WP3: Gradual improvement of predictive capabilities of *in vitro* tests

WP3 is a central work package of the ACuteTox project, which aims to reduce step by step the outliers from the *in vitro/in vivo* correlations, by examining the underlying reasons for deviations, such as the quality of *in vivo* and *in vitro* data, lack of information on ADME and/or target organ specificity. Consequently, WP3 interacts intensively with all the other WPs to identify a set of assays that will provide valuable alerts and corrector factors. The selected assays will be further introduced into a testing strategy.

The objectives in WP3 have been to:

1. Provide a database platform (AcuBase) to facilitate storage of SOPs, transfer of data from all partners (*in vivo* human and animal data, *in vitro* experimental data) and statistical analyses of larger data sets
2. Identify outliers from the *in vivo-in vitro* comparisons of data obtained from WP1 and WP2
3. Support with competence in multivariate modelling as performed in the MEIC (Multicenter Evaluation of In Vitro Cytotoxicity) project (Ekwall et al., 1998, 2000) and to carry out modelling of new data generated in WP1, WP2, and WP4-7.
4. Adapt the *in vitro* methods, selected for inclusion in the testing strategy, to robotic platforms for high-throughput screening (HTS)

1. AcuBase

A pilot version of AcuBase was opened to all ACuteTox partners in March 2006.
The database consists of the following sections:

- Chemicals section – contains the list of 97 ACuteTox reference chemicals with information about the provider (company, Cat number), a set of physico-chemical properties and a short description of the chemical (common use, reported target organ toxicities, some information on human biokinetics, etc.)
- Animal *in vivo* section - contains oral acute toxicity data from animal experiments collected from literature on the 97 reference chemicals
- Human poisoning cases - gathers available data on human acute poisoning cases
- *In vitro* section – is the largest part of the database. An accurately designed, standardized template enables to report practically any experiment performed *in vitro*, including detailed information about the protocol used, the conditions in which the particular experiment was carried on, and the raw data. The in vitro part contains also a section where the SOPs for all in vitro assays used in this project, and truthfully revised by experts in the field, are stored.
- Kinetics - includes data from *in vitro* kinetics experiments and in silico modelling
- Reports – enables to generate summaries of all data in form of Excel files

At the end of June 2007 AcuBase contained a full set of data regarding the selected 97 reference chemicals, including molecular structure, physicochemical properties and summary descriptions. Moreover, 2204 data from acute oral toxicity studies *in vivo*, 2902 data sets from *in vivo* human blood poisoning reports, 6512 files from *in vitro* experiments and 80 SOPs were introduced into the database by partners and/or administrators.

In summary, AcuBase has proven to be a very useful platform for data management and data exchange in such a large international project as ACuteTox.

2. The robotic platform for HTS

One of the endeavours of the ACuteTox project is to provide an *in vitro* testing strategy composed of methods that are amendable to robotic HTS platforms and thus, could be easily automised . In WP3, the 3T3/NR uptake and HepG2/MTT assays have successfully been adapted to two commercially available HTS robotic platforms. Automation of a new cell system assay based on the use of human blood-derived cells -Neo-hepatocytes - has started.

3-4. Multivariate modelling and identification of outliers

In vitro-in vivo modelling of LC50 values for humans and LD50 values for rats have been performed using different combinations of *in vitro* tests. The models show a number of outliers (17 in the comparison with human LC50 values and 16 in the comparison with rat LD50 values) (Table 1).

Furthermore, the *in vitro-in vivo* linear regression analysis has shown that the *in vitro* basal cytotoxicity assays correlated better with human peak lethal blood concentrations (LC50) than with LD50 rat values. From this analysis it was also concluded that the *in vitro-in vivo* correlation based on mol/l and mol/kg was superior to g/l and g/kg.

The identified outliers are now being tested by Partners in WP4-7. In July 2007 approximately 30 best performing *in vitro* assays have been selected for further testing of 41 additional reference compounds (including both outliers and non-outliers). The testing will be finalized in the end of December 2007. The analysis of all the available data will take place in March-April 2008, and shall provide sufficient information to allow WP8 to design a simple *in vitro* testing strategy for the assessment an classification of acute oral toxicity of chemicals. The pre-validation of this strategy should start in May-June 2008.

References:

Ekwall, B. et al (1998) MEIC evaluation of acute systemic toxicity: Part VI. The prediction of human toxicity by rodent LD50 values and results from 61 *in-vitro* methods. *ATLA* 26: 617-658.

Ekwall B. et al (2000). MEIC evaluation of acute systemic toxicity. Part VIII. Multivariate partial least squares evaluation, including the selection of a battery of cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals. *ATLA* 28, 201-234.

Table 1. Outliers identified by comparing mean IC50 values of 3T3 assay with animal LD50 and human LC50 values

Chemical name	Outliers animal LD50	Outliers human LC50
(-)-epinephrine	x	No human data
2,4-dichlorophenoxyacetic acid		x
5-fluorouracil		x
acetaminophen		x
atropine sulfate monohydrate		x
cis-diammineplatinum (II) dichloride		x
codeine		x
cyclosporine A		x
D-amphetamine sulfate	x	No human data
digoxin	x	x
diquat dibromide		x
formaldehyde	x	No human data
lindane		x
malathion		x
methadone hydrochloride		x
nicotine	x	x
ochratoxin A	x	No human data
parathion	x	No human data
pentachlorophenol		x
phenobarbital	x	
physostigmine	x	No human data
potassium cyanide	x	
sodium chloride		x
sodium selenate	x	
strychnine	x	x
thallium sulphate	x	
warfarin	x	