounds **
1 to 3	38%	1 to 10	61%
3 to 10	29%	10 to 30	26%
10 to 100	26%	> 30	13%
> 100	7%		
* n=463		** n=441	
Empirical data for Clint and Fup via personal communication (J. Wambaugh, 2015)			

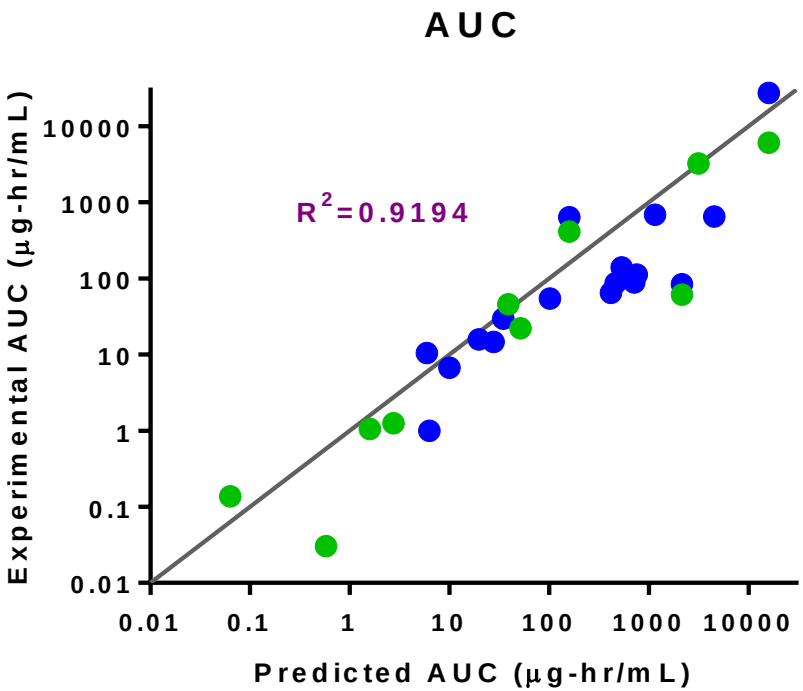
Metabolic clearance and Fup predictions by GastroPlus are quite acceptable:

- ***67% of predicted Clint values within 10x of empirical data***
- ***87% of predicted Fup values within 30% of empirical data***

Pharmacokinetic Data Evaluation

Oral Exposures





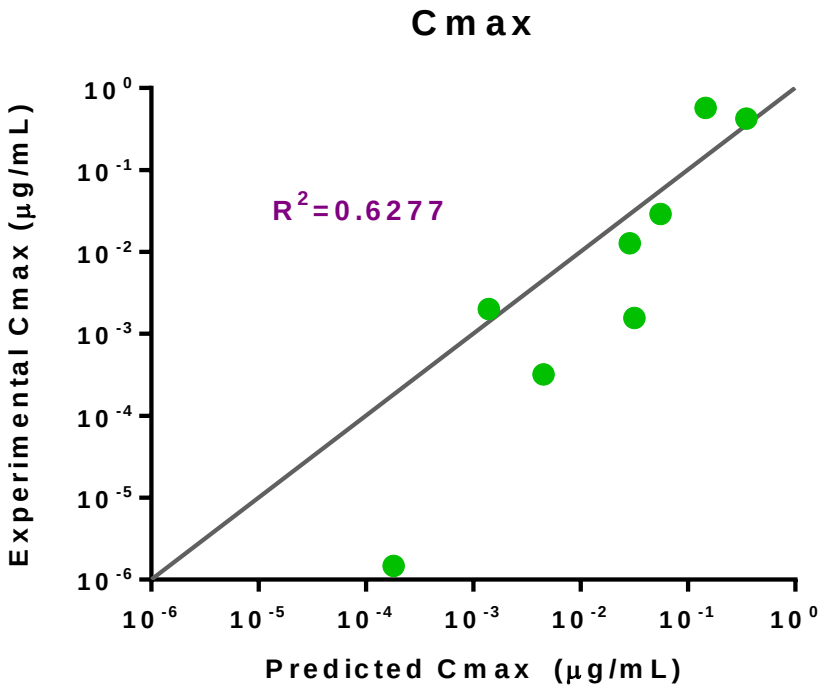
The predicted pharmacokinetic values from GastroPlus correlated well with the literature data

