Grosser grüner Papagey mit gelb und rothen Flecken.
Psittacus major viridis maculis luteis et rubris.
Grand Papegaut vert avec marques jaunes et rouges.

Grauen Papagey mit gelbem Kopf gelben
Flügel schildern und Schenkeln.
Psittacus viridis alius capite luteo.
Papegaut vert avec tête jaune.

Alternative methods in risk assessment and kinetics in paediatric pharmacology: A common need for PBPK modelling.

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ecvam.jrc.it

- ECVAM
- Directive 1986 (86/609/EEC) on the protection of animals used for experimental and other scientific purposes
- Established 1991, 61 staff
- Missions: Validation, Database, Research, Communication.
- ESAC.
- The 3 Rs:
  - Refinement,
  - Reduction,
  - Replacement.

Alternative methods: Reduction, Refinement, and Replacement (3 Rs)

• **Reduction** is any means of lowering the number of animals used to obtain information of a given amount and precision.

• **Refinement** is any development that refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being.

• **Replacement** is any scientific method employing non-sentient material which may replace methods which use conscious living vertebrates.

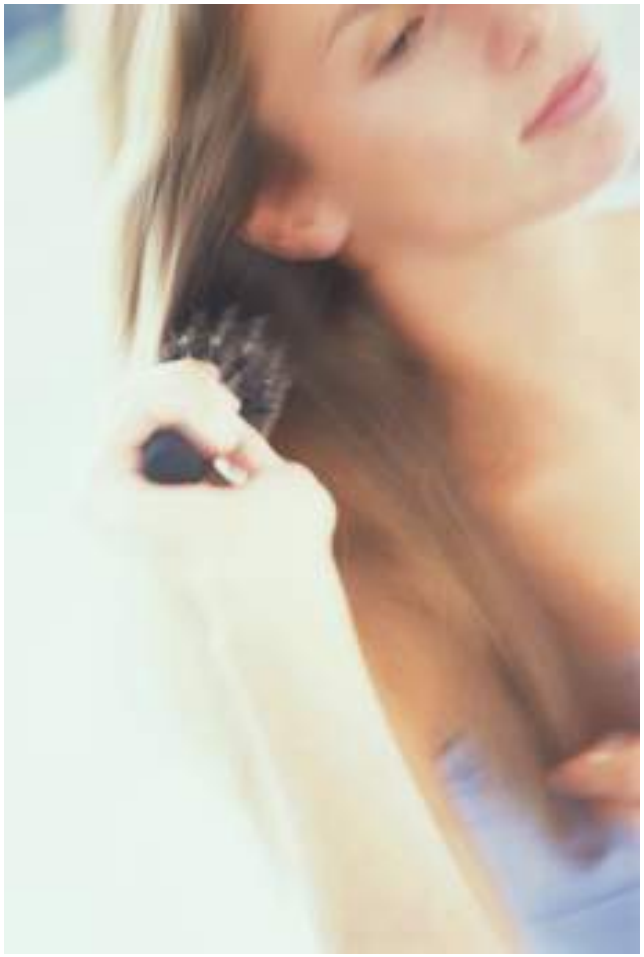
The regulatory context shifts the frame of testing towards an increased need for extrapolation.

- 2003: 7th amendment to the Cosmetics Directive. Phasing out animal testing for cosmetic ingredients. Target dates in 2009 and 2013 for various endpoints.

- December 2006: REACH regulation for Chemicals. Production volume-dependent regulatory requirements. Sheer number of substances to be evaluated creates testing challenges.

- More generally, there is a growing political and regulatory pressure to reduce animal testing.

