Framework for Establishing an Internal Threshold of Toxicological Concern

Presentation for the In Vitro to In Vivo Extrapolation for High Throughput Prioritization and Decision Making Webinar Series

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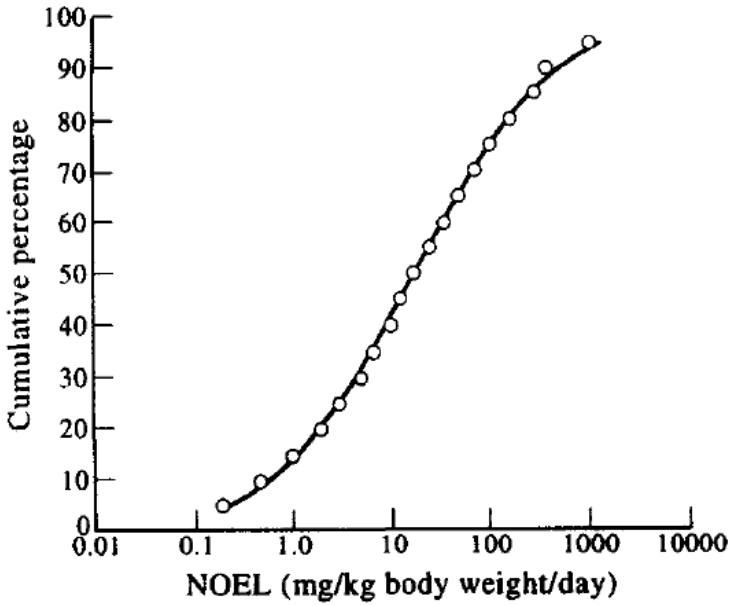
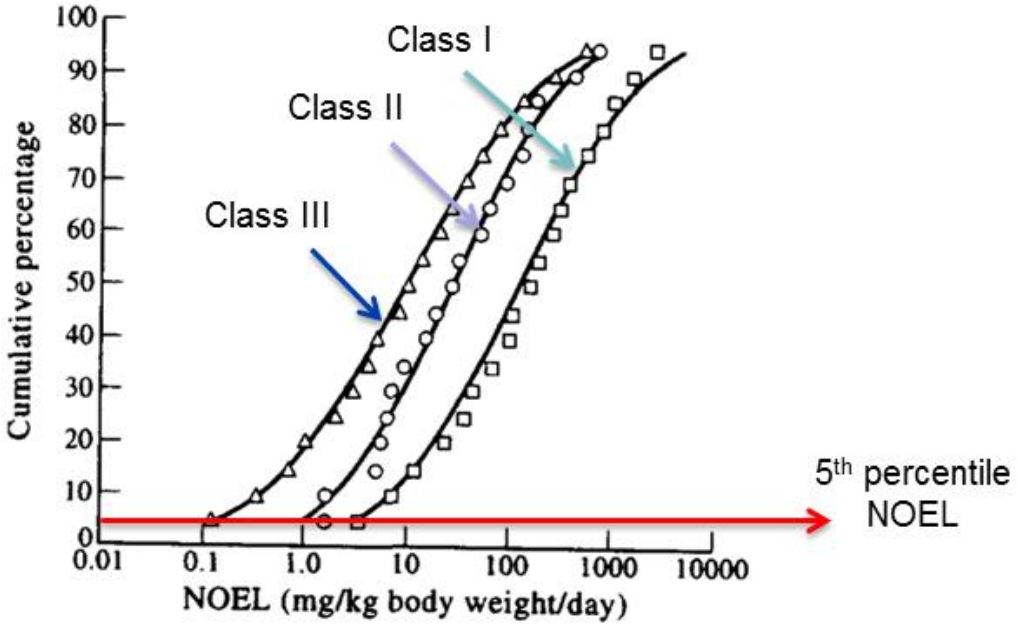
Threshold of Toxicological Concern (TTC)