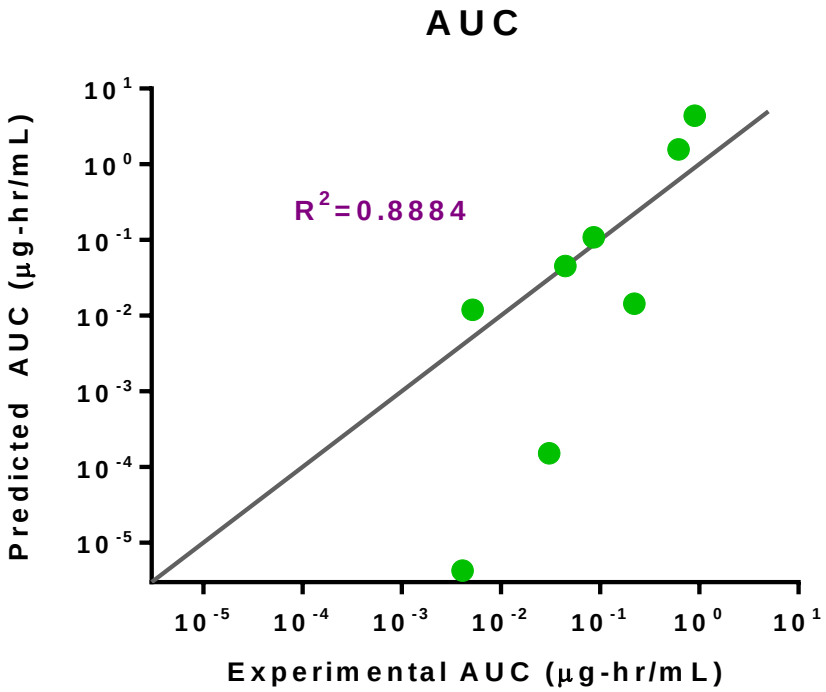
Cmax: 69% within 3-fold, and 88% within 10-fold of experimental data

