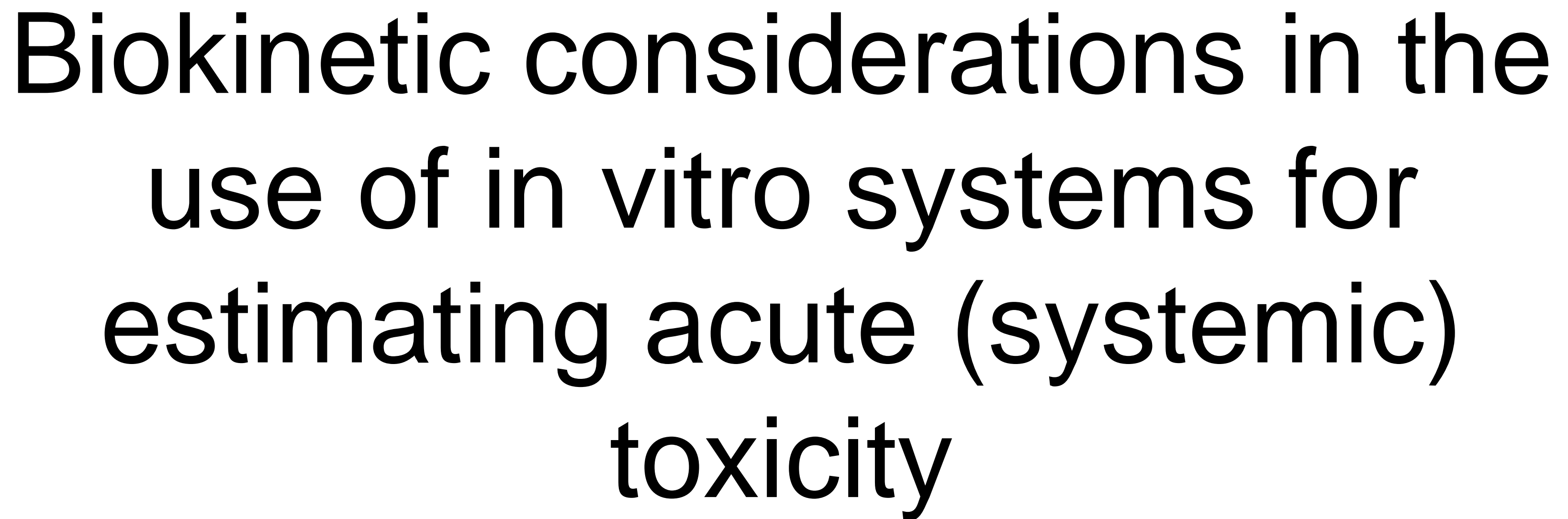

Biokinetic considerations in the use of in vitro systems for estimating acute (systemic) toxicity

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The new paradigm in toxicology

toxicity is determined by:

the *critical* concentration and time of exposure (dose metric)

to

the *critical* compound (metabolite?)

at

the *critical* site of action

use of in vitro cytotoxicity data in estimating in vivo acute toxicity

- **Assumptions:**
- basal cytotoxicity (EC50) is a good predictor for acute toxicity in vivo
- cytotoxic concentrations in vitro mirror blood plasma concentrations
- blood plasma concentrations mirror target tissue concentrations

- **but**….there are complicating factors

Local vs systemic effects

- If a compound would quickly act on each and every cell type, then the effects would be the most obvious on the place of application (skin, lungs, gi tract: oesophagus, stomach): local effects.
- Systemic toxicity will appear if the process of toxicity is less fast or if other cell types are more sensitive/less well protected.

reasons for deviations

- **quality of in vivo and/or in vitro data**
- **cytotoxic concentration is irrelevant for in vivo target tissue concentration**

relevance of in vitro concentrations (1)

- specific organ or tissue toxicity

  main organs relevant for acute toxicity: brain, liver, kidney, lung (local ?)

relevance of in vitro concentrations (2)

- *biokinetic* behaviour of the compound in organism:
- 1. <u>absorption</u>: if limited: in vitro concentrations will be higher than in vivo plasma concentrations: <u>overestimation</u> of toxicity