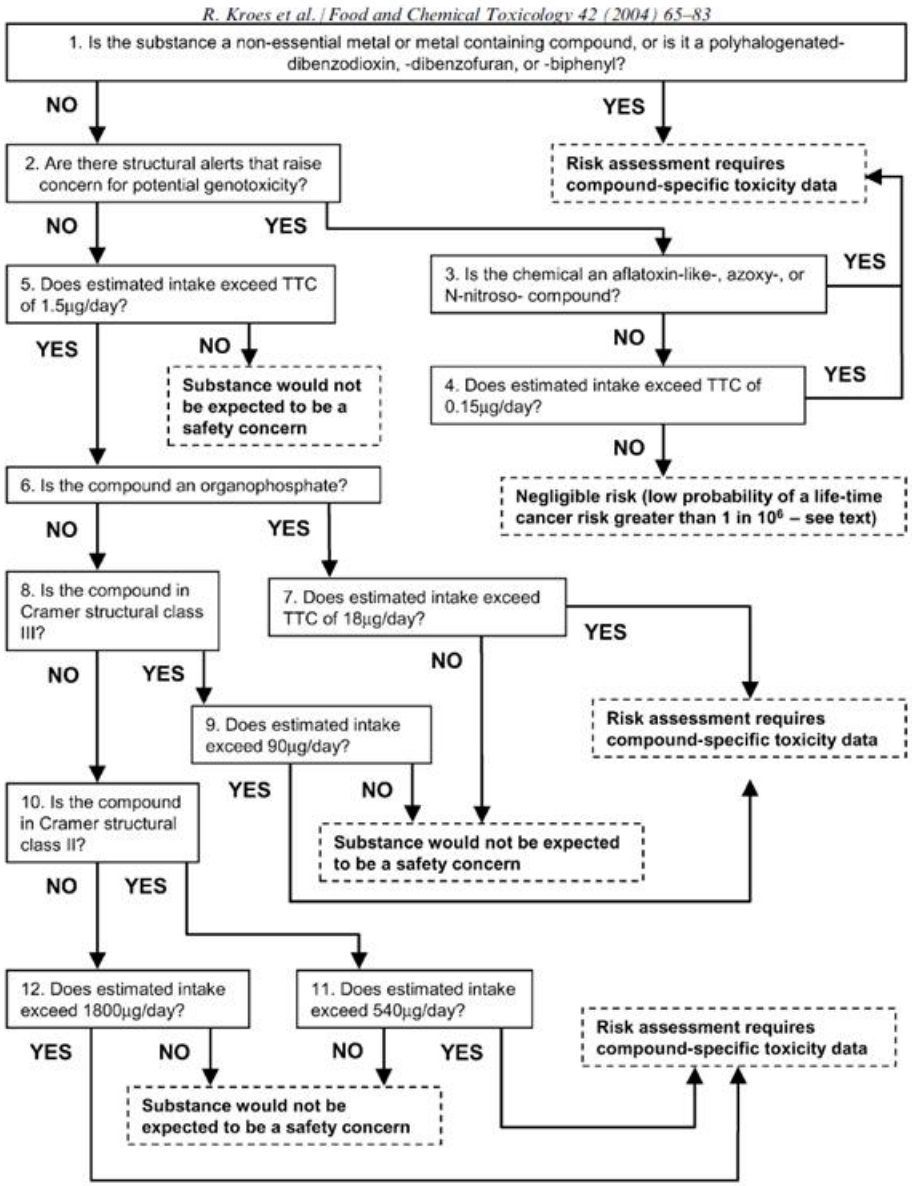
- TTC is a risk assessment tool that establishes acceptable low level exposure values for chemicals with limited toxicological data
- TTC databases are based on systemic effects after oral exposures
- Non-cancer TTC databases consist of distributions of chemical specific oral No Observed Adverse Effect Levels (NOAELs)
- Chemicals in existing TTC databases have been categorized using Cramer classification criteria as an indicator of systemic toxicity
- TTC threshold limits established by identifying a low percentile NOAEL value (e.g. 5^{th} percentile) from the database and applying appropriate uncertainty factors

Cumulative Distribution of Oral NOAELs





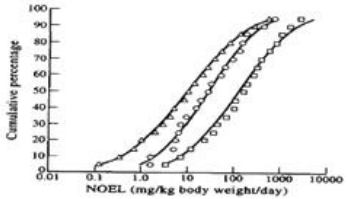
Application of TTC in a Risk Assessment



Internal TTC – Why it's Relevant

- Multiple situations in risk assessment where it is more appropriate to address internal exposure rather than external dose
  - Metabolism based read-across assessments
    - Tox assessment is based on metabolite(s) for a parent compound that lacks direct tox data (see example later in presentation)
  - Exposure-based waiving of toxicity data
    - Establishing a dermal penetration threshold below which it would not be necessary to have tox data
  - Low level chemical exposure from more than one exposure route
- Partosch (2015) converted external NOAELs to "internal" NOAELs by multiplying by in silico oral bioavailability estimates for each chemical
  - Good initial first steps
  - Still results in an external dose metric
- The need remains for development of an internal TTC utilizing internal exposure metric (e.g. concentration in blood, area under the curve)

