Mini-Seminar on Alternatives and Risk Assessment

Cefic
The European Chemical Industry Council,
4 Avenue E. Van Nieuwenhuyse
B-1160 Brussels

18th May 2009

Organised by;

Cefic-LRI and the EPAA organised a mini-seminar on 3R alternatives and risk assessment. The event was hosted by Cefic at their offices in Brussels on May 18th 2009. The objective of the seminar was to provide a forum for the dissemination and exchange of information on the status of alternatives as tools for risk assessment within the USA and Europe.

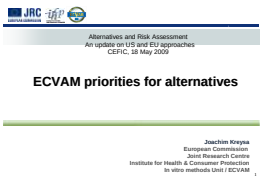
Programme

1. Alan Goldberg (CAAT, John Hopkins University)

   Update on the status of the 3Rs in the USA (40 mins)

2. Joachim Kreysa (ECVAM, Joint Research Centre)

   ECVAM priorities for alternatives (20 mins)



3. Watze De Wolf (Dupont)

   Industry efforts and perspective on alternatives in REACH (20 mins)

4. Andrea Tilche (DG Research, European Commission)

   Research support for 3Rs alternatives in Europe (20 mins)

5. Deborah Declercq (Straticell)

   SMEs experiences and needs in Europe (15 mins)

6. Syed Ahmed Mustafa (Ceetox Inc.)

   SMEs experiences and needs in the USA (15 mins)

7. Moderated by Dick Philip (Exxon Mobil Chemicals and chair Cefic-LRI SIG)

   Panel[1] Discussion (20 mins)

[1] Composed of speakers and Karin Kilian from DG Health & Consumer Protection

Summary

The keynote presentation - "Update on the status of the 3Rs in the USA" - was given by Alan Goldberg from the John Hopkins Center for Alternatives to Animal Testing (CAAT). The main highlight was the suggestion that the landscape in the US has reached a “tipping point” and that a rapid change in the acceptability and implementation of 3Rs alternatives was about to occur. This shift is a consequence of “toxic ignorance”, significant proliferation of diseases of unknown aetiology, a growing legislative demand to address these first to factors and the availability of a new vision of what future toxicology might actually look like (i.e., the National Toxicology Programme Roadmap and the National Academy of Sciences Vision and Strategy for the 21st Century).

Joachim Kreysa (Head of the In Vitro Methods Unit of from ECVAM) gave a presentation on "ECVAM Priorities for Alternatives". Priorities will be driven by EU legislation such as REACH, Cosmetics, Pesticides and the revision of EEC/86/609. There was the recognition that whilst problematic complex endpoints will require complex test methods (i.e., entailing intelligent testing strategies) and complex validation, progress can only come when such methods are applied and accepted by users and competent authorities. A new vision for organization of ECVAM activities was also shared.

Watze De Wolf from Dupont provided an outline of "Industry efforts and perspective on alternatives in REACH". Cefic has supported considerable range of activities to aid compliance with REACH. This is particularly the case for computational tools to support ITS, QSARs and Read-Across.

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Andrea Tilche from DG Research provided an overview of "Research Support for 3Rs Alternatives in Europe". In particular, the substantive efforts supported under the FP6 and FP7 Programmes.

The final two speakers delivered new perspectives from contract research organizations (CRO) from both sides of the Atlantic. Deborah Declercq from Straticell and Syed Ahmed Mustafa from Ceetox Inc provided views of "SME Experiences and Needs” in Europe and the USA respectively. Demand for alternatives is ultimately driven by legislation and expenditure in the development of alternative methods or test method capacity is necessarily determined by the expectation of return on investment. The opportunity to employ alternatives is therefore partly driven by marketing considerations.

A panel discussion was moderated by Dick Phillips from Exxon-Mobil, who is also the chair of the Cefic Long Range Research Initiative (LRI) Strategic Implementation Group (SIG). Topics of discussion included the regulatory acceptance of alternatives, effectiveness and translation of research investment into practicable methodologies and the landscape for SMEs.