ACute Tox

Research Project for Alternative Testing

relevance of in vitro concentrations (3)

- *biokinetic* behaviour of the compound in organism:
- **2. <u>distribution</u> of compound leads to higher or lower concentrations on target site**
- example: fat tissue, CNS

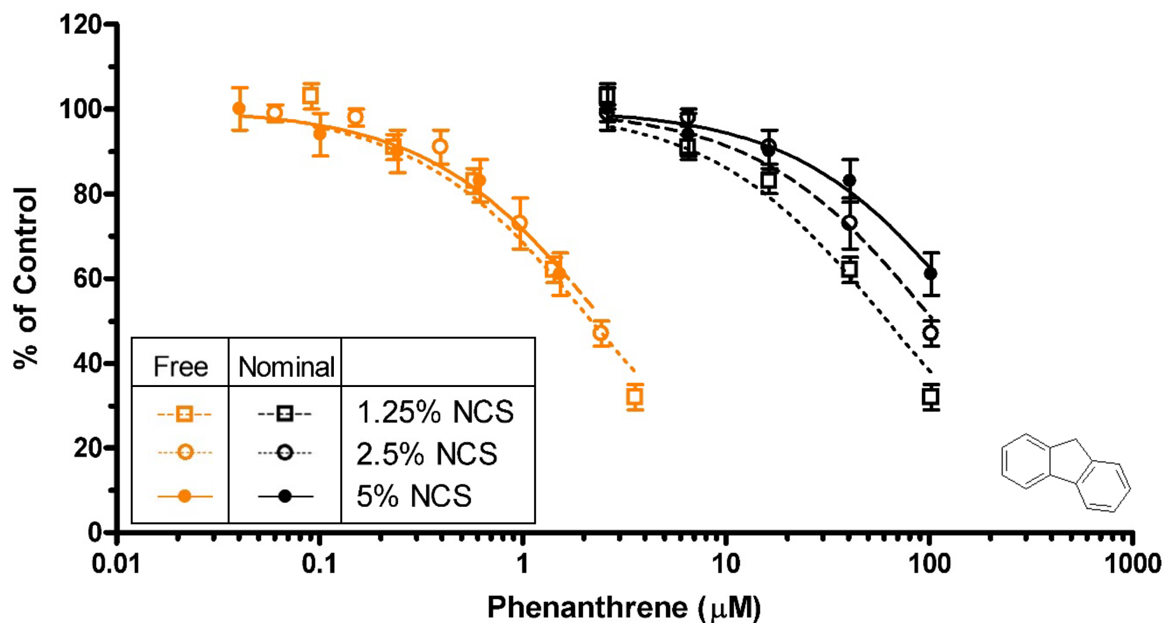
relevance of in vitro concentrations (4)

- *biokinetic* behaviour of the compound in organism:
- 3. <u>elimination</u>:
- fast excretion/exhalation
- fast metabolism:
  - fast elimination or formation of toxic metabolite

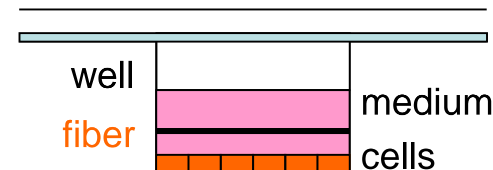
relevance of in vitro concentrations (5)

- **quality of in vitro data:**
- **“biokinetics in vitro”**
- **protein binding, medium vs cells, binding to plastic, evaporation**
- **nominal vs real free concentration**

Cytotoxicity vs Serum Level



NCS (%)	1.25		2.5		5	
Log EC_{50}	1.79 ± 0.06	0.33	2.02 ± 0.04	0.40	2.22 ± 0.03	0.40
EC_{50} (μM)	61.3	2.15	104.1	2.49	165.5	2.48