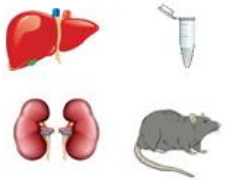
Internal TTC Proposed Approach



TTC data set:
NOAELs in mg/kg/day

CHEMICAL SPECIFIC ADME DATA

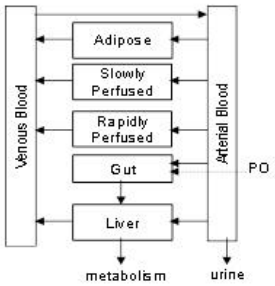
Existing Literature Data



Plasma protein binding
Hepatic clearance
Renal clearance
Oral absorption
Partition coefficients

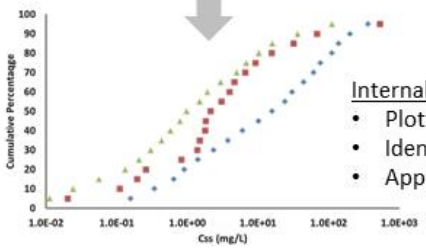
In Silico Estimates

PK MODELING



Steady state blood concentration (Css) in animal

INTERNAL TTC IDENTIFICATION



Internal TTC values identified by:

- Plotting cumulative distribution of Css for Cramer classes
- Identify 5th percentile Css values
- Apply appropriate uncertainty factors to 5th percentile Css values

Application of Internal TTC to a Risk Assessment

Human exposure assessment

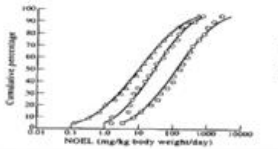
- Risk assessment based on metabolites (e.g. rapid & complete metabolism; SOI produces one metabolite not covered by any analog)

OR

- Exposure based waiving of toxicity data

Utilize appropriate modeling method to estimate blood concentration following consumer product exposure scenario

Compare human blood concentration to internal TTC value to determine MoS



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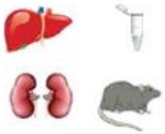

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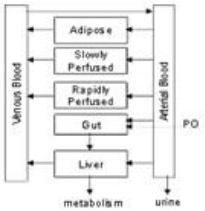
In Silico Estimates

PK MODELING



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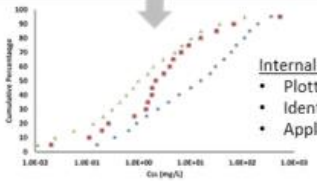

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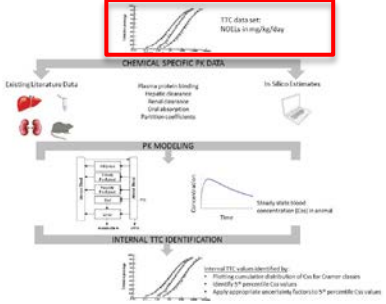
Approach to Develop an Internal TTC

- Base modeling on as much compound specific data as possible
- Use *in silico* tools to estimate parameters not found in the literature
- Recommend experimental work only for key chemicals and key parameters
- Focus verification on chemicals that drive the internal TTC threshold



TTC Databases

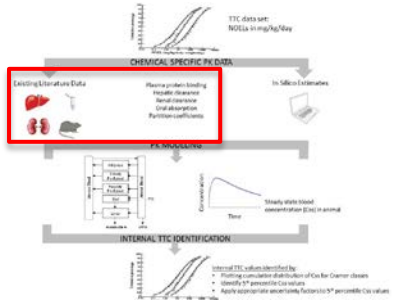
- Munro et al. (1996)
  - 613 chemicals
  - Species: rat, mouse, rabbit, hamster
  - Routes: gavage, diet, drinking water
  - Durations: subchronic & chronic
  - NOELs identified (mg/kg/day)

- COSMOS project
  - 553 chemicals
  - Species: rat, mouse, dog, primate, rabbit
  - Route: oral
  - Durations: studies ≥ 28 days
  - Chronic NOAELs preferred (mg/kg/day)

SEURAT-1

COSMOS
Integrated *In Silico* Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety

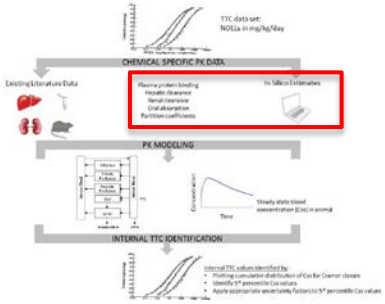
http://www.cosmostox.eu/home/welcome/



Literature Search

- Manual PubMed search “pharmacokinetics [chemical name]”
  - Manual review of title and abstracts for papers of interest
  - ~600 papers collected
  - ~60% of TTC chemicals had a paper available
  - Available papers distributed approximately equally across Cramer Classes
  - Manual review of papers needed to extract PK parameters (in process)
- Opportunity for more robust search using analytics approach
- Literature search will help
  - Identify existing ADME data
  - Prioritize what chemicals need more data for modeling
  - Identify in vivo data to support verification of models

