



# Joint Research Centre

## Optimisation and pre-validation of an *in vitro* test strategy for predicting human acute toxicity: progress of the “A-Cute-Tox” project

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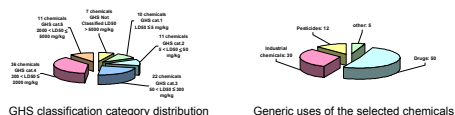
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A-Cute-Tox is a large integrated project under the EU 6FP where 35 partners from 13 European countries are working together for 5 years with the aim to develop and pre-validate an *in vitro* testing strategy for the prediction of human acute systemic toxicity. The project aims to improve the already established correlation (over 70%) between *in vitro* basal cytotoxicity and rodent LD50 values, as well as human lethal blood concentrations, to a level sufficient enough to ensure a valid prediction of human acute toxicity. This will be achieved by integrating cytotoxicity data with additional information on biokinetics, metabolism and target organ toxicity.

### WP1: Selection of 97 reference chemicals

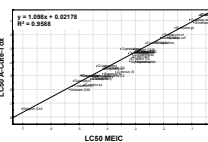
The 97 reference chemicals were selected within a wide range of acute toxicity and generic uses:



### WP1: Evaluation of *in vivo* animal data

- Variability:** log-transformed LD50 have SD <0.5 for majority of chemicals
- Inter-species comparison:** rat vs mouse mean LD50 highly correlated
- Relevance:** regression of MEIC human LC50 vs ACuteTox rat LD50 give coherent correlation similar to the MEIC correlation (human vs animal).
- Reliability:** no trend associating LD50 range with presumed quality rank
- Predictive capacity for classification:** for ~90% chemicals evaluated the LD50 ranges were within 2 adjacent classifications.

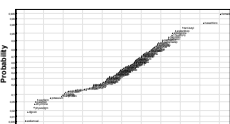
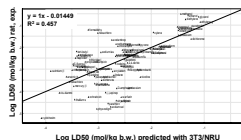
### WP1: Evaluation of human data – calculation of LC50 values



The LC50 values derived for human acute poisoning cases collected from literature within the ACuteTox project are consistent with the LC50 peak values calculated in the MEIC study

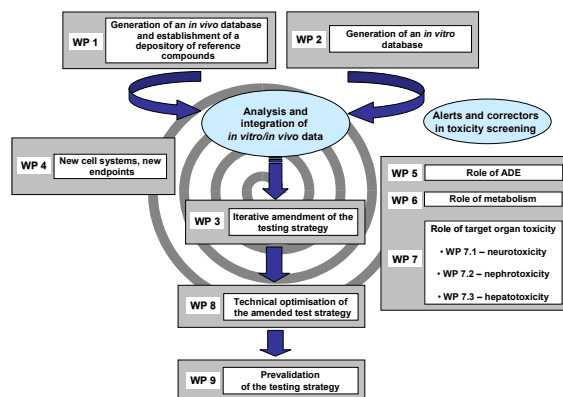
### WP2 & 3: Evaluation of *in vitro* cytotoxicity data

- The seven basal cytotoxicity tests evaluated (3T3/NRU, NHK/NRU, HL-60/ATP, HepG2/NRU, Fa32/NRU, HepG2/total protein, Fa32/total protein) provide similar information (except from colchicine, cycloheximide, hexachlorobenzene, digoxine, 5-fluorouracil).
- In vitro/in vivo* correlation with human data [LC (mol/l)] is better than with LD50 rat values (mol/kg)
- In vitro/in vivo* correlation based on mol/l and mol/kg are superior to g/l and g/kg.