7th amendment to the Cosmetics Directive



- E.U: 2.000 companies, 60 billion € turnover
- EU: 5.000 new products per year, 25% turnover with products released within last 6 months.
- since 2003: Marketing ban if animal testing of finished products or not using ECVAM-validated methods.
- Phasing out animal testing of ingredients with test and marketing bans in 2009 and 2013.
- Critical need for alternatives

Chemical industry and REACH

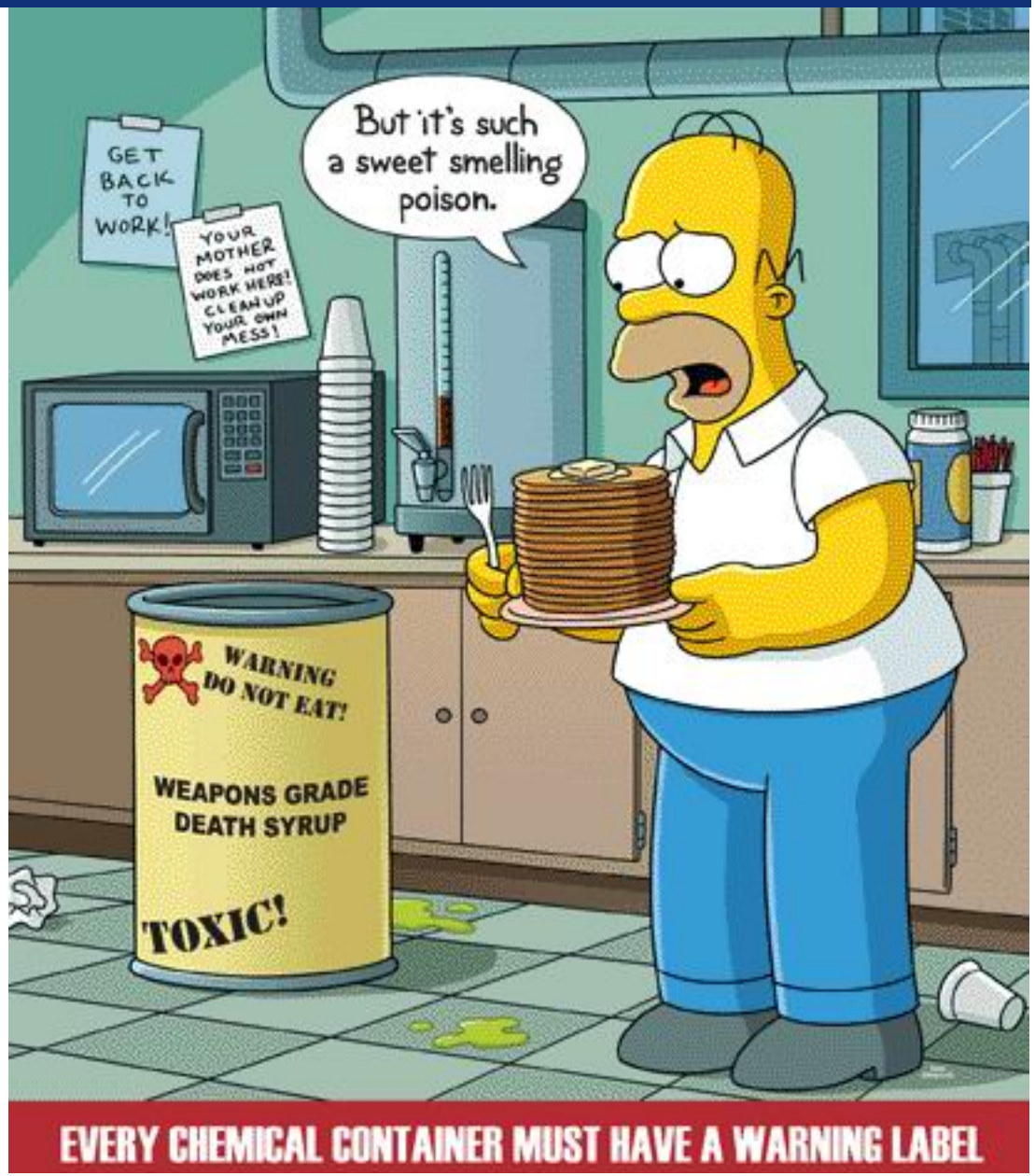
- **EU: 27.000 companies (96% SME), 590 billion € turnover = 33% of world market, 1,7 million employees**
- **86% of toxicological data on 'old' chemicals are lacking**
- **30.000 existing chemicals marketed >1 ton/year will be assessed under REACH regulatory frame.**
- **Expected 140.000 dossiers in the first 11 years of REACH**
- **Expectation: Alternative methods not only reduce animal experimentation, but also increase test through-put and reduce costs.**

REACH in a nutshell

picture
© ChemSec

Risk assessment: The ~~39~~ Four steps

- Hazard identification
- Hazard characterisation
- Exposure assessment
- Risk characterisation



Risk Assessment: a qualitative and quantitative process

Hazard identification: qualitative statement whether or not the compound can potentially cause a given (toxic) effect. High potential for in vitro methods.