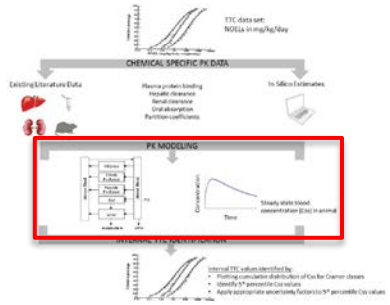
In Silico Prediction of Parameters



- Various options for predicting ADME parameters
  - Swiss Institute of Bioinformatics provides summary of software, web services & databases
    http://www.click2drug.org/index.html
  - Multiple published algorithms for different ADME input parameters

- Robust *in silico* approaches for predicting metabolism are not currently available
  - QSARs developed to date have limited applicability domain

PK Modeling Approaches



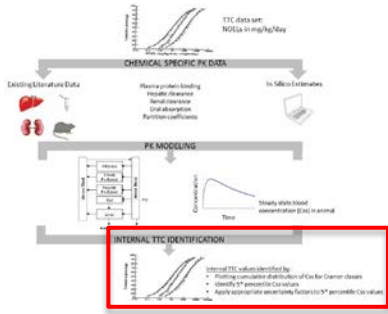
- Multiple pharmacokinetic approaches available as options to use in framework
  - C_{ss} equation

$$C_{ss} = \frac{k_0 \times F}{(GFR \times F_{ub}) + \left[\frac{(Q_l \times F_{ub} \times Cl_{int})}{(Q_l + F_{ub} \times Cl_{int})}\right]}$$

Wilkinson and Shand (1975)

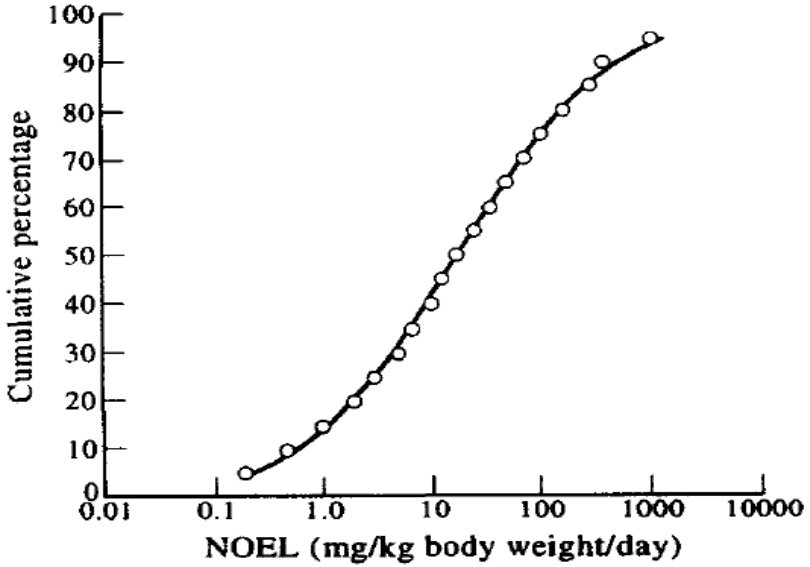
  - Commercially available generic PBPK models
    - GastroPlus ™ (Simulations plus)
    - ADME WorkBench ™ (Aegis Technologies)
    - SimCyp ™
  - Freely available generic PBPK models

