Main achievements and impact of the project on industry and research sector

In the ACuteTox project undertook **for the first time** a challenging goal **to create an integrated testing strategy** to **replace the animal testing used today for predicting human acute oral systemic toxicity** which is based exclusively on *in vitro* and *in silico* methods, with the aim.

This project represents also the **first attempt to pre-validate a testing strategy** based exclusively on non-animal methods, therefore it provides an excellent case study for ECVAM and helps progressing the discussions on validation of testing strategies which are currently ongoing at several levels (ECVAM, European Partnership for Alternative Approaches to Animal Testing (EPAA), COLIPA, ECHA and others). Indeed, this case study has been already presented at the recent EPAA/ECVAM workshop on Validation of Integrated Testing Strategies (Ispra, Italy, 12-13 October 2009).

In the first phase of the project, a very large number of *in vitro* test methods (approximately 75 endpoints) have been evaluated in terms of their within-laboratory variability (and in some cases also the between-laboratory variability), preliminary predictive capacity and the potential to identify alerts for organ-specific toxicity, The outcome of this phase of the project is a **large toolbox of *in vitro* methods** with associated optimised protocols, some of them evaluated to the level of prevalidation.

An in depth statistical analysis of the large dataset generated in this project resulted in a **list of 8 *in vitro* and *in silico* methods,** which resulted to be the most promising for inclusion in proposal of potential testing strategies. Protocols of all these methods will be available to the public as INVITTOX protocols, through the ECVAM database on alternative methods (DB-Alm).

The last phase of the ACuteTox project focused mainly on the assessment of the predictive capacity of the proposed tiered testing strategies and the identification of assay combinations that give the best prediction in terms of classifying chemicals into the official acute oral toxicity categories (GHS and EU CLP system). Five **proposals for *in vitro* tiered testing strategies** were formulated and evaluated in terms of predictivity.

The outcome of this analysis reinforced previous results obtained with the **3T3/NRU assays** and supports the use of this validated cytotoxicity assay to identify unclassified substances (LD_{50} > 2000 mg/kg), as a **first step in a tiered testing strategy**.

Several ***in vitro* assays** have proved to be **useful to identify alerts for tissue specific toxicities** such as neurotoxicity and nephrotoxicity. However, the results of the classification analysis showed that complementing the 3T3/NRU assay with those *in vitro* assays is not improving significantly the classification of compounds in toxicity categories 1-4. This outcome is largely linked to the fact that the current classification systems are based on arbitrarily assigned cut-offs for the rat LD_{50} values, and do not include more detailed scientific (mechanistic) information on the compounds. Thus, a revision of the current classifications schemes might be advisable and should be put forward to the European regulatory agencies.

Most of the results obtained in the course of the ACuteTox project have resulted in **peer review publications**, which proves the scientific quality of the data generated. Some of the knowledge derived from the project has been already applied in the daily activities of companies which are ACuteTox partners (e.g. pharmaceutical sector). This goes beyond the main objective of the ACuteTox project (prediction of acute oral toxicity) and helps improving European competitiveness. Examples include, for instance, the case of Noscira, which increased the confidence in the results obtained by using some of the neurotoxicity models that were optimised during the project.

In the same direction, the project allowed to establish interesting **specific collaborations** with individual partners covering e.g. aspects of hepatotoxicity and metabolism; not only at the experimental level, but also in the preparation of dossiers and documents being presented to both the European Medicinal Agency and the US-FDA, which in the end is a definitive success of the project.

Recommendations

The results obtained from the classification analysis performed in the ACuteTox project lead us to question the scientific motivation for the current classification systems for acute oral toxicity and to suggest the revision of the GHS/EU CLP systems.

The outcome of the analysis of consistency in classification (GHS and EU categories) corresponding to reported ranges of LD_{50} respective of individual 97 substances included in the ACuteTox project give an indication of potential consequence for ambiguity in corresponding classification. The analysis showed that (with at least 90% probability) ~50% of the substances would be unequivocally classified by a single category, ~40% would ambiguously occur within the limits of two adjacent classification categories, and ~10% of the substances have LD_{50} ranges of sufficient scope to span three or more different classifications. This analysis reinforced previous findings by Rudén and Hansson (2003) and leads to the same recommendation of revision of the GHS/CLP system.

The estimation of the oral dose by including kinetic parameters needs to be further evaluated, in particular the availability of well established in house validated analytical methods for non-drug like compounds is at present a limiting factor, and requires further investment in future strategies.

If more data on kinetic parameters were available, a better evaluation of the impact of the different kinetic factors, i.e absorption, distribution (e.g. lipophilicity, protein binding) needs to be made.