•Hazard characterisation: Establishing a quantitative relationship between:

- **external dose, and effect: "dose-response relationship".**
- **internal dose (concentration-related), and effect: "PKPD model".**

•The relationship needs to be built for the experimental model, whether *in vivo* or *in vitro*, and for the species of interest, e.g. human.

Risk Assessment: a qualitative and quantitative process

- Exposure assessment: estimating the potential external doses and internal doses in various populations.
  - Uses exposure models;
  - Unwanted exposure vs prescribed dose regimen.

- Risk characterisation: integrating available information in order to predict effects in exposed populations. Methodology includes several modelling frames: Modelling of in vitro tests, data-based PK, PBPK, Population PK-PD, PKPD modelling.

Extrapolations and Predictions

- Are necessary to make decisions.

- In many situations:
  - from in vitro to in vivo
  - between species
  - between doses
  - between routes of administration
  - between groups within species: age groups, gender, disease groups, genetic groups, etc.

- This is particularly true in relatively “data-poor” situations such as: Cosmetics, Chemicals, Paediatrics…

Alternative testing: Extrapolation from in vitro tests to in vivo effects

- In vitro testing: biological targets are in contact with concentrations of potential toxicant in the test system conditions.

- In vivo: cells of animals and people will or may be in contact with concentrations in extra cellular fluids.

- The only possible link between in vitro findings and in vivo observed or predicted effects is the concentrations, corrected for differences in biological conditions. (Ex: 10% FCS in vitro vs blood and extracellular fluids in vivo).

ECVAM – IHCP workshops: PBPK, Kinetic modelling of in vitro systems.

- Blaauboer BJ, Bayliss MK, Castell JV, et al. The use of biokinetics and *in vitro* methods in toxicological risk evaluation. The report and recommendations of ECVAM workshop 15. ATLA 1996; 24: 473-497.

- Bouvier d'Yvoire M, Prieto P, Blaauboer BJ, et al. Physiologically-based modelling: meeting the 3Rs agenda. The report and recommendations of ECVAM workshop 63. ATLA 2007; 35: 661-671.

- ECVAM Workshop : In vitro kinetics. Factors affecting the movement, transformation and effects of chemicals in *in vitro* testing systems. April 18-20, 2007. Chaired by: Pelkonen O, Rostami Hodjegan A, Blaauboer BJ. Report in preparation.

PBPK prediction from "*non in vivo*" data: An evaluation problem.