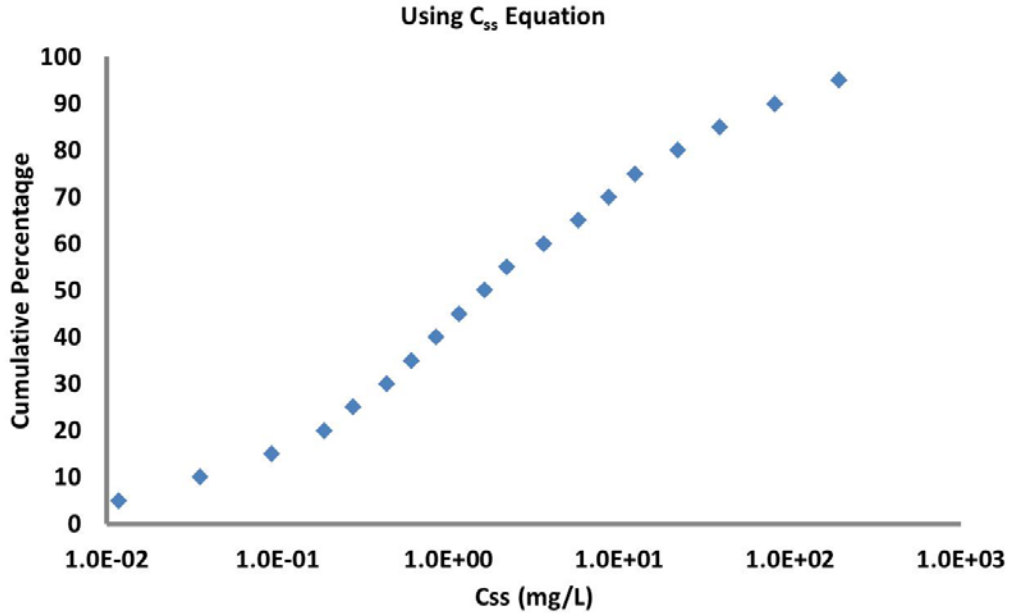
Initial Model Evaluation



- Identify a PK modeling approach with ability to process large batches of chemicals and generate steady state concentrations in blood
  - Batch mode approach needed to support large size of TTC dataset and the number of anticipated loops through the process
- GastroPlus ™ and C_{ss} equation
- Chemical specific input parameters (e.g. metabolism, protein binding) were all *in silico* estimates derived from ADMET Predictor ™
  - Due to use of all *in silico* input parameters, estimates of C_{ss} are not expected to be quantitatively accurate. Current objective is not to derive accurate estimates of C_{ss}, rather to identify approach to be utilized within an internal TTC framework.
- Dosing scenario was representative of the tox study where the NOAEL was derived (e.g. species, dose, route)

