WP5: Alerts and correctors in toxicity screening (I): Role of ADE (P5, P6, P13, P15, P27, P34 and P35)

One of the reasons why a compound will be an outlier in the comparison between its EC_{50} value and its LD_{50} is its kinetic behaviour. Two aspects can be distinguished:
1) the kinetics of the compound in the *in vitro* system ("biokinetics *in vitro*"); and
2) the use of kinetic models in extrapolating the *in vitro* dose metrics to the *in vivo* situation.

Biokinetics in vitro

In many *in vitro* experiments, toxic effects or biotransformation rates are related to the concentrations of the compound added to the medium, i.e. the amount added divided by the volume of the culture medium. This nominal concentration can to a great extent deviate from the actual, free concentration of the compound in the system and also change over time, as a result of binding to proteins, binding to the culture plastic, evaporation, or uptake in the cells. Since it usually is the freely available concentration that is the driving force for toxic reactions on the (sub) cellular level, these processes will influence the free concentration and thus the effect. It is therefore necessary to estimate or measure this free concentration, especially when on the basis of the physico-chemical properties (e.g. lipophilicity) it can be expected that the free concentration will differ from the nominal concentration. One technique to do this is to sample the culture medium with solid-phase micro-extraction (SPME) devices and to analyse the compound (Kramer *et al*., 2007). These devices consist of small rods covered with material absorbing the compound in equilibrium with its free concentration.
This technique allows the identification of processes influencing the free concentration. This in turn enables the modelling of the *in vitro* system. The application of these techniques showed that for some compounds the free concentration could differ up to two orders of magnitude from the nominal concentration, showing the importance of understanding, measuring and modelling the "biokinetics *in vitro*" (Kramer *et al*., 2009).
However, for calculating the acute toxicity of chemicals, it became clear that only in extreme cases this influences the estimations of the *in vivo* toxic dose, since many of the factors influencing the free concentration *in vitro* are also of influence on the free concentrations of compounds *in vivo*.

Biokinetics in in vitro-in vivo extrapolation (IVIVE)

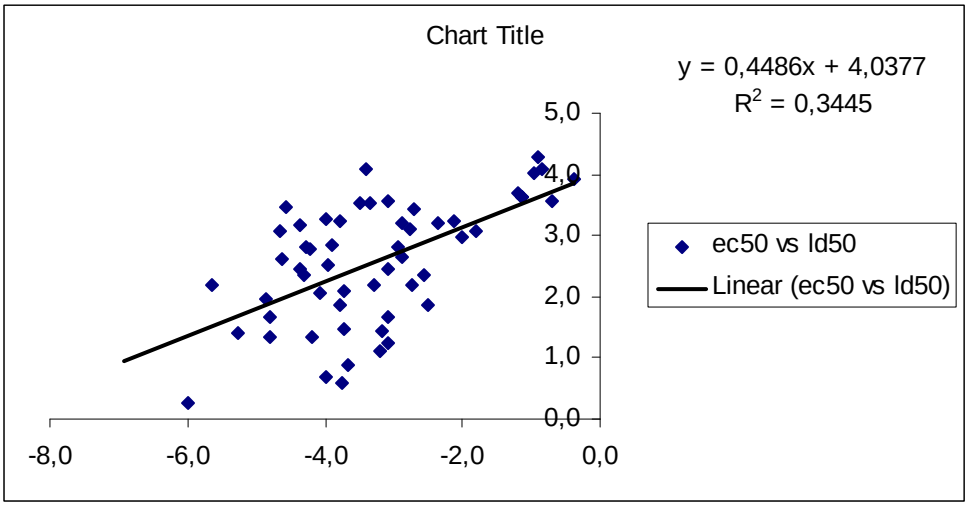
Physiologically based biokinetic (PBBK) models are essential tools in the evaluation of *in vitro*-derived data on dose-(or concentration)-response relationships for the situation in intact organisms.
For the purpose of predicting as to whether a compound would be an outlier, an evaluation of the most prominent factors for its kinetic behaviour was made. An eight compartment PBPK model, representing the main routes of elimination, was used. The model describes the biokinetics of a substance after its oral uptake into the system.
It was concluded that the most important parameters are:
- the extent of oral absorption of the compound;
- the distribution over the tissues, as governed by its lipophilicity,
- its intrinsic clearance ($CL_{t,int}$),
- the free fraction (i.e the fraction that is not bound to protein)