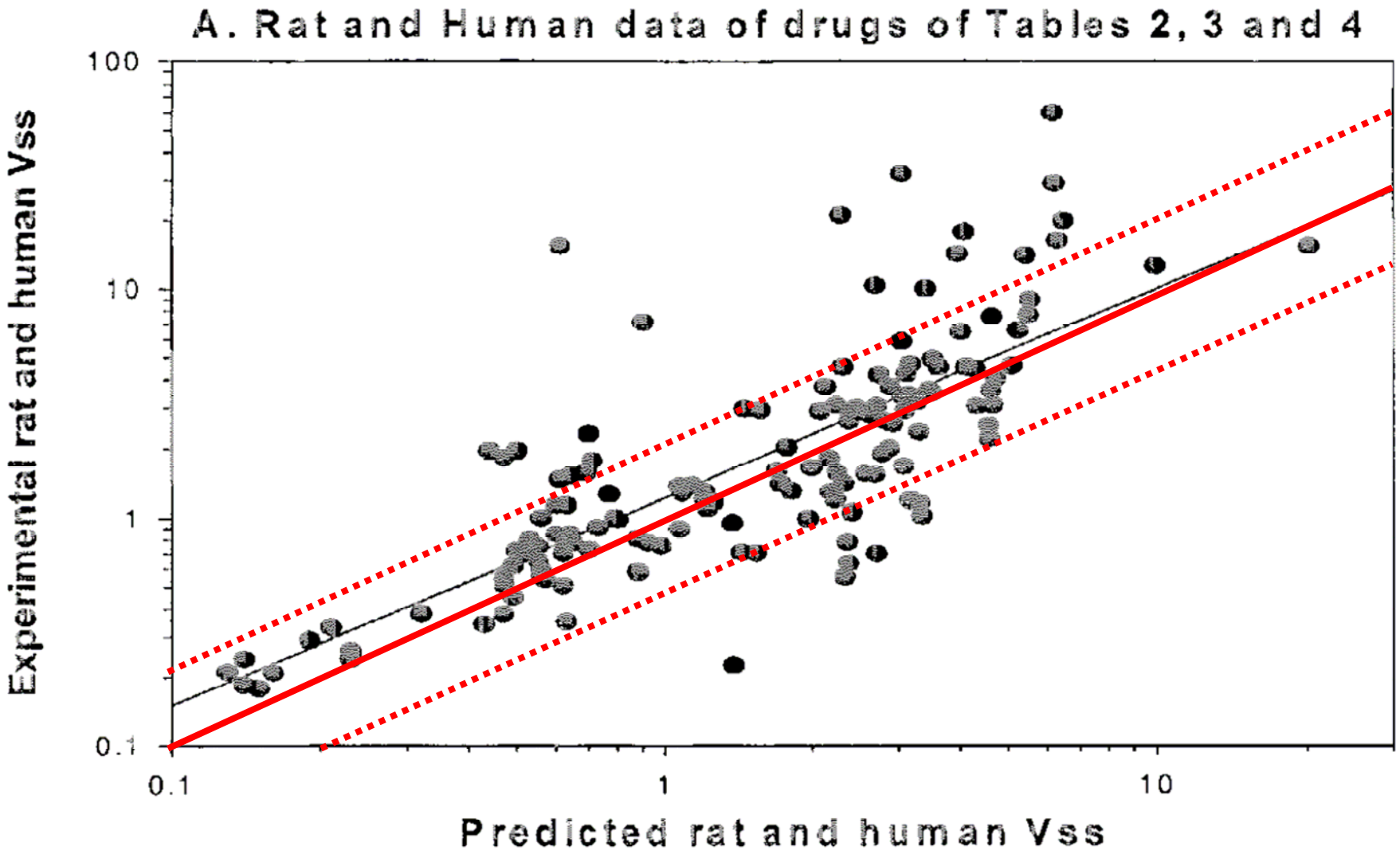
- How do we evaluate the quality of a PBPK prediction ?
  - For a single compound
  - For a set of compounds (evaluate a method or a strategy)

- In particular:
  - Quality of input data ?
  - Quality of modelling exercise ?
  - What compound set ?
  - What reference data ?
  - What parameters ?
  - What statistics ?

Example 1: Vdss estimation.

Poulin P, Theil FP. Prediction of Pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. *J. Pharm. Sci* 2001; 91(1): 129-158.

- **Set: 123 structurally unrelated compounds**
- **Ref data: Intravenous kinetic data**
- **Parameter: Vss**
- **Defined Fold-error (FE) as Obs/Pred or Pred/Obs, whichever is the greater (Max[O/P, P/O])**
- **Compounds Classified according towhether FE > 2 or FE < 2**
- **Average ratio and correlation in: all data, group FE > 2, group FE < 2.**


A. Rat and Human data of drugs of Tables 2, 3 and 4
Experimental rat and human Vss
Predicted rat and human Vss
100
10
1
0.1
0.1
1
10

Example 2: full PK prediction, mostly oral route

Jones HM, Parrott N, Jorga K, and Lavé T. A novel strategy for Physiologically Based Predictions of Human Pharmacokinetics. Clin. Pharmacokinet. 2006; 45(5): 511-542

- Set: 19 compounds

- Ref data: mostly oral

- Parameters calculated: Ratio of predicted over observed:
  - Plasma concentrations,
  - Cl or Cl/F, Vss of Vz/F, apparent terminal $T_{1/2}$, AUC, Cmax, Tmax.