Initial Model Evaluation Results

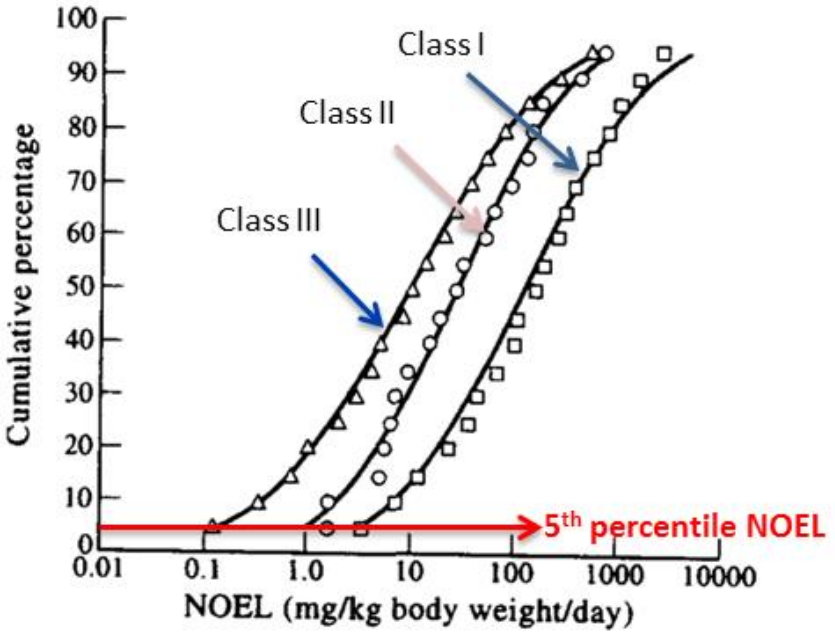


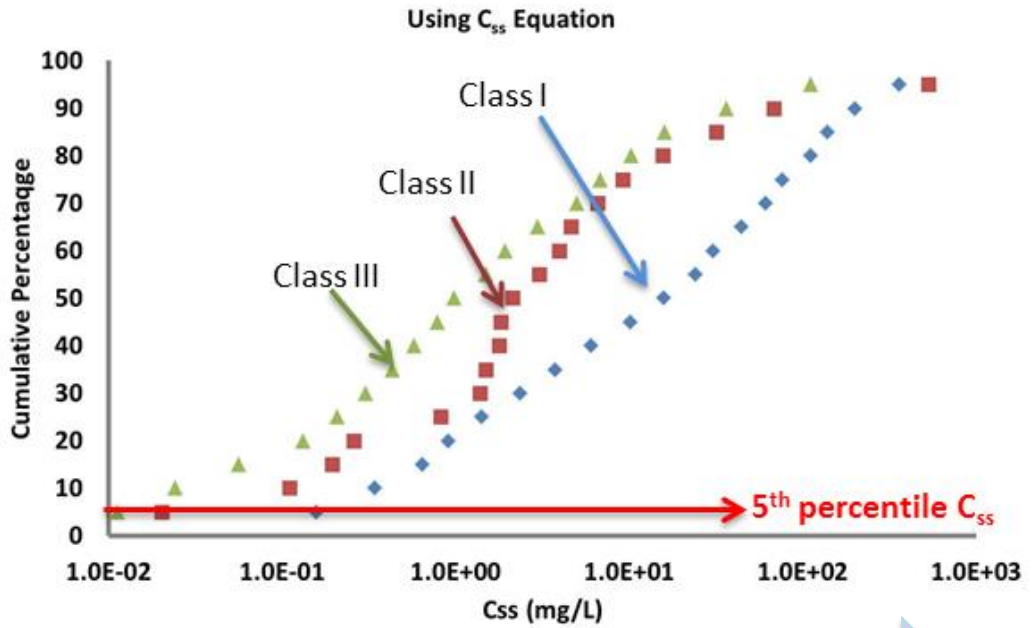


External dose → Kinetic modeling → Internal exposure

Similar results achieved using GastroPlus ™

Initial Model Evaluation Results





External dose → Kinetic modeling → Internal exposure

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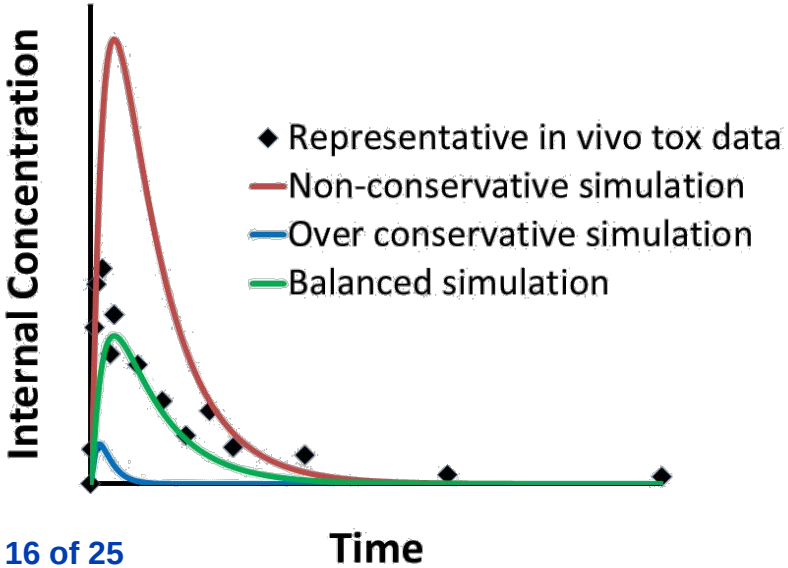
Fit-for-Purpose Approach

Important to understand amount of conservatism in modeling assumptions/approach

(High)	Oral absorption	(Low)
(Low)	Hepatic metabolism	(High)
(Low)	Non hepatic systemic clearance	(High)
	Etc.…	



Ex. Metabolism Based Read-across

Hypothetical assessment for chemical XYZ	
Usage scenario	0.5% chemical XYZ in a facial moisturizer
Exposure	0.1 mg/kg/day [Conc. prod. * Amt. applied * Freq. * (1/Body wt.)] (SCCS 2012)
Tox data	Data to support lack of genetox hazard but no additional tox data or suitable analogs. Predicted metabolite has full tox dataset
Dermal penetration	Anticipated to be high (assumed 100%)
Protein binding	Determined to have relatively low protein binding (20%)
Metabolism	Predicted to be quickly metabolized to metabolite A. This is further confirmed via a metabolism assay (CL_{int} = 80 L/h)
Estimated Css	Estimated internal exposure is 0.007 mg/L[a]
Risk value for QRA	Compare to appropriate internal TTC value
QRA	Internal exposure to XYZ < internal TTC → utilize tox data for metabolite A Internal exposure to XYZ > internal TTC → further evaluation needed; possible need for new tox data

a

$$C_{ss} = \left[\frac{k_0}{(GFR \times F_{ub}) + \left[\frac{(Q_l \times F_{ub} \times Cl_{int})}{(Q_l + F_{ub} \times Cl_{int})} \right]} \right] \times BW$$

K_0 = 0.004 mg/kg/hr (SCCS (2012) H&Ps), 24 hr exposure, BW = 70 kg, F_{ub} = 0.8, CL_{int} = 80 L/h, Q_l = 87 L/h, GFR = 7.5 L/h (Davies 1993)

Published Case Stud

Regulatory Toxicology and Pharmacology 68 (2014) 212–221

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The challenge of using read-across within the EU REACH regulatory framework; how much uncertainty is too much? Dipropylene glycol methyl ether acetate, an exemplary case study

Nicholas Ball [a,*], Michael Bartels [b], Robert Budinsky [b], Joanna Klapacz [b], Sean Hays [c], Christopher Kirman [d], Grace Patlewicz [e]

- Registrants attempted to use metabolism based read-across to support their chemical
  - parent half life in blood ~ 15 minutes
  - PBPK modeling demonstrated that parent AUC was <1% of metabolite AUC following exposure to parent chemical (i.e. predominant systemic exposure is to metabolite)
- Registrants were unable to adequately justify why the low level, short term systemic exposure to the parent would not represent a human safety concern. As such, they had to perform a developmental toxicity study in rodents.
- Availability of an internal TTC may have allowed for comparison of the systemic exposure to an internal exposure threshold.

