The SCARLET workshop

SCARLET discussed, during a workshop organised in April 2008 in Milan, the state-of-the-art of *in silico* models for carcinogenicity and mutagenicity. The discussion started with an overview of the new *in vitro* experimental approaches. A major issue is the occurrence of false negatives and positives. The precautionary principle is pointing towards a conservative approach, which tends to eliminate chemicals with positive results, but the use of batteries of *in vitro* methods is progressively eliminating most of the compounds, with an increasing number of false positives.

Nevertheless, *in vitro* methods are used for regulatory purposes, and they are required information within many evaluation protocols and laws. It was mentioned that when the Ames test was originally introduced, it was severely criticised, as not suitable to replace carcinogenicity studies. Today it is widely accepted that mutagenicity studies are a valid support to the general evaluation of the genotoxicity studies. It is expected that a similar evolution will happen also for *in silico* methods. Indeed, there is a knowledge gap on the carcinogenicity and mutagenicity evaluation of the chemical compounds. Any useful piece of information should be used to limit toxic effects. Alternative methods, such as *in vitro* and *in vivo* methods, can contribute to cover this knowledge gap.

The positions of industry and the regulation frameworks

At the workshop the positions of industry and regulators were discussed. Pharmaceutical companies, chemical, cosmetics, and food industries have needs, which are partly similar, partly different. Different regulations apply, which vary in the countries. In Europe the regulation for cosmetics is progressively banning all animal experiments. Thus, only alternative methods will be used. Different is the situation for pharmaceutical companies, which will continue using animals. The REACH regulation is promoting the use of methods alternative to animal models. However, their reliability has to be proved.

Furthermore, in USA certain *in silico* models are used when providing data for the Food and Drug Administration, while they are not so popular in Europe.

Regulators and industry may have different attention to false negatives (of high concern for regulators) and false positives (which cause ban of certain compounds which have industrial interest, but no toxicity).

Furthermore, the overall evaluation of a certain chemical compound may be different in the different industrial sectors, considering for instance the balance between the risk and the benefits, or if the compound is a natural food component.

The different *in silico* methods

More and more *in silico* methods are appearing. They differ for the theoretical basis and for the purposes. Thus it is not appropriate to evaluate them with a single perspective. Conversely, the practice in several industries is to adopt a battery of *in silico* tools.

Several commercial/public software and databases are available, and a number of methods have been published in the literature. Some methods are based on the human expert knowledge, codified into rules, while other methods are based on knowledge engineering techniques. Some methods use chemical descriptors, others fragments, in particular active sub-structural components. Some methods predict toxicity categories, others predict the toxicity potency. Some methods are global (ideally useful for a large set of compounds, even if restrictions exist), others apply to a much more specific skeleton/chemical class.

The accuracy of the models for mutagenicity is higher, around 75-80%, while for carcinogenicity it is lower (65%).

It is important to consider the reproducibility of the experimental methods (for instance for mutagenicity it is 85%).

The purposes of the model can be different, and thus its evaluation procedure. For screening/prioritisation purposes all methods can be useful, provided that they enrich a certain component (for instance they identify many of the genotoxic compounds). For a more precise evaluation, stricter criteria apply, and many methods may not be suitable.

The perspectives

In such a broad scenario, with different components, not only theoretical, but also related to the application and use, complex, multiple solutions exist. The challenge is to tackle the problem in its complexity, taking advantage of the new tools offered by new assays and the omics techniques. The concept of toxicity profiles has gained new prominence. *In silico* methods can benefit from this increased information, and better contribute to carcinogenicity studies.