AUC: 54% within 3-fold, and 85% within 10-fold of experimental data

Pharmacokinetic Data Predictions

Inhalation Exposures

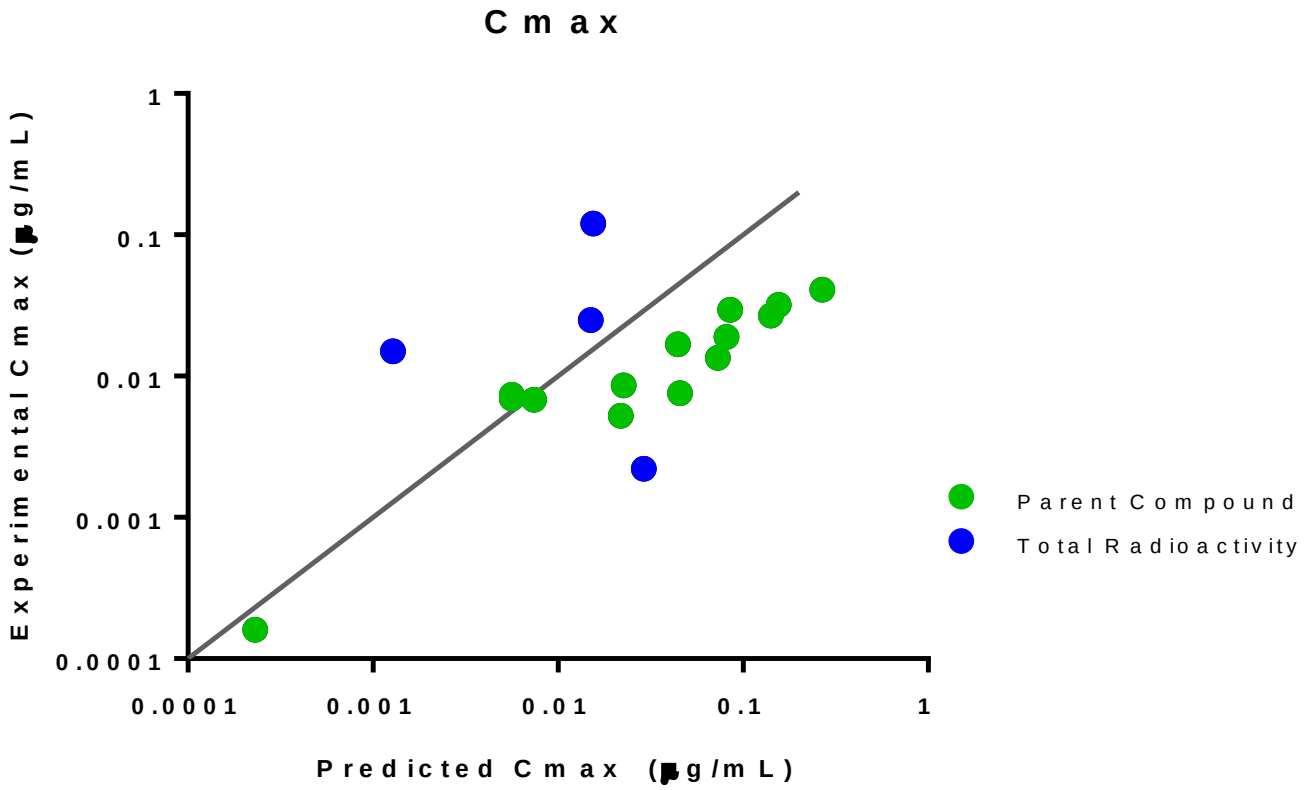




Cmax: 50% within 3-fold, and 63% within 10-fold of experimental data
AUC: 50% within 3-fold, and 63% within 10-fold of experimental data
- generally over-predicted (conservative)

Pharmacokinetic Data Predictions

Dermal Exposures



Cmax: 44% within 3-fold, and 89% within 10-fold of experimental data
- generally over-predicted (conservative)

Accuracy of Steady-State Systemic Exposure Evaluation

Comparison of GastroPlus Prediction Results with Published IVIVE Modeling Results (oral route)				
Chemical Name	Reference PK or PBPK derived (Css µM) *	Restrictive hepatic clearance (Css µM) *	GastroPlus Predicted (Css µM)	GastroPlus Predicted with Empirical Clint* and Fup* (Css µM)
2.4-D	9.05-90.05	43.27	64.56	57.95
Cacodylic acid	1.8	3.06	9.53	7.37
Carbaryl	0.03	0.07	1.13	0.47
Fenitrothion	0.03	17.92	0.84	15.7
Lindane	0.46	13.21	7.96	6.68
Parathion	0.17	24.64	1.66	17.28
Perfluorooctane sulfonic acid	19,990	153.23	143.68	155.42
Perfluorooctanoic acid	20,120	53.16	89.57	61.34
Picloram	0.27	57.63	39.27	67.96
Thiabendazole	0.45	13.76	11.76	15.8
Triclosan	2 to 10	1.56	7.67	1.36
Bisphenol A	<0.13	0.35	2.60	2.49
* *Data from Wetmore, et al. 2012 (Toxicol Sci 125(1): 157-174)*				

Steady state blood level predictions from GastroPlus consistent with those obtained with SimCYP and overall conservative vs. Reference data

Predicted Css values generally improve with inclusion of measured Clint and Fup

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Applications of GastroPlus™

Toxicology Study Design

- Dose level selection for animal toxicity studies based on IVIVE (*In Vitro-In Vivo* Extrapolation) comparison to *in vitro* endpoints
- Inhalation study waiver
- Dose route selection for chronic toxicity study

Exposure Assessment

- HEAT (High-Throughput Exposure Assessment Tool)

Applications of GastroPlus™

- **Dose level selection for animal toxicity studies based on IVIVE comparison to *in vitro* endpoints**