What Needs to be True for Success

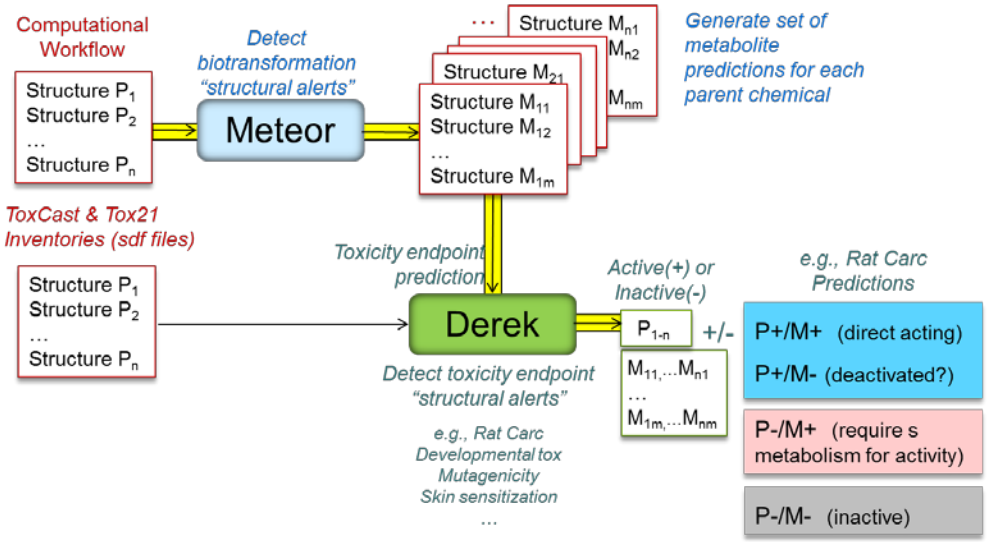
- Clear and transparent documentation
  - Needs to be easily understood by a non-PK expert
  - Well documented so that critical stakeholders can easily understand the strengths and limitations
- Easily reproducible
  - Allows critical stakeholders to have the opportunity to test and become familiar with approach
- Easy access to tools
  - Utilize tools that are easily accessible and available at a reasonable cost to critical stakeholders so that those interested can have a ‘hands-on’ experience
- Publish case studies
  - Case studies that demonstrate the development, progression and utility of the approach may help with its acceptance
- Cross sector collaboration
  - Will increase the diversity in perspectives

Anticipated Challenges

- Predicting if hepatic metabolism is activating or inactivating
  - Will determine if a hepatic metabolism rate is conservative or not
- Other factors that could impact internal concentration
  - Extrahepatic metabolism, renal clearance, transporters
- Regulatory acceptance of PBPK modeling
  - Not all Regulatory agencies have accepted the use of PBPK modeling
- Verification work
  - Limited *in vivo* PK data for comparison to estimates
- Confidence in existing literature data
  - Data from multiple sources over decades will include data that is of poor quality
- Need for additional *in vitro* data
  - Requires time, money, analytical methods

Predicting Impact of Hepatic Metabolism

An example of a preliminary workflow for predicting impact of metabolism



Work by Patra Volarath (former post doc of Ann Richard,US Environmental Protection Agency)

<u>More work is still needed</u> due to limitations of Meteor and Derek

- Many Derek alerts are based on metabolites
- Meteor doesn't necessarily predict the toxicologically important metabolites

Next Steps for Internal TTC Work

- Proposed internal TTC framework presented at Cosmetics Europe workshop in Sept 2015
- Internal TTC to be a part of the Cosmetics Europe Long Range Science Strategy (LRSS) research program 2016-2020
- Cosmetics Europe working group will form in early 2016 to begin executing internal TTC work
  - Thorough literature search for existing ADME data
  - Identify ADME data gaps for TTC chemicals and selectively generate new in vitro data
  - Evaluate different PK modeling approaches
- There is still a need for strategic partners. If interested contact either:
  - Corie Ellison (ellison.ca@pg.com)
  - Harvey Clewell (hclewell@scitovation.com)