- Statistics:
  - "Judgment variable" defined as Fold error (FE) = Max[O/P, P/O]
  - The % where FE < 2, FE < 3, FE < 5 were tabulated
  - Geometric mean of FE was calculated.

- For all compounds, and for the subset fulfilling the conditions for the pre-defined PBPK human PK prediction strategy described in the paper.

Table VII. Prediction accuracy for pharmacokinetic parameters using physiologically based pharmacokinetic (PBPK) modelling

Parameter	Average-fold error[a]	Within 2-fold error (%)[a]	Within 3-fold error (%)[a]	Within 5-fold error (%)[a]
CL or CL/F[b,c]	2.7 (1.7)	71 (83)	76 (92)	76 (92)
V_{ss} or V_z/F[b]	2.8 (2.3)	41 (50)	65 (75)	76 (83)
$t_{½}$	2.2 (1.9)	71 (75)	76 (83)	88 (92)
AUC[c]	2.6 (1.7)	76 (92)	76 (92)	82 (92)
C_{max}	2.3 (1.9)	47 (67)	71 (83)	94 (100)
t_{max}	1.4 (1.2)	94 (100)	94 (100)	94 (100)

a Values in parentheses represent prediction accuracy only for those compounds that fulfilled the criteria outlined in the strategy.

b This prediction accuracy refers to CL and V_{ss} when intravenous data were available, and CL/F and V_z/F when oral data were available.

c The slight discrepancies in prediction accuracy between AUC and CL result from the fitting of the individual observed data separately and meaning the parameters.

AUC = area under the plasma concentration-time curve; **CL** = clearance; **CL/F** = apparent oral clearance; C_{max} = peak plasma concentration; $t_{½}$ = terminal elimination half-life; t_{max} = time to reach C_{max}; V_{ss} = volume of distribution at steady state; V_z/F = apparent volume of distribution during terminal phase after non-intravenous administration.

A few selected Difficulties

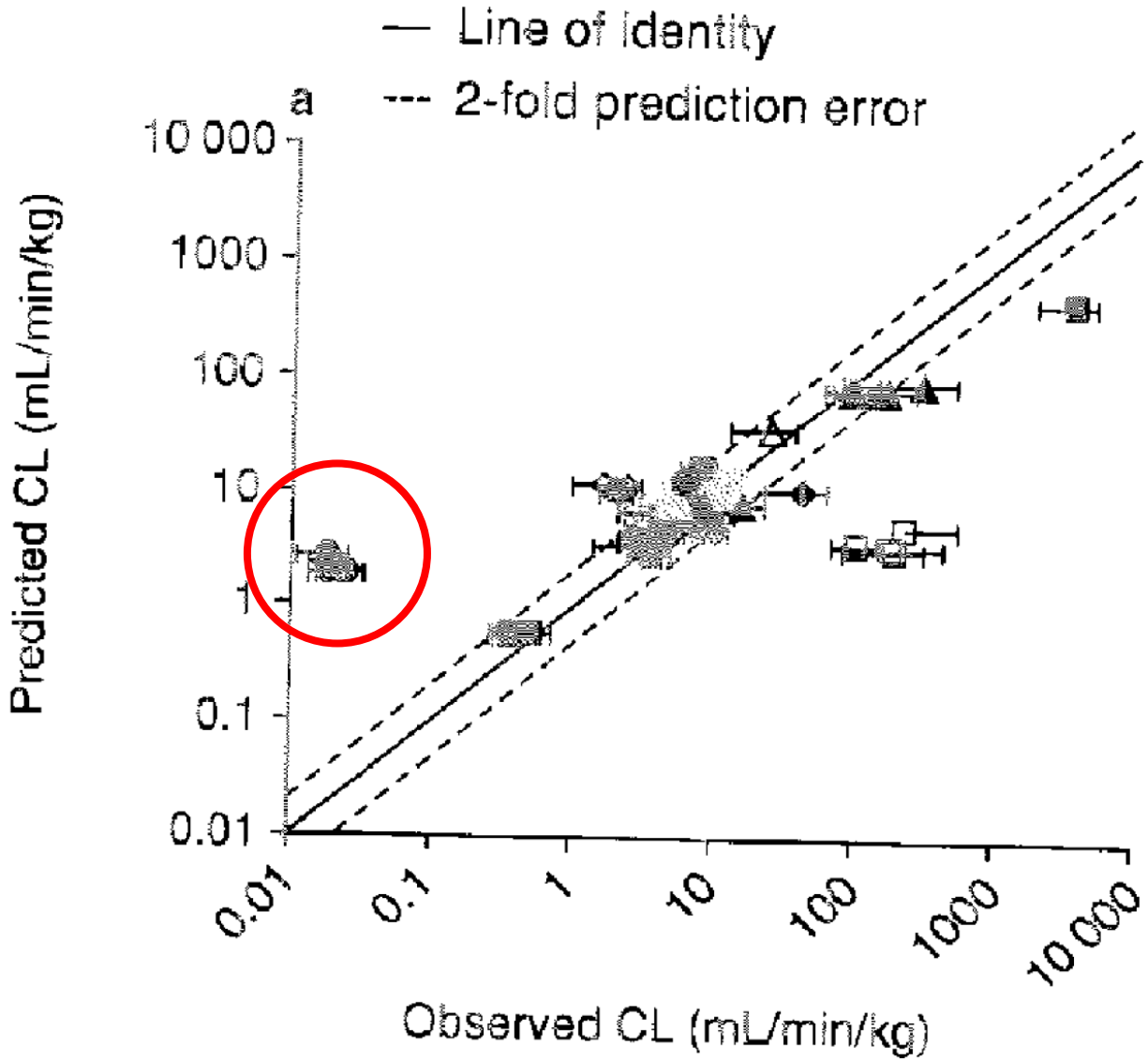
- Problem of the applicability domain of the prediction strategy, depends on the "chemodiversity" of the set of compounds used for validation.