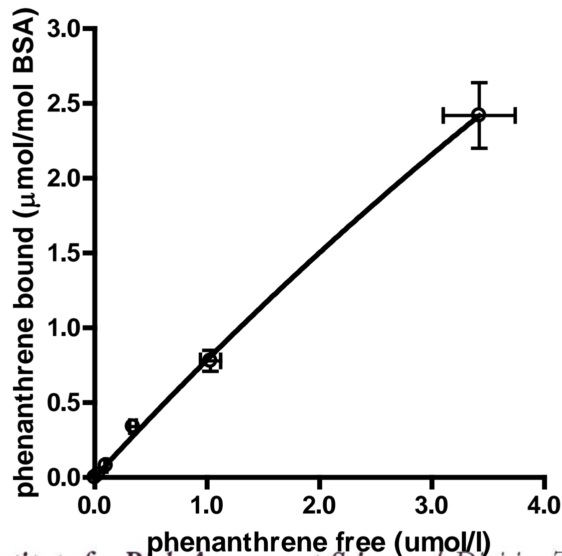


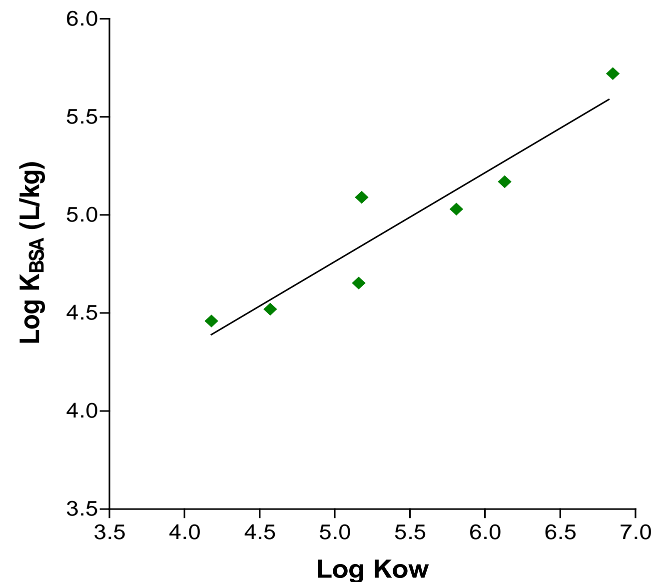
% NCS	% Free	
	Measured	
1.25	6.85 ± 0.75	
2.5	4.55 ± 1.01	
5	2.70 ± 0.20	