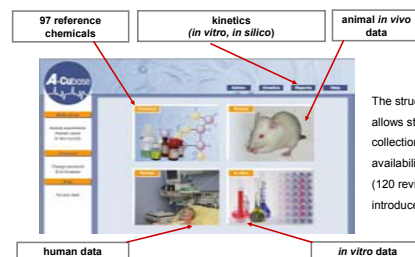


Outliers (n = 14) were identified by normal probability plots of *in vitro* - *in vivo* model residuals

### The structure of the A-Cute-Tox project



### WP3: Development of Acubase



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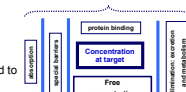
### WP4: New Cell Systems, New Endpoints

Two models:

- Cytokine (IFN- $\gamma$ , IL-5 and TNF- $\alpha$ ) secretion** in human blood-derived mononuclear cells
- CFU-GM assay** performed in human cord blood-derived cells show a very good correlation with the rat oral LD50 values ( $R^2 = 0.843$  and  $R^2 = 0.855$ , respectively; n=25).
- Cytomic multiparametric assays** in HepG2, SH-SY5Y, A.704 cell lines show a very good correlation with the rat oral LD50 values ( $R^2 = 0.77 - 0.89$ , n=5-16).

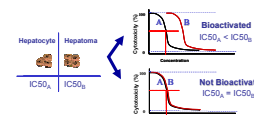
### WP5: Role of ADE (in vitro/in silico)

Measurement of the transport across the **intestinal barrier** and the **blood-brain barrier** using *in vitro* models and **neural networks** (n=21)  
The *in silico* models for oral absorption and BBB passage classify the compounds (n=16) with a 73% and 72% accuracy, respectively, as compared to the *in vitro* models.



Measurement of **protein binding**, **microsomal stability**, **lipophilicity** (n=42)  
Measurement (n=3) and modelling of free concentration of compounds in the *in vitro* systems.  
Generic biokinetic model for the interpretation of *in vitro* toxic concentrations in relation to the *in vivo* acute toxic dose – under development

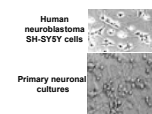
### WP6: Role of metabolism



Compound	Comparison hepatocytes vs HepG2	Reported bioactivation
Albendazole	More toxic to hepatocytes than HepG2	YES
Allyl isothiocyanate	More toxic to hepatocytes than HepG2	NO
Permethrin	Slightly more toxic to hepatocytes than HepG2	YES
Rotenone	Slightly more toxic to hepatocytes than HepG2	NO
Tetrahydrocannabinol	Slightly more toxic to hepatocytes than HepG2	NO
Chlorpyrifos	Similar toxic to hepatocytes and HepG2	NO
Fluazacyn	Similar toxic to hepatocytes and HepG2	NO
Malathion	Similar toxic to hepatocytes and HepG2	YES
Acetaminophen	Similar toxic to hepatocytes and HepG2	NO
Endosulfan sulfate	Similar toxic to hepatocytes and HepG2	YES
Dibutyltin dilaurate	Less toxic to hepatocytes than HepG2	NO
Diacyl	Less toxic to hepatocytes than HepG2	NO
Vanilic hydrochloride	Less toxic to hepatocytes than HepG2	NO

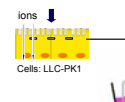
### WP7: target organ toxicity

#### WP7.1 NERVOUS SYSTEM



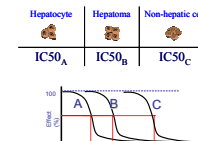
- Assays selected based on the results obtained with 24 compounds - *in vitro* data were compared with human LC50 values:
- GABA-A receptor function** in rat primary cortical neurons (IC50/EC50)
  - Cell membrane potential** in SH-SY5Y cells (LOEC)
  - AChE inhibition** using the pure enzyme assay (IC50)
  - GFAP & neurofilament mRNa expression** in rat brain aggregates (LOEC)
  - Caspase-3 mRNA expression** in mouse cerebellar granule cells (LOEC)

#### WP7.2 KIDNEY



- Trans-epithelial electrical resistance (TER)** is a sensitive indicator of nephrotoxicity and showed greater sensitivity for nephrotoxic chemicals compared to non-nephrotoxic chemicals (n=21)
- Compounds requiring metabolism (diethylene glycol) did not show toxicity at concentrations used

#### WP7.3 LIVER



$IC_{50}(A) < IC_{50}(B) = IC_{50}(C)$ :  
“hepatotoxic” (bioactivable) → **alert**  
 $IC_{50}(A) = IC_{50}(B) < IC_{50}(C)$ :  
“hepatotoxic” → **alert**  
 $IC_{50}(A) = IC_{50}(B) = IC_{50}(C)$ :  
no hepatotoxic → **no alert**



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