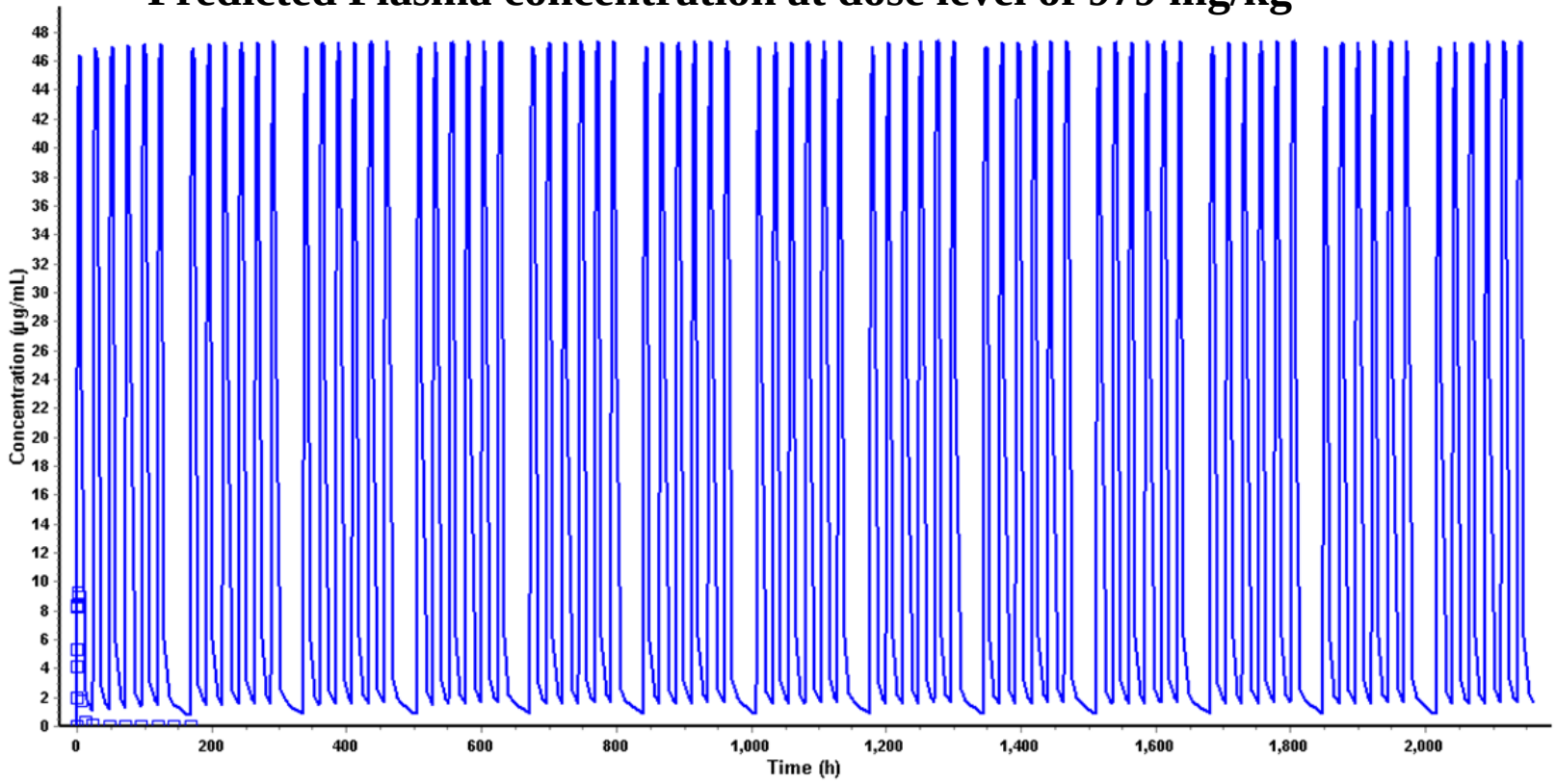
In vitro mouse hepatocyte dose (µM) for Compound A	Mouse Dose (mg/kg/day Compound A)	Predicted Cmax (µM)		
		4 Days	7 Days	14 Days
0	0	0	0	0
1	3.00	1.02	1.03	1.04
3	10.0	3.50	3.52	3.54
10	30.0	11.3	11.3	11.4

The predicted *in vivo* dose levels (3, 10, and 30 mg/kg/day) that reach the corresponding *in vitro* concentrations.