Modeling Free Concentration

$$F = \frac{1}{1 + K_s[S] + K_p[P] + K_c[C] + K_a \cdot \frac{V_a}{V_m}}$$

- Serum protein binding

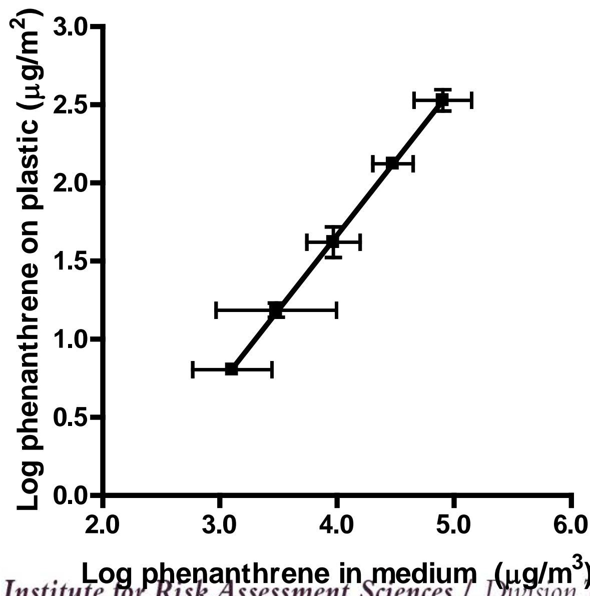


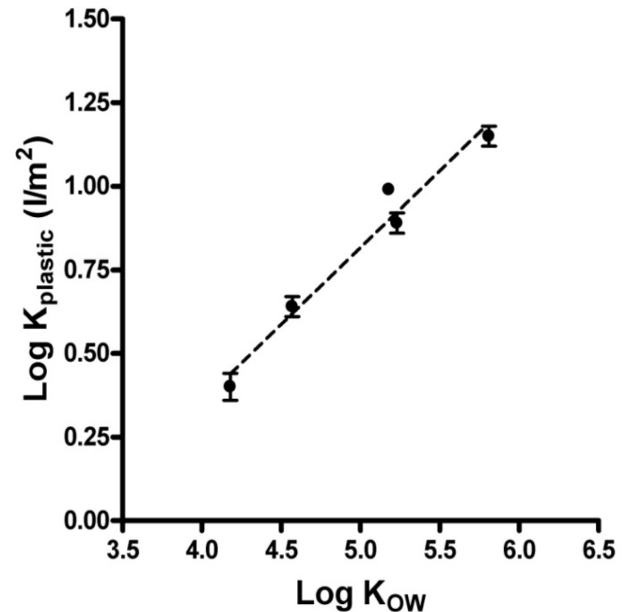


Modeling Free Concentration

$$F = \frac{1}{1 + K_s[S] + K_p[P] + K_c[C] + K_a \cdot \frac{V_a}{V_m}}$$

- Plastic binding





biokinetics in ACuteTox

- prediction of biokinetics from PhysChem
- in vitro absorption
- in vitro blood-brain barrier
- PBBK modelling

- collect data for prediction of alerts and correctors

biokinetics in ACuteTox

- find alerts and correctors for improving interpretation of basal cytotoxicity data
- incorporate these in logical and transparent strategy

