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ACuteTox
– Research Project For Alternative Testing

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Summary of results from ACuteTox scientific work packages

It is a great joy to present this year's first newsletter from the project ACuteTox. Our foremost aim is to develop a testing strategy for predicting acute oral toxicity of chemicals without the use of laboratory animals. This strategy could replace the animal acute oral toxicity tests used today for regulatory purposes. Several important steps were taken during the past year to realise this goal. This newsletter summarises our main results achieved in 2006.

Three highlights from the results obtained in the past year

- Generation of high quality animal, human and *in vitro* databases on acute toxicity of 97 reference compounds;
- A number of test assays were improved and adapted to two commercially available screening robotic platforms.
- The concept of the testing strategy has been developed.

Background

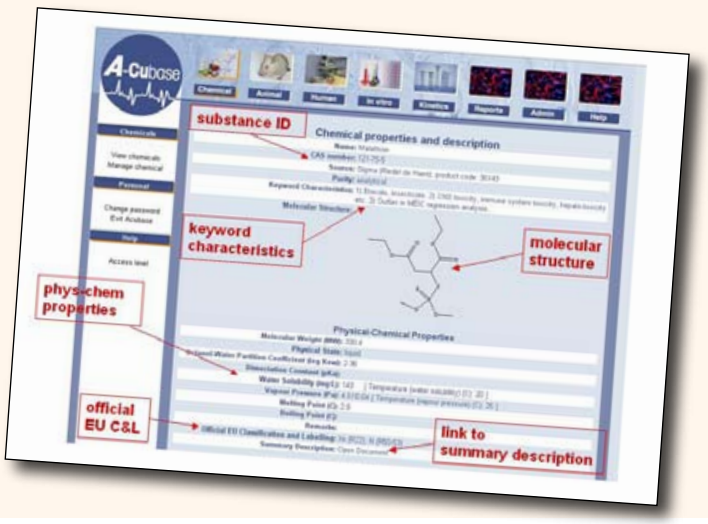
The extensive amount of work performed since the 70´s has led to development and optimisation of a large number of assays for the assessment of acute oral toxicity testing, based entirely on the use of cell cultures *in vitro*. Many studies have shown that a good correlation (70%) exists between data from cell culture tests and animal testing, as well as human lethal blood concentrations in the MEIC project (Multicenter Evaluation of *In Vitro* Cytotoxicity). This means, however, that when using the existing tests, a certain number of misclassifications will occur. ACuteTox, an EU integrated project that started in January 2005, aims to identify the factors that can improve the correlation between *in vitro* and *in vivo* data for acute oral systemic toxicity testing and screening.

The project is divided in 9 scientific work packages (WP)

WP1: The generation of a high quality in vivo (animal and human) database
WP2: The generation of a high quality in vitro database
WP3: Iterative amendment of the testing strategy
WP4: New cell systems and new endpoints
WP5: Role of adsorption, distribution and elimination
WP6: Role of metabolism
WP7: Role of target organ toxicity (brain, kidney and liver)
WP8: Technical optimisation of the modified testing strategy
WP9: Prevalidation of the testing strategy

The efforts to establish the *in vitro* testing strategy are gradually yielding results.

WP1 and WP2: The generation of high quality *in vivo* and *in vitro* databases



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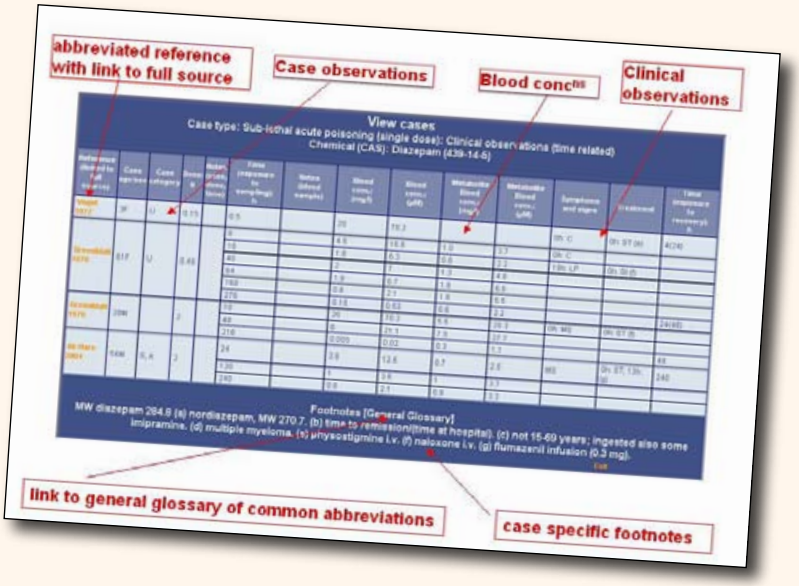
We have generated high quality databases on acute toxicity of the 97 ACuteTox reference compounds for both in vivo (animal and human) and *in vitro* data. The project database (AcuBase) contains LD50 (lethal dose required to cause death of 50% of the animals in the tested population) values from 2206 animal studies, as well as information on human toxicity from 2902 cases reports, including acute sub-lethal and lethal blood concentrations. We have also completed testing of the reference chemicals using six cell culture systems and basal toxicity as endpoint. The cytotoxicity data for most of the 97 reference compounds are now available in AcuBase.