Applications of GastroPlus™

Inhalation study (90-Day inhalation) waiver for Compound B

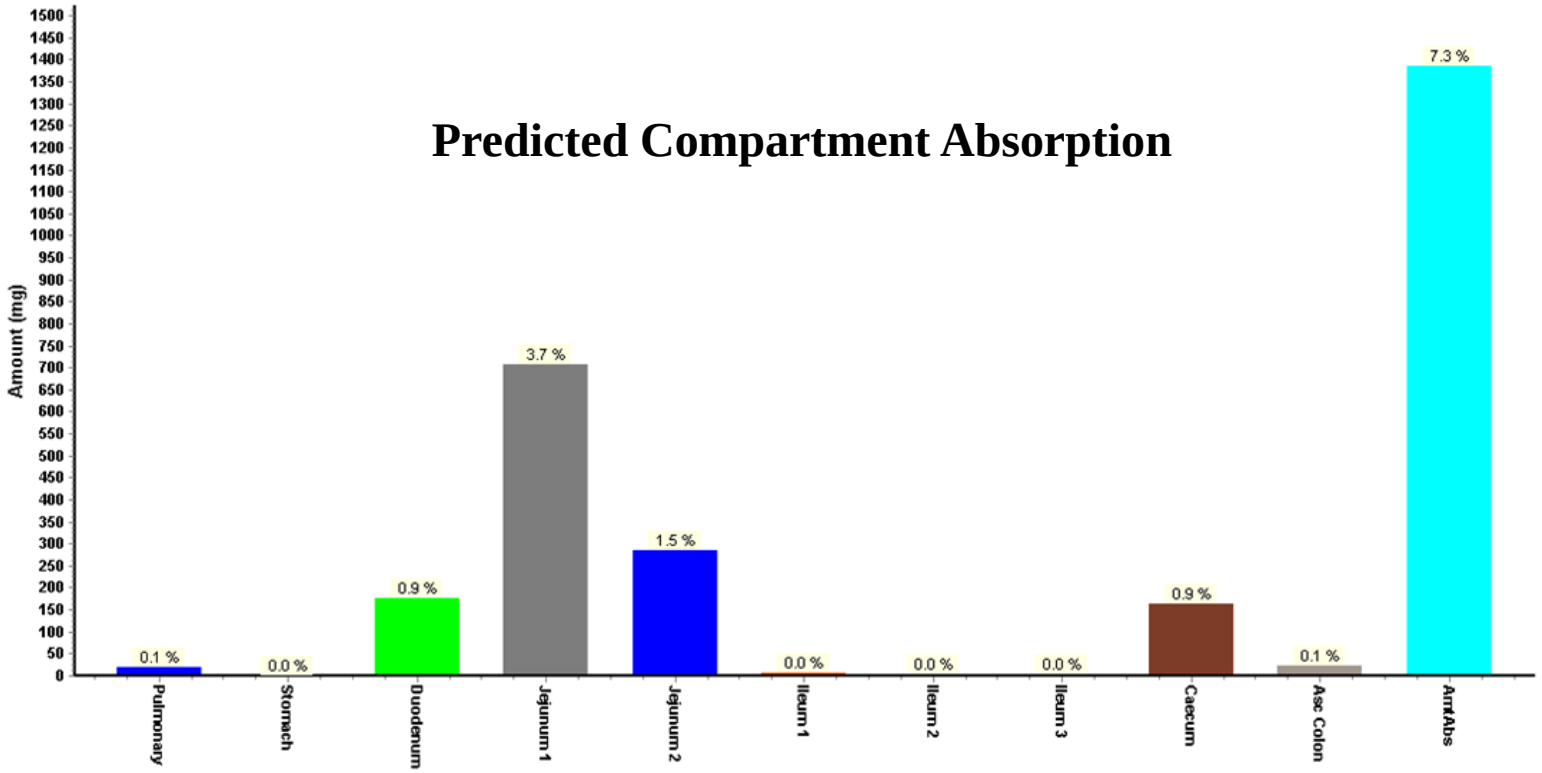
Predicted Plasma concentration at dose level of 979 mg/kg



The predicted plasma concentration that reaches the steady state after one week exposure and the bioaccumulation factor is around 1.

Applications of GastroPlus™

Inhalation study (90-Day inhalation) waiver for Compound B



The total absorption for compound B by the inhalation route is predicted high (73%)

- however, fraction absorbed through the pulmonary tissue is predicted low (0.1%)
- These data support the rationale for waiving the inhalation study

Applications of GastroPlus

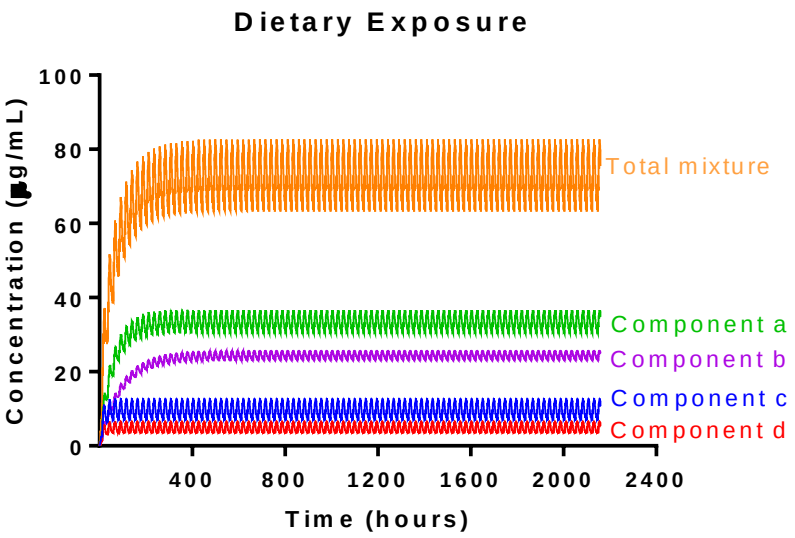
Justification for the selection of administration route for 2-year rat chronic study of Compound C (Total mixture containing four similar components)