Qp

alveolar space

Qp

lung blood

Qc

fat

Qf

RPtissue

Qr

SPtissue

Qs

brain lipids

brain water

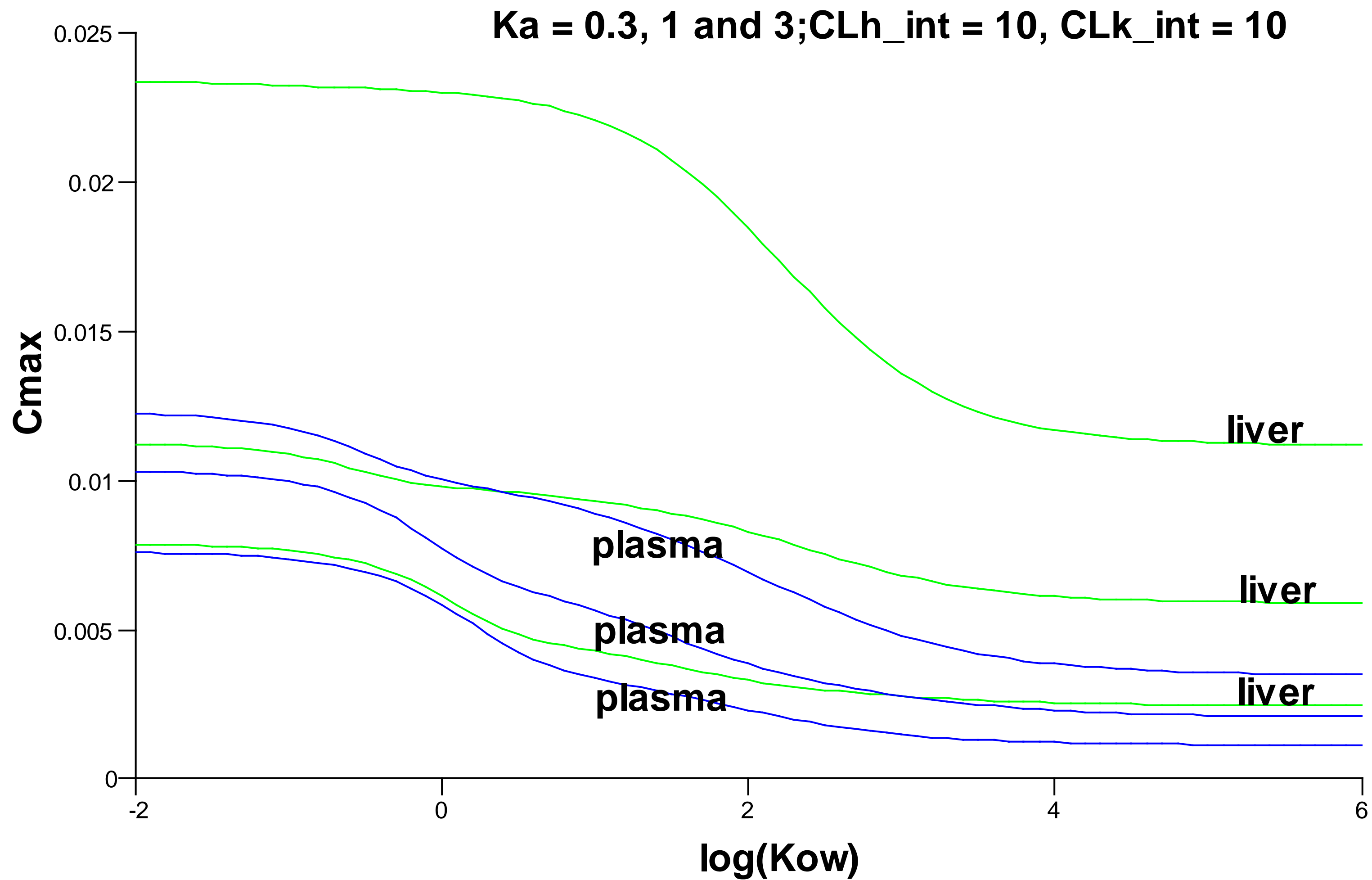
Qb

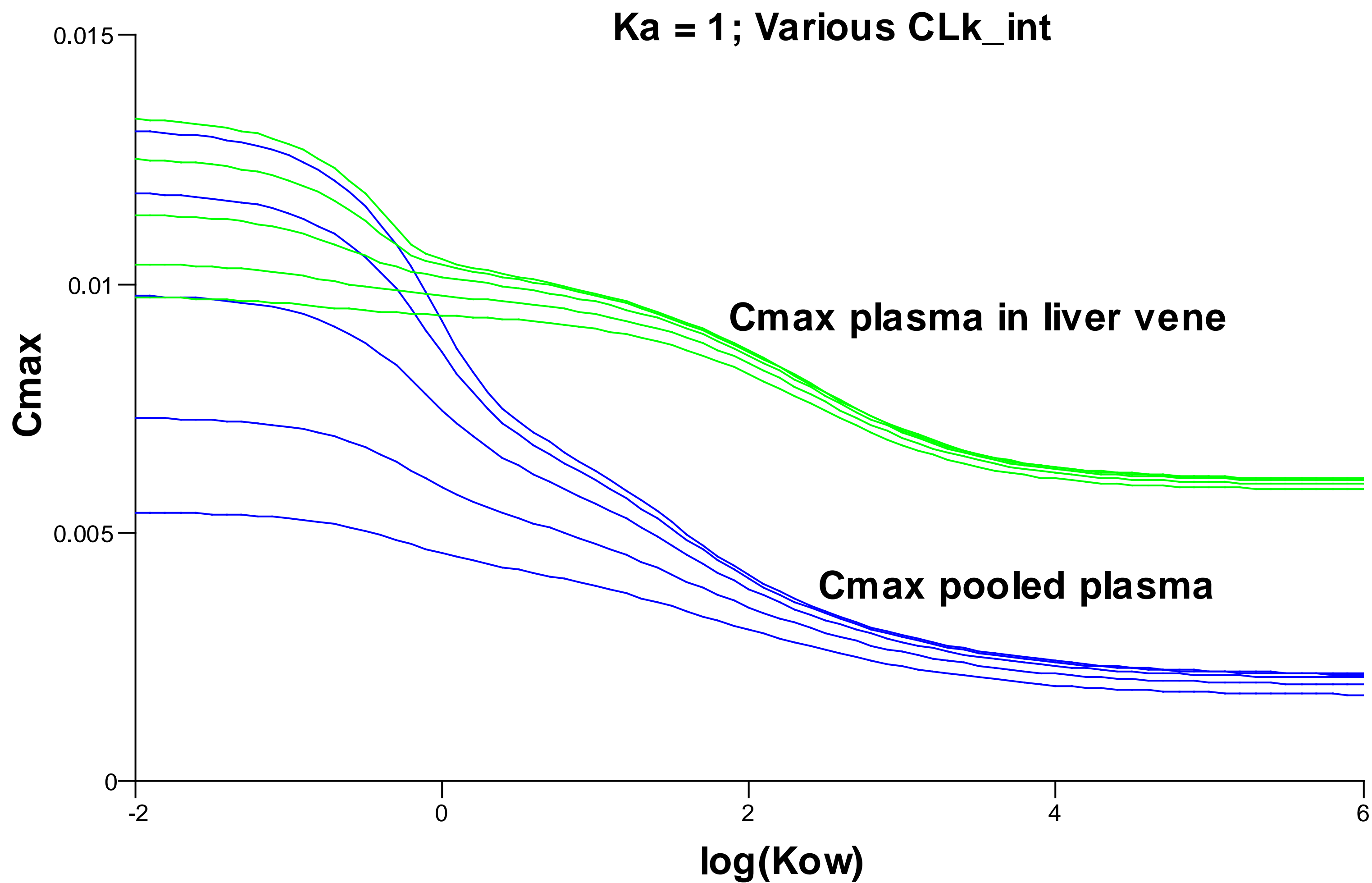
liver

Qrl

Vmax

Km


Ka = 0.3, 1 and 3;CLh_int = 10, CLk_int = 10
Cmax
0.025
0.02
0.015
0.01
0.005
0
-2
0
2
4
6
log(Kow)
liver
liver
liver
plasma
plasma
plasma


Ka = 1; Various CLk_int
Cmax plasma in liver vene
Cmax pooled plasma
Cmax
log(Kow)
0.015
0.01
0.005
0
-2
0
2
4
6

NOW: how to make this
practical

Toolboxes

1. Pre-existing data
2. Physico-chemical properties (theoretical)