Extending Beyond Internal TTC

The experience gained through this work will be applicable to broader issues as well

- Single chemical PBPK model development
- Balancing conservatism in estimates
- Model verification with limited or no chemical specific in vivo PK data
- Group PBPK read across approaches
- Route to route extrapolation
- In vitro to in vivo extrapolation

References

ADME WorkBench - http://www.admewb.com/

Ball N, Bartels M, Budinsky R, Klapacz J, Hays S, Kirman C, Patlewics G (2014) The challenge of using read-across within the EU REACH regulatory framework; how much uncertainty is too much? Dipropylene glycol methyl ether acetate, an exemplary case study 68, 212–221.

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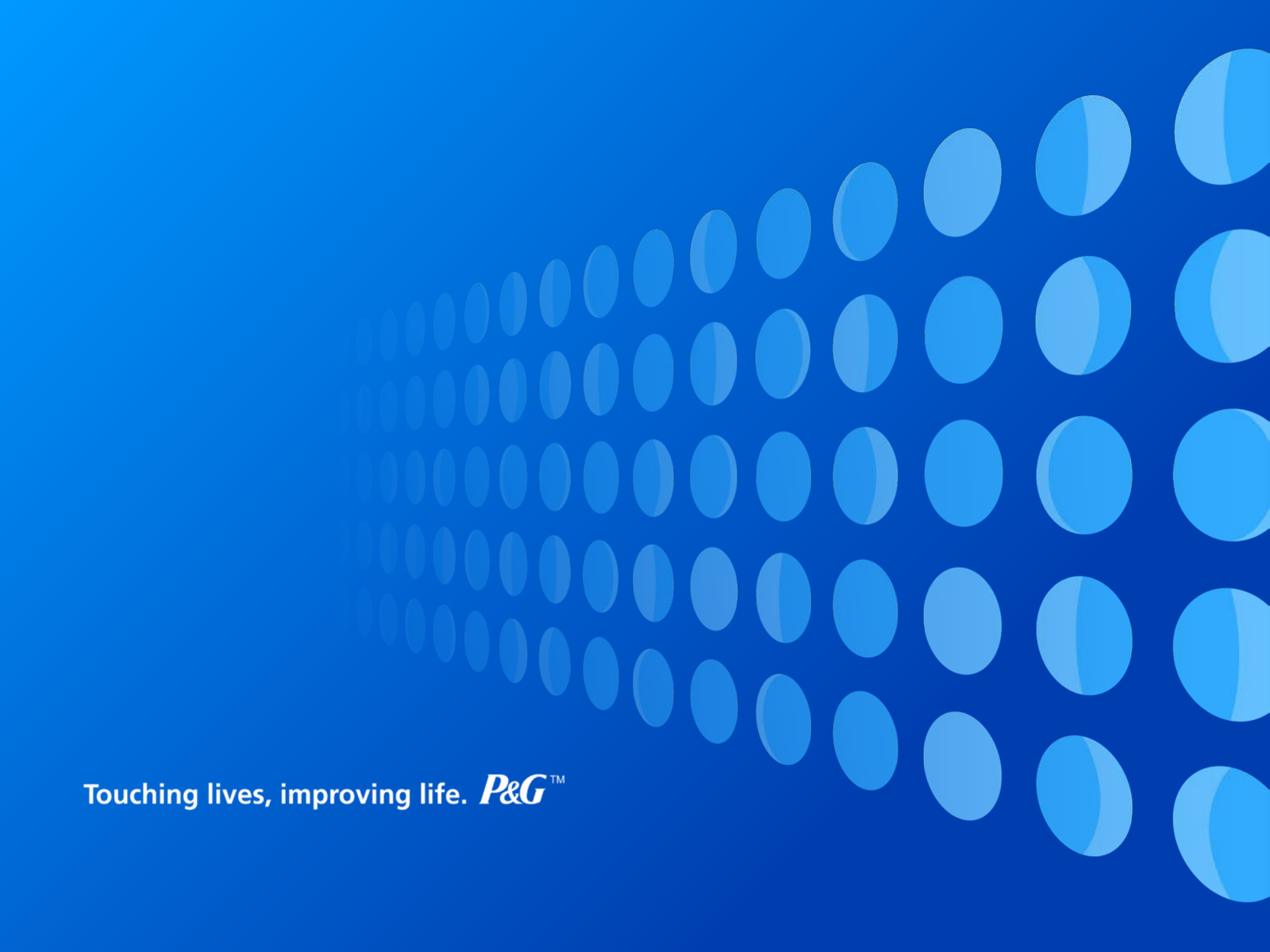
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