In WP5, the different partners have contributed *in vitro* data on these parameters, including:

- estimates of oral absorption by measuring transport over Caco-2 cell layers and over artificial membranes and by estimating the transport by use of *in silico* neural networking techniques on the basis of structural properties of the compounds under study.
- estimates of metabolic stability by measuring loss of compound in different metabolising systems, including rat and human microsomes.
- estimates of transport over the blood-brain barrier, by also using *in silico* neural networking techniques and by measuring the transport over *in vitro* systems representing the barrier.

On the basis of these data and considerations, the steps in the kinetic modelling of EC_{50} values for estimating LD_{50} values were developed and applied in a set of algorithms. These algorithms first took into account the calculation of the internal dose, based on the effective concentration of compounds (e.g. as EC_{50}s), the parameters for the distribution of the compound (lipophilicity, metabolic clearance, protein binding). The next step is then the calculation of the external dose (i.e. the oral absorption estimates) and the conversion from the molar dose to a dose in mg/kg bw.

The final outcome of the work in WP5 was that the corrections for the kinetic parameters led to an improvement of the estimates of LD_{50}s. If for example a comparison is made on the correlation between in vitro data based on EC_{50}s for basal cytotoxicity (3T3-NRU test) with data on LD_{50}s, R^2 values increased from 0.34 to 0.55 on the inclusion of kinetic parameters (Figure 3). The algorithms developed were used in the prevalidation exercise. A drawback, however, was the fact that it was not possible to have *in vitro* or *in silico* data for all compounds. This was mainly due to the lack of sufficient analytical techniques.



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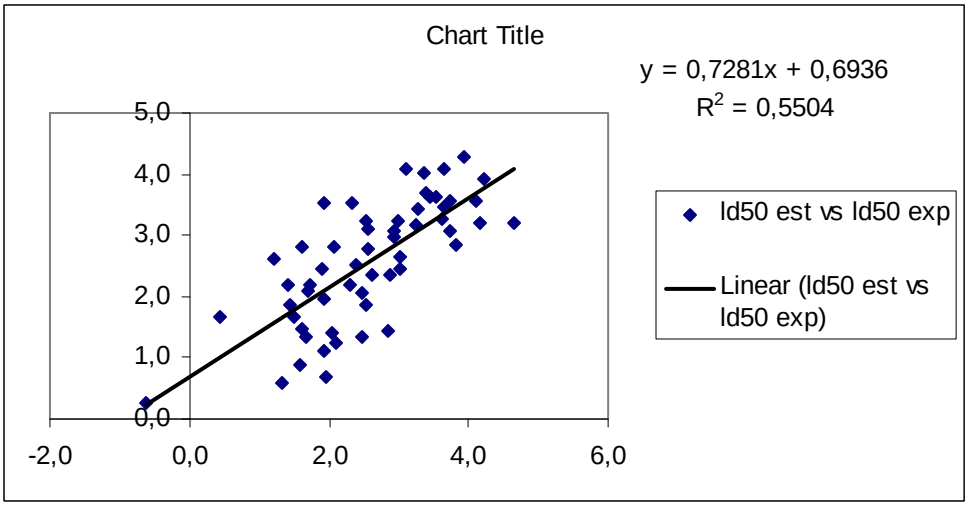

B

Figure 3: EC_{50} for basal cytotoxicity (3T3-NRU test) (X-axis) vs. rat LD_{50}s (Y-axis)

A) Comparison based on log EC_{50} (mM) vs log LD_{50} (g/kg bw)

B) Comparison based on estimated log LD_{50} (g/kg bw), including kinetic parameters vs log LD_{50} (g/kg bw). Note that the comparison is based on the basal rather than specific toxicity. The under-predicted outliers in C are almost all neurotoxic compounds.