3. Simple test battery using cell lines
4. PBPK modelling
5. Target-specific toxicity
6. Verify acute toxicity

First step:

- When is basal cytotoxicity sufficient:

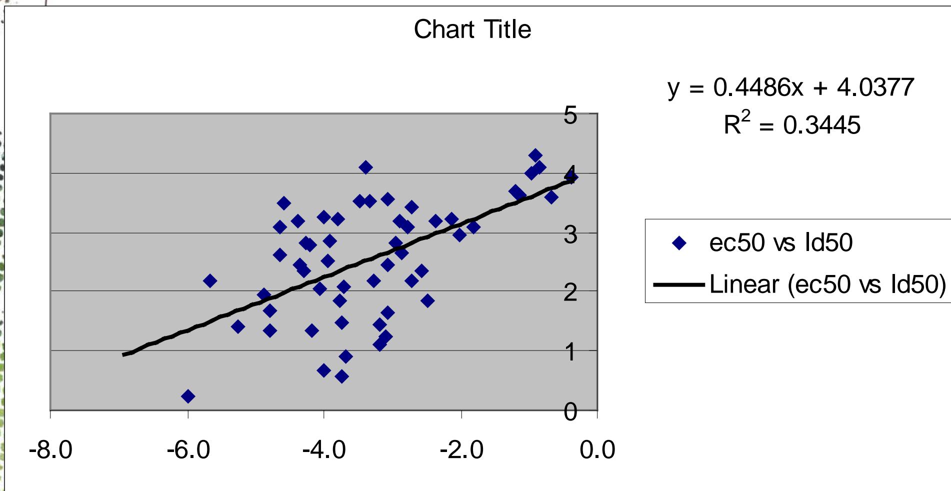
- When the compound is rapidly absorbed;
- When no aberrant distribution pattern is encountered
- When no fast (metabolic) clearance occurs
- When acute tox is not the result of something else than basal cytotox: specific target tox.
- When in vitro freely available concentrations do not deviate much from nominal concentrations

