Session II-5: Validation of Three Rs alternative methods

Session II-5: Oral presentations

II-5-561

Validation of the 21st Century Toxicology Toolbox: challenges, opportunities, and the way forward

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Increasing efforts are being directed at finding improved innovative methods for assessing whether chemicals may cause adverse health effects. Collectively referred to as the 21st Century Toxicology Toolbox, these methods includes a wide range of tools that increasingly incorporate understanding and detection of the molecular, genetic, structural, and cellular perturbations of pathways and mechanisms that may lead to adverse health outcomes. Applications include toxicogenomics, metabolomics, proteomics, cell based assays, biochemical activity profiles, and computational models. These tools are used to create complex biological activity profiles, with an expectation that these will eventually predict toxicity and safety without the use of animals. Much of this profile data will initially be used for prioritizing chemicals for further testing in validated test methods, decisions on product development, as mechanistic data to inform weight of evidence decisions on chemical safety, hazard, and risks, and to reduce uncertainties in risk assessment. Using such data to make regulatory risk assessment decisions will require validation to demonstrate that the proposed decision strategies can provide equivalent or improved protection of consumers and workers compared to existing test methods. Flexibility in the validation of these new tools and strategies is essential, and will vary depending on the intended purpose, applicability domain, and existing data for the proposed tools. Consideration and use of appropriate validation strategies early in the test method development process is expected to expedite acceptance of new tools and approaches that will provide improved predictions of safety and hazard and reduce and replace animal use.

II-5-688

Post-approval validation issues: Experience with the 3T3 NRU *in vitro* phototoxicity assay

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Validation of alternative toxicological tests is historically done by comparing results generated by the alternative test against results generated by the accepted "gold standard". However, due to many limitations the number of compounds that can be used for validation remains rather small in comparison to the entire chemical universe. It is therefore logical to assume that the practical application of a validated test will generate information that can and should be used to review the test performance and to improve the original method, if needed.

Such a "post-validation" review was initiated in 2009 by EFPIA, when it informed ECVAM that it would have data from industry that indicate that the 3T3 NRU phototoxicity test, when applied to oral intake, generates an inacceptable high number of false positives, hence triggering a large number of confirmatory animal tests. ECVAM then proposed to organise a workshop with EFPIA where industry could present its data and phototoxicity experts could discuss how to approach the issue.

This workshop took place in October 2010 and the workshop report will most likely be published before the WC8. However, this paper will not address the specific finding of the workshop but discuss the overall usefulness of such reviews and the conditions to make these a success.

As a second example some cell transformation assays will be discussed for which ECVAM proposes that standardised protocols, developed in the course of a prevalidation study concluded in 2010, should be used for future application of that type of test. ECVAM also invites test users to provide ECVAM with the test results in order to allow a future review of the methods.

II-5-289

Reduction of animal use through validation of a chemical method of detection for paralytic shellfish toxins

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Shellfish toxins are produced by algae and accumulate in filter-feeding shellfish. Paralytic shellfish toxins (PSTs) are particularly serious since toxin levels can rise quickly and cause death. The Canadian Food Inspection Agency (CFIA) analyses more than 11,000 samples of Canadian shellfish for PST levels each year. Until recently the reference method for PSTs was a mouse bioassay (MBA) which used three mice/sample for a total of approximately 40,000 mice/year. In 2005 a collaborative, multi-year project was initiated with the National Research Council Canada to develop and validate a chemical PST method to replace the MBA.

Available methods were evaluated. The best alternative was optimized for a high-throughput regulatory laboratory and used in parallel with MBA testing for one summer as a pilot project to overcome challenges with high sample load. A single lab validation was then completed at the CFIA Dartmouth Laboratory, followed by approval from Canadian and US officials to use this method for screening during a transition period. During this transition period a small percentage of samples received by the laboratory (10%) and all results requiring regulatory action were confirmed with MBA. Animal use was decreased by 75%. To gain international acceptance of the new chemistry-based procedure a collaborative study was carried out among labs from ten countries. This study confirmed the method could be implemented successfully in different laboratories and has led to the method being granted official method status by AOAC International. Animal testing for PSTs in all CFIA labs has now been reduced or eliminated.