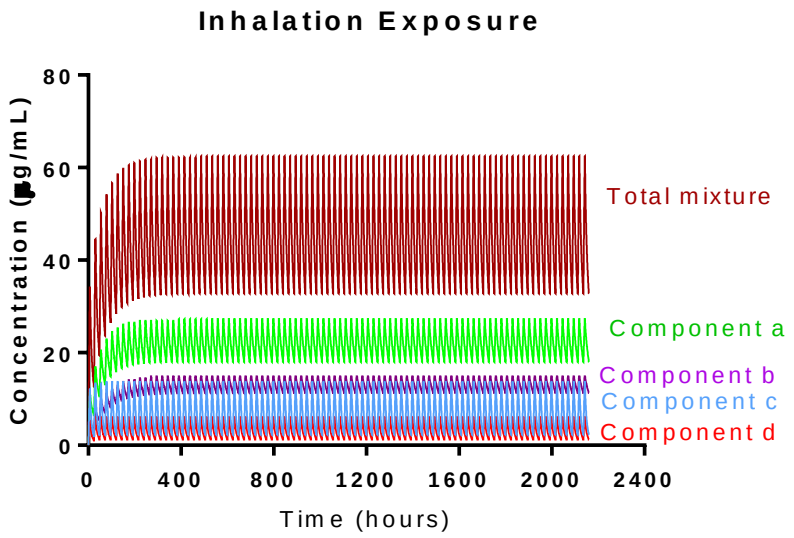
Name	C_{max} in blood (µg/mL)	C_{max} in reproductive tissues (µg/mL)	AUC0-t in blood (µg-h/mL)
	90-Day dietary exposure		
Component a	36.9	222	70470
Component b	26.0	154	50400
Component c	13.0	61.7	20670
Component d	6.98	32.2	10430
Total mixture	**82.9**	**470**	**151970**
	90-Day inhalation exposure		
Component a	28.3	164	46790
Component b	15.2	89.9	27320
Component c	13.7	64.8	14320
Component d	6.34	29.5	6985
Total mixture	**62.5**	**348**	**95415**

MKD = 300 mg /kg

Applications of GastroPlus

Justification for the selection of administration route for 2-year chronic study of compound C





The predicted total steady Cmax from dietary was much higher than that from inhalation.

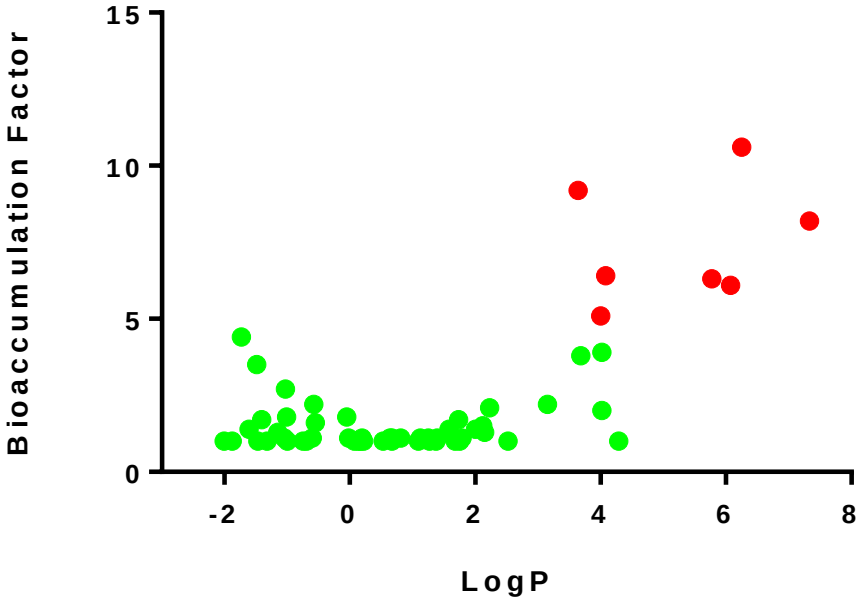
Applications in HEAT

Methods for High Throughput Exposure assessment Tool (HEAT)