- The parameters are not independent, and most are influenced by bioavailability.

- Within N-fold error means that for a "true" value of X, prediction may vary between X over N and N times X, i.e. an N-squared-fold. Within 3-fold means a 9-fold range. This is close to safety factors used in risk assessment for inter-species extrapolation (commonly 10).

- The geometric mean purposefully gives the same weight to over- and under-prediction. But these are not biologically equivalent:
  - inverse effects of bioavailability and clearance on AUC;
  - inverse effects of clearance and volume on Half-life.

- Different combinations of under- and over-prediction of parameters create a large variety of pictures.


Line of Identity
2-fold prediction error
a
Predicted CL (mL/min/kg)
Observed CL (mL/min/kg)
10 000
1000
100
10
1
0.1
0.01
0.01
0.1
1
10
100
1000
10 000

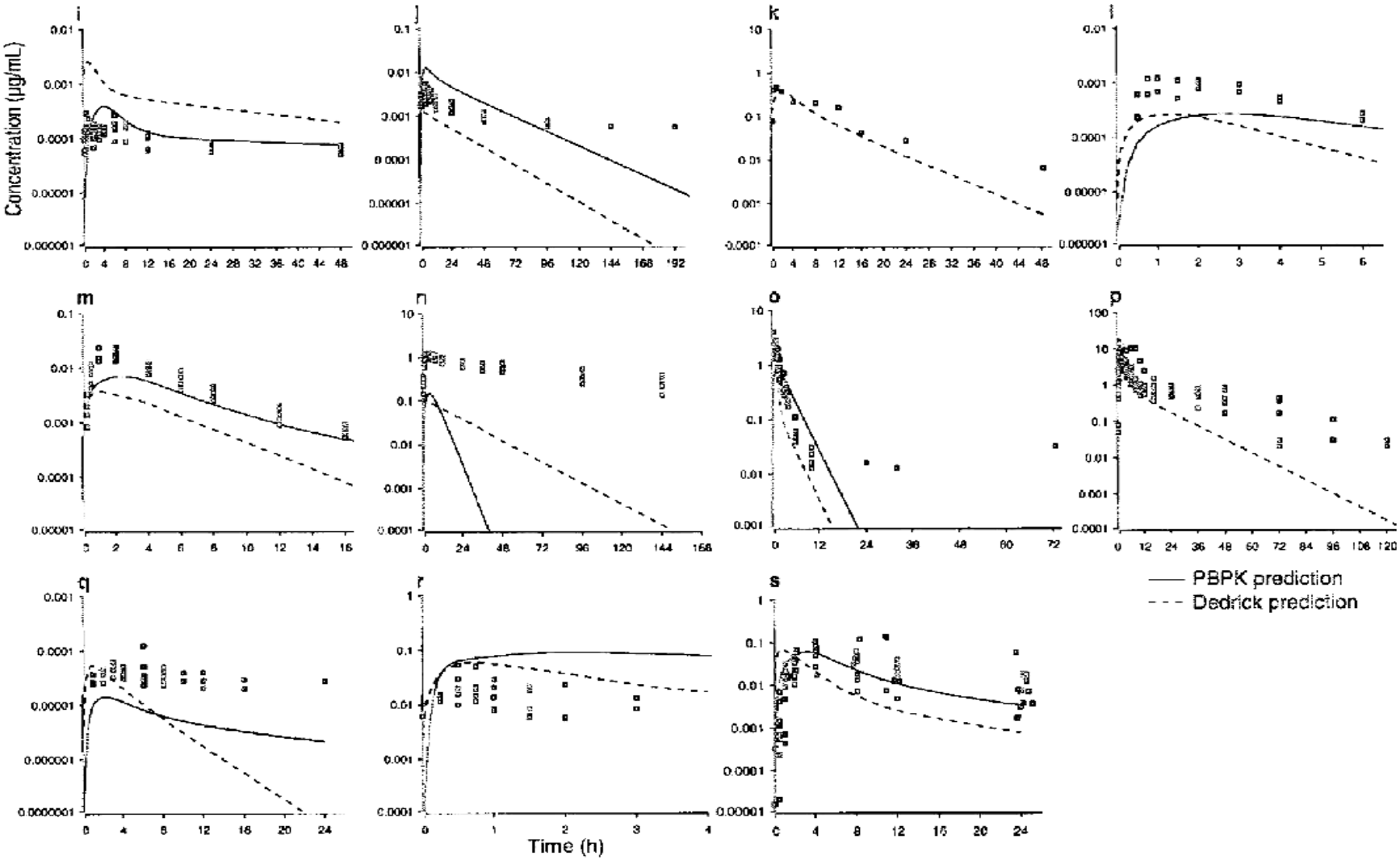


Fig. 2. Prediction of plasma concentration-time profiles using physiologically based pharmacokinetic (PBPK) and Dedrick approaches for all compounds: (a) CPD1; (b) CPD2; (c) CPD3; (d) CPD4; (e) CPD5; (f) CPD6; (g) CPD7; (h) CPD8; (i) CPD9; (j) CPD10; (k) CPD11; (l) CPD12; (m) CPD13; (n) CPD14; (o) CPD15; (p) CPD16; (q) CPD17; (r) CPD18; (s) CPD19. Lowest dose selected in all cases. No PBPK predictions are available for (k) and (p).

Conclusion

- Extrapolation from in vitro to in vivo using PBPK is a powerful tool to support decisions in drug development as well as in risk assessment.

- The prevalent methodology still needs animal (and / or human) data to validate the chosen models.

- Similarly, at least some clinical pharmacokinetic data in children need to be collected in paediatric development. PBPK modelling is one of the tools to improve the strategy for gathering and interpreting such data.