II-5-203

The limited value of acute toxicity tests in safety assessment

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A collaboration, led by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and AstraZeneca, analysed data from 70 compounds across therapeutic areas and demonstrated that acute toxicity studies had no value in assessing risk before the first clinical trials in humans. Additionally, consensus between clinicians, toxicologists, regulators and directors of Poison Centres has been reached that acute toxicity studies are not used for managing overdose of pharmaceuticals and are of little value in treating human poisoning from chemicals. Therefore, the last remaining driver for acute toxicity studies for pharmaceuticals has been removed.

The impact of the pharmaceutical sector initiative has stimulated efforts to explore the value of acute toxicity testing for other sectors. An expert working group, led by the NC3Rs, has highlighted circumstances where acute toxicity testing of non-pharmaceutical chemicals is redundant and may be avoided.

In addition, the European Partnership for Alternative Approaches to Animal Testing (EPAA), has established a multi-stakeholder team (including AstraZeneca, NC3Rs, ECVAM, the Humane Society and representatives of industry sectors) which has demonstrated that the primary regulatory driver for conducting acute toxicity studies across non-pharmaceutical sectors is for classification and labelling. Further work into the value of acute toxicity studies for classification purposes is ongoing.

These collaborations demonstrate the opportunities provided by creating a forum for a wide range of stakeholders to review whether animal toxicity studies are providing the information needed to make assessments of risk to human safety. The results will enable consensus to be reached on how to reduce the number of animals used and make the drug and chemical development process more efficient.

Session II-5: Poster presentations

II-5-192

Developing regulatory acceptable *in vitro* alternatives to established *in vivo* assays

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In the last 15 years there has been a significant change in the safety assessment paradigm that has seen the introduction and regulatory acceptance of an increasing number of alternative *in vitro* methods as replacement for, or supplement to, existing *in vivo* test strategies. This has been driven by both the search for scientific advancement and ethical considerations. Within the EU, in particular, this has also been driven by changes in the regulatory legislation relating to chemical (REACH) and consumer products (7th Amendment to the Cosmetics Directive). A major challenge in developing alternative methods is the need to validate against a known human endpoint and not *in vivo* animal data. In this presentation we will discuss some of the assays (e.g. dermal absorption, skin and eye irritation, phototoxicity, drug transporter and hepatic metabolism) that have been developed over the last 15 years. We will highlight how these have assisted in achieving the goals of the 3Rs and discuss some of the issues facing the validation of new assays for more complex toxicological endpoints.

II-5-321

InVitroJobs – communication network and job platform presents "Working group – a Portrait"

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Against the backdrop of European legislation (REACH, Cosmetics Directive, new EU Directive on animal experiments), the development of replacement methods gains particular significance. The Federal Association People for Animal Rights has creating the internet platform InVitroJobs, providing support via the publication of job offers, thesis assignments and internships for budding scientists and students interested in working with animal-free methods. InVitroJobs also offers a forum to many international and renowned working groups with promising approaches to replacing animal experiments in research, development or service.

Since its online launch in 2009, InVitroJobs has grown continuously, with job offers, working groups and online visitors on the rise (over 90,000 visitors so far, more than 400 per week). Approximately 50% come from Germany, followed by visitors from the United States. 15 percent of all clicks were job offer-related, followed by searches for in-depth information on *in vitro* and *in silico* working groups.

As the results of the working groups' research is often known only to professionals, and above all because there is a need for an overview of contents and methods in the area of replacement methods, InVitroJobs has recently started a regular description of scientists and their innovative research, "Working Group – a Portrait". The focus is on newly-developed methods, their evaluation and a forecast as to which animal experiments they can replace. The presentation of the first working group immediately generated considerable interest in participation. This indicates the need for public education and presentation.

II-5-371

An evaluation of the Reconstructed human Epidermis (RhE) method for predicting skin corrosivity of chemical products with extreme acid pH