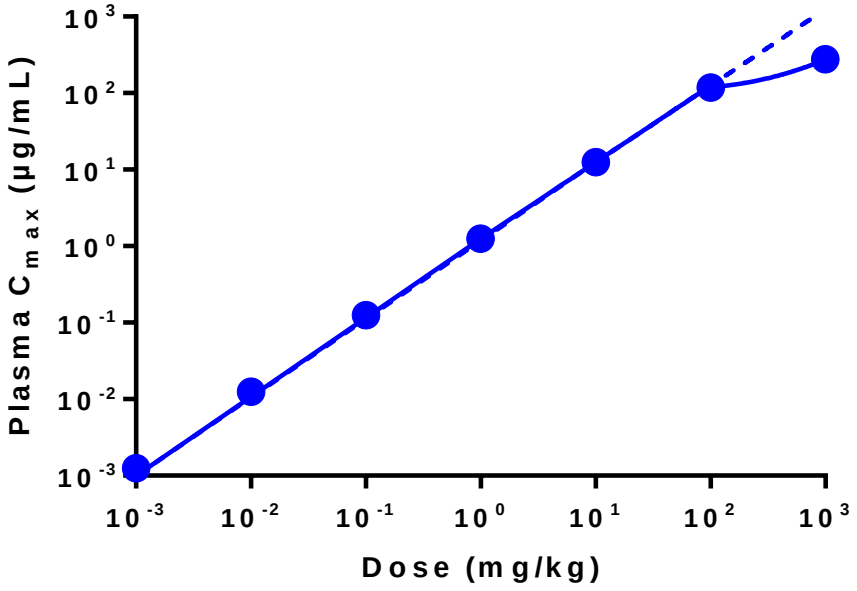
- **Determine external exposures for Dow products**
  - Using formulation data and validated Occupational or Consumer exposure models
- **Pre-define predictions of blood levels across a range of external exposures (0.001-1000 mg/kg)**
  - Oral, Inhalation and Dermal routes
  - Select most conservative formulation types (highest C_{max} values) and exposure conditions for each route

Applications in HEAT

Trends in Systemic Exposure Predictions with GastroPlus



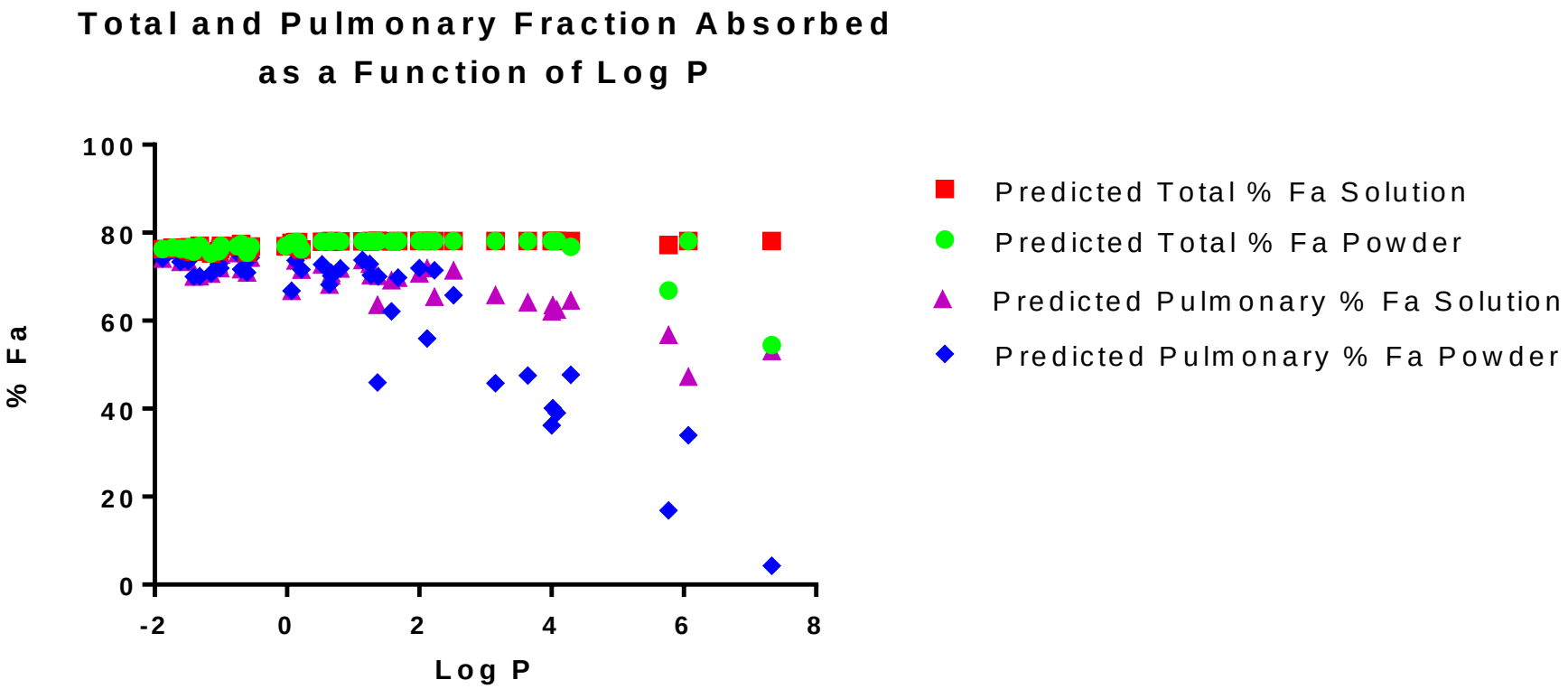
Bioaccumulation after 28 days oral exposure