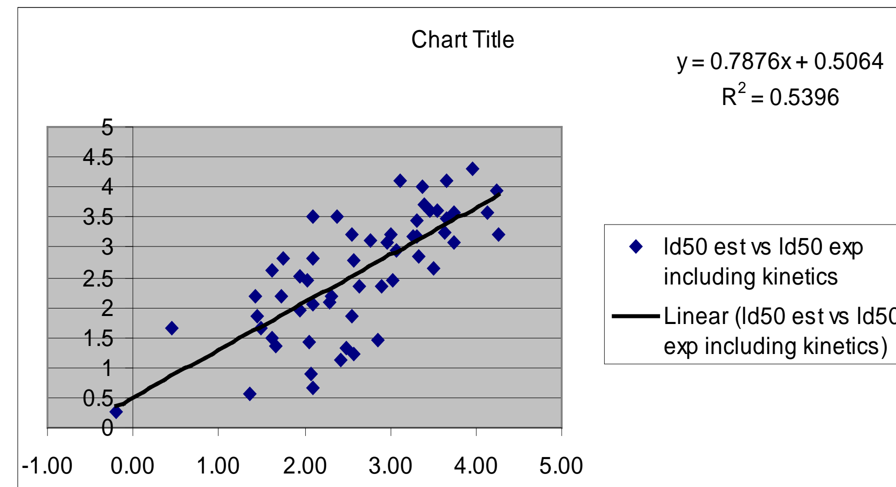
Next step:

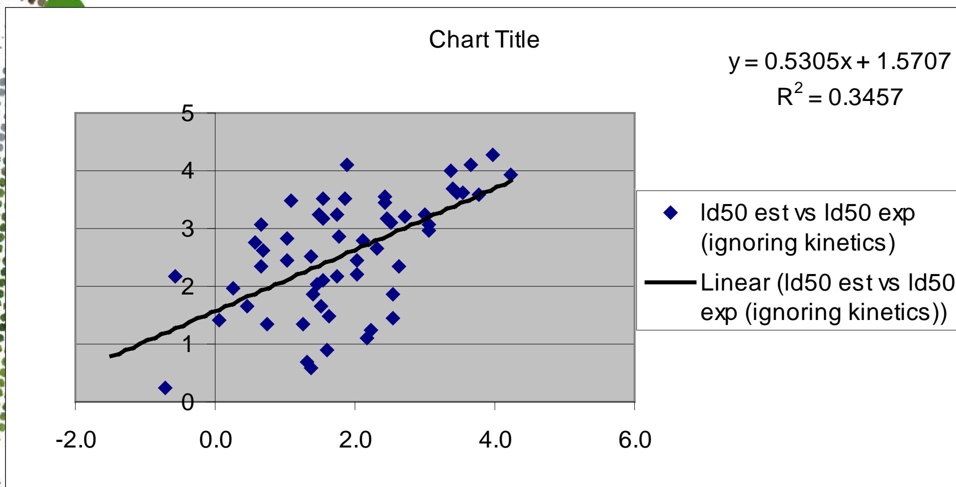
- When do we decide that the criteria for inclusion in the “ideal” algorithm are NOT fulfilled?
- i.e. when does absorption become a reason for deviation?
- Can we decide on the basis of
  -phys-chem properties
  -in vitro data

Same exercise for:

- Distribution
- Metabolism
- Excretion
- Neurotox
- Hepatotox
- Nephrotox
- Hematotox
- In vitro biokinetics







r^2:

EC50 vs LD50: 0.3445

estLD50 ign kinetics vs LD50: 0.3457

estLD50 incl kinetics vs LD50: 0.5396

Conclusion

1. Need to take in vitro biokinetics into consideration: will improve quality of in vitro toxicity data
2. Use kinetic parameters to correlate in vitro effective concentrations to a dose in vitro: QIVIVE.
3. Use approach in improving the applicability of in vitro data in risk assessment

Acknowledgement

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