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The purpose of this analysis was to evaluate the Reconstructed human Epidermis (RhE) model as an *in vitro* method to predict skin corrosivity (OECD 431) for acid products with extreme pH (< 2) when compared with *in vivo* data and the AISE Method (The Worst Case Table) of classification. Extreme pH can be a useful predictor of irritation but may lead to over classification in weakly buffered systems. Our objective was to determine whether the RhE model could accurately identify corrosive and non-corrosive acid products. When compared with the *in vivo* data, 2/7 products tested using the RhE method predicted the same skin classification. The skin classification of the remaining five formulas was over-predicted when compared with the *in vivo* data. There were no products for which the RhE under-predicted the skin classification when compared to the *in vivo* results. When compared with the AISE Method (which considers the results of the EU conventional method calculation and pH/acid reserve), 8/23 products tested using a RhE method predicted the same skin classification. The skin classification of the remaining fifteen formulas was over-predicted when compared with the AISE Method. There were no products in which the RhE under-predicted the skin classification when compared to the AISE method. Overall, the RhE did not reliably identify non-corrosive formulations when compared to either the *in vivo* data or the AISE method. This presents significant challenges under hazard classification guidelines such as the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) which recommends testing with a validated *in vitro* method to confirm a non-corrosive classification for an extreme pH product.

II-5-534

Update on validation status and industry utilization of normal human 3D (NHu-3D) tissue models in toxicology

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Progress in *in vitro* tissue engineering over the last several decades has contributed to a significant decrease in animal use for industrial risk and safety assessment of products and ingredients. MatTek Corporation has a long-standing involvement in development, production and validation of animal alternative *in vitro* models produced from normal human cells (NHu-3D models). The currently available suite of NHu-3D models includes: Skin (EpiDerm, MelanoDerm, EpiDerm-FT), Ocular (EpiOcular, EpiOcular-FT), Airway (EpiAirway, EpiAirway-FT), Vaginal (EpiVaginal, EpiVaginal-FT), Oral (EpiOral, EpiGingival, EpiGingival-FT) and Dendritic Cells (DC-100). EpiDerm NHu-3D skin models are widely utilized in preclinical toxicology and efficacy applications during industrial product development (e.g. skin penetration, skin lightening, irritation, corrosion, ingredient efficacy and wound healing). In addition, the EpiDerm model is formally validated by ECVAM and accepted by OECD for regulatory use in skin corrosion (OECD TG 431, 2004), and irritation (OECD TG 439, 2009) and is pre-validated for phototoxicity testing. The EpiOcular model is widely utilized for ocular irritation studies during industrial product development. The EpiOcular EF50 assay is currently an EPA accepted method for testing of antimicrobial cleaning products. In addition, an EpiOcular eye irritation test (EIT) is currently undergoing formal COLIPA-ECVAM validation for regulatory use. Additional NHu-3D models and assays for toxicity, allergenicity, genotoxicity, and other animal alternative applications are under development. Thus, NHu-3D models have made significant contributions to reduction of animal use in industrial product development and regulatory testing. MatTek will continue to devote focused efforts toward further elimination of animal use in biological science and regulatory testing.

II-5-645

Multi-study validation trial for cytochrome P450 induction providing a reliable human-metabolic competent standard model or method using the human cryoHepaRG® cell line and cryopreserved human hepatocytes

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The Institute IHCP-JRC of the European Commission organised under the auspices of ECVAM a stakeholder meeting in the field of Toxicokinetics and Metabolism in which the basis for the current multi-study validation trial for cytochrome P-450 (CYP) induction was established. The main objective of this trial is the provision of a reliable human-metabolic competent standard model or method for use in integrated testing strategies. It was agreed to initially assess the potential for CYP induction at clinically relevant doses in selected *in vitro* test systems since CYP induction is a sensitive indicator of *de novo* protein synthesis. In this way human clinically obtained data on CYP induction could be used as the reference data. Based on the new legislation for cosmetics (Directive 2003/15/EC) and chemicals (REACH Regulation 1907/2006/EC) emphasising the need for alternative methods and integrated non-animal test strategies concerns have been raised over the need for regulatory purposes of a reliable and relevant human metabolic competent source modelling the process of xenobiotic biotransformation.

The CYP induction validation trial will assess the reliability (reproducibility within- and between-laboratories) and relevance (ability to assess *in vivo* human CYP induction) of two test systems (cells in culture) with a challenging set of test items (chemicals) for which high quality *in vivo* data are available. In this validation study the test systems cryopreserved HepaRG® and cryopreserved human hepatocytes will be used. Five test facilities are involved including, the CRO's Pharmacelsus and Kaly-Cell as the lead laboratories, the pharmaceutical companies Astra Zeneca and Janssens Pharmaceutica, a division of Johnson and Johnson and the European Commission JRC ECVAM *In vitro*-Methods Unit and Systems Toxicology laboratories. The result of this validation trial will be the starting point for a novel *in vitro* platform for assessing biotransformation and toxicity.