WP3: Iterative amendment of the testing strategy

The main achievement during 2006 was the establishment of a central database, AcuBase, for coherent management of all data collected and generated during the project.
One of the aims of the ACuteTox project is to adapt the successful toxicity testing methods to an industrial scale. This is done in order to be able to test thousands of chemicals and compounds. In ***WP3*** we have effectively improved and adapted some cell models to commercially available screening robotic platforms.

WP4: New cell systems and new endpoints

In WP4, our goal is to provide an alternative way to improve the prediction of acute toxicity by using more specific parameters, and/or cell models from blood cells. We have succeeded in obtaining data for 20 reference chemicals and the results are now being analysed. The results show good correlation with human blood LC50 values.



WP5: Role of administration, distribution and elimination

Some kinetic aspects of the reference chemicals (adsorption, distribution and elimination) have been studied using cell culture models and/or computer-based kinetic modelling. We have developed a multiprocessor

computer system (neural) that is useful to estimate oral drug uptake and passage through the blood-brain-barrier (BBB), the natural barrier protecting the brain.

Results from three variants of the Caco-2 model for the prediction of drug uptake, used in three different laboratories, have been compared. The results obtained with 20 reference chemicals show good agreement between the different cell models used. Toxicity studies and permeability studies using *in vitro* BBB models have been performed for 22 and 19 compounds, respectively. They show relatively good correlation with human data. We have also successfully used a technique that measures the partitioning of a number of polycyclic aromatic hydrocarbons within different compartments of a cell culture system. In addition, data on protein binding have been obtained. The data obtained in WP5 are now the basis of further modelling of dynamic interaction between chemicals.

WP6: Role of metabolism

In order to evaluate whether compound's toxicity is dependent on its metabolism, the effects of 21 reference compounds have been studied in a metabolically active and a non-metabolically competent cell culture model and toxicity was compared between the two cell culture models.

In WP6 we also have investigated computer programs that estimate the toxicity and metabolic fate of compounds with known metabolism. Fourteen ACuteTox reference compounds were analysed. One program seems to be a promising alternative for the prediction of metabolism while the other program can give important information of the toxic profile of the tested substances.

WP7: Role of target organ toxicity

Neurotoxic chemicals have been studied in several human and animal cell culture models by using more

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than 20 different specific endpoints. The results show that many *in vitro* assays investigated could correctly identify the specific mechanisms of neurotoxicity of the selected reference compounds. However, the challenge is to find more general assays that could pick up several different neurotoxic mechanisms.

The toxicity in kidney was measured by a functional assay in a renal proximal tubular cell line. Twenty one reference chemicals were tested and the results show that the assay is a promising model for measuring nephrotoxicity.

We are also in the process to identify a set of markers for acute liver toxicity. A protocol for testing potential liver toxic chemicals is being developed.

Summary

During the first half year of 2007 has the development of methods and end points continued. The just concluded midterm meeting decided to test 40 new reference chemicals in the most relevant assays. Testing of the selected reference compounds will continue within WP4-WP7the different work packages until December 2007 when the evaluation of all data will be performed.

Testing of the selected reference compounds will continue within the different work packages until December 2007 when the evaluation of all data will be performed. Based on the outcome of this evaluation we will select the best performing tests, which could improve the prediction of acute oral toxicity. Our aim is, however, not just to improve the prediction, but also to identify which compounds require further testing because their acute oral toxicity cannot be properly predicted. Using the first data generated by the partners a draft of the testing strategy has been proposed in ***WP8***. The strategy, which consists of a stepwise approach, will be further developed during 2008.

To facilitate the prevalidation process during the two last years of the project, the work in WP9 has already started. Common Standard Operating Procedure templates have been prepared and are used by all partners in ACuteTox. Furthermore, a list of criteria for the selection of promising methods that will be included in the testing strategy have been prepared as well as a common template, which will be used by work package leaders to report the results of the promising models that will enter evaluation before the prevalidation exercise starts.

It is expected that a formal validation of the final testing strategy will lead to regulatory acceptance and its incorporation into the set of standardized test guidelines for chemicals hazard assessment.

More information about the latest progress and the midterm meeting will soon be published on the *http://www.acutetox.org* and the next newsletter will be distributed in October.

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