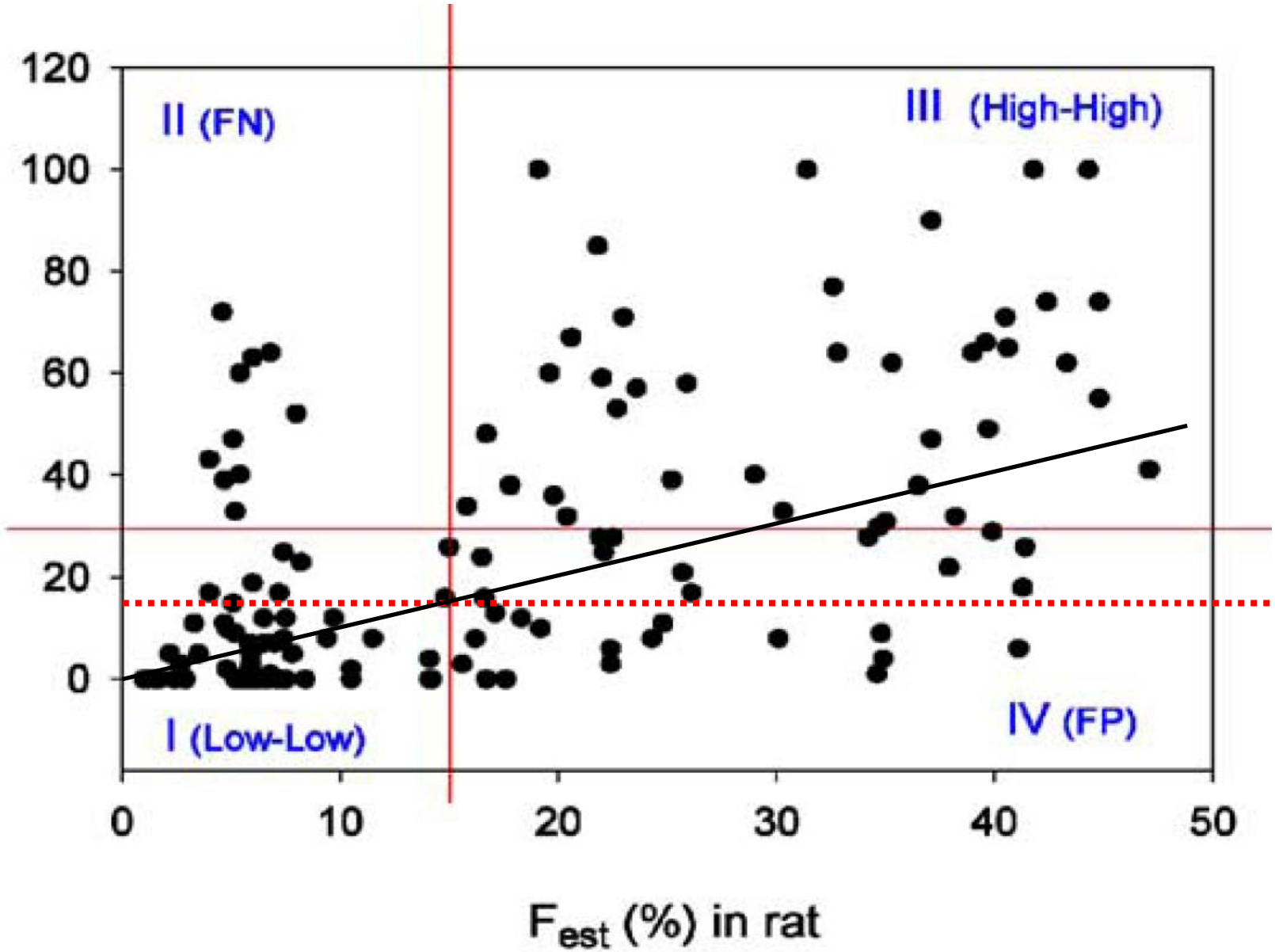
- The methodology to evaluate PK prediction methods, i.e. on sets of compounds, needs to be further developed and standardised.

Der IVten Hauptart IIIte Abtheilung. I Platte.

Der Rothe Papagey mit schwarzer Platte. grünen Flügeln und blauen Schenkeln.
Psittacus ruffus vertice nigro alis viridibus femoribus caeruleis
Perroquet rouge à Sömet noir.

Der IVten Hauptart IIIte Abtheilung II^te Platte.

Der ganz Rothe Papagey mit grünen Flügeln und Schenkeln.
Psittacus ruffus femoribus et alis viridibus.
Perroquet tout rouge les ailes vertes.


120
100
80
60
40
20
0
II (FN)
III (High-High)
I (Low-Low)
IV (FP)
0
10
20
30
40
50
F_{est} (%) in rat