Saturation of oral absorption

Applications in HEAT

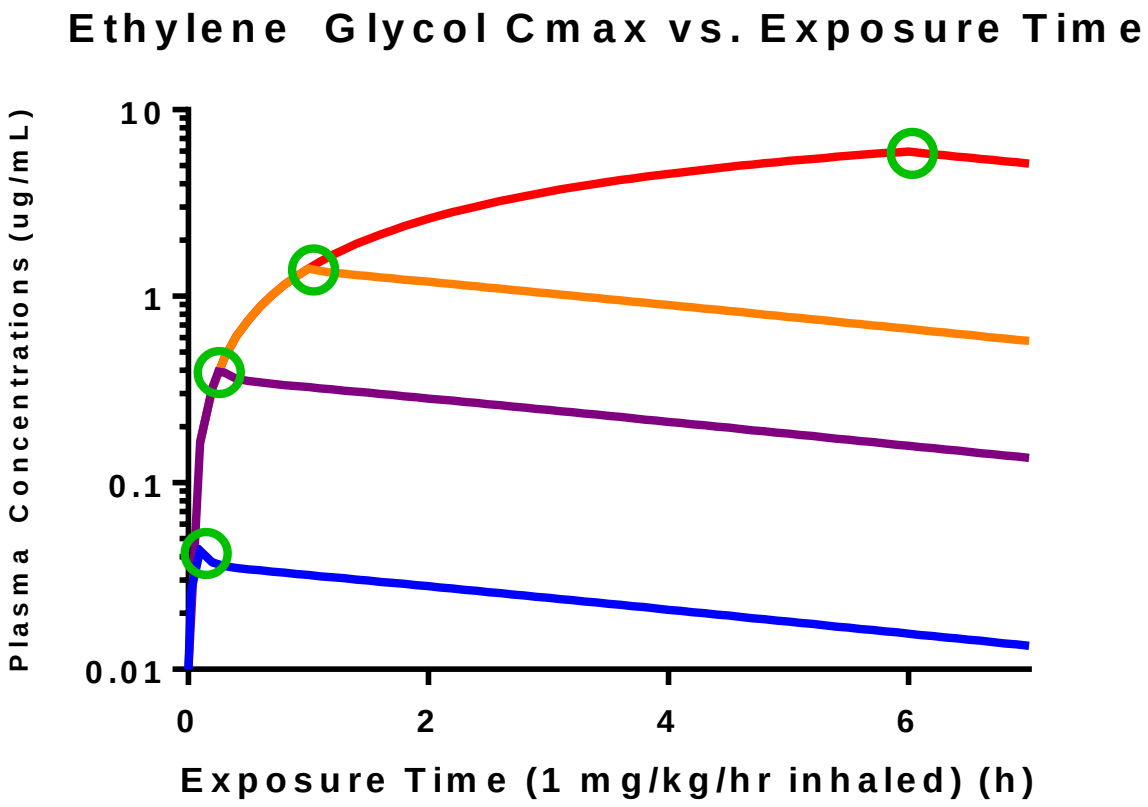
Trends in Systemic Exposure Predictions



Trends towards lower uptake of inhaled chemicals through pulmonary tissue
- trend enhanced for solid formulations vs. solutions

Applications in HEAT

Selection of Optimal Exposure time for *de novo* Inhalation modeling



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Conclusions

- **The prediction for Physico-chemistry properties was assessed and the experimental data correlated well with the predicted data**
- **GastroPlus™ was assessed for systemic exposure prediction *via* oral, dermal and inhalation routes**
- **Based on the validation results, GastroPlus™ is deemed acceptable for IVIVE evaluation by the oral, inhalation, and dermal routes.**
- **GastroPlus™ should be used for high throughput toxicokinetic predictions**
- **GastroPlus™ will allow for optimum implementation of animal alternatives in novel high throughput safety assessment programs (Tox21)**