Presentation Reports

Key Note Speaker: Alan Goldberg (John Hopkins Center for Alternatives to Animal Testing, CAAT)

Title: Update on the status of the 3Rs in the USA

The central premise was the suggestion that the landscape in the US has reached a "tipping point" and that a rapid change in the acceptability and implementation of 3Rs alternatives was about to occur. This shift is a consequence of "toxic ignorance", significant proliferation of diseases of unknown aetiology, a growing legislative demand to address these first to factors and the availability of a new vision of what future toxicology might actually look like (i.e., the National Toxicology Programme Roadmap and the National Academy of Sciences Vision and Strategy for the 21st Century).

Toxic ignorance was used to describe the situation where a lack of publicly available hazard data prohibits meaningful risk assessment of the use of a significant proportion of chemicals in commerce (including HPV substances). Solving toxic ignorance is in itself problematic. A lack of resources (i.e., human capital, laboratory infrastructure, finance) combined with animal welfare concerns would tend to preclude a large scale comprehensive testing programme intended to address the very many data gaps. This necessitates a new approach. The rising incidence of many diseases (e.g., breast cancer, prostrate cancer, autism etc) has often been attributed or related to environmental exposure to chemicals. Without full knowledge of the toxicological profile or effective exposure, the reality of such associations remains unknown.

Various legislative developments in the USA - either at state level (e.g., in California) or at federal level – have highlighted the fact that relatively few materials have been fully characterised. Increased legislation oversight will clearly be a significant driver to expanding knowledge on the hazard profile of the majority of chemicals in commerce (i.e., 80,000). An argument was also advanced that more humane science can correspond to better science. One example, cited was the FDA requirement to employ human cell cultures for *in vitro* metabolic studies. This has significantly reduced the failure rate for subsequent clinical studies.

The notion of a "tipping-point" point being reached was illustrated by highlighting recent developments in toxicological sciences in the USA. Namely, the "National Toxicology Programme (NTP) Roadmap for the 21st Century" (2006), the NAS report on "Toxicity Testing in the 21st Century: Vision and Strategy" (2007) and the EPA-NIH Memorandum of Understanding on "Developing the Science for the NAS 21st Century Vision" (2008). Collectively, these initiatives emphasis the role of the 3Rs, the need for investigator training in humane techniques, the potential limitations of animal studies (i.e., time, expense, predictability etc.), the benefits of a systems biology approach, the role of a mechanistic studies and the importance of the use of human cells. Together this will translate to a shift away from primarily *in vivo* animal studies to *in vitro* assays, *in vivo* assays with lower organisms and computational

modeling for toxicity assessments. As such these major initiatives mark the start of a significant policy change and will come to be characterized as “beginning of the end” of animal testing.

Speaker: Joachim Kreysa (ECVAM, Joint Research Centre)

Title: ECVAM priorities for alternatives

Details of ECVAM’s mission, a “vision” for the near future and a report on the status of current activities were presented. The validation of 3Rs alternative methods as tools for risk assessment and risk management is the central aspect of ECVAM’s mission. The promotion of the development, the application by industry and regulatory acceptance of such methods is also within the remit of ECVAM. Priorities are determined by evolving European legislation (i.e., REACH, cosmetics, pesticides, animal protection and welfare etc.). REACH will be a significant driver as animal testing should be a last resort under this legislation. The complexity of the endpoints that need to be addressed have implications for (i) the approaches and strategies employed in test methods development (i.e., ITS), (ii) the validation process and (iii) the application and acceptance of the alternatives once developed. Plans are underway to strengthen the organisation structures related to independent scientific review, stakeholder dialogue, regulatory dialogue and international cooperation.

The current workload includes activities on genotoxicity (Comet Assay, 3D Skin), carcinogenicity (cell transformation assays), reprotoxicity (LumiCell, extended F1 generation study) and acute toxicity (Balb 3T3/NRU cytotoxicity, EPAA WG on classification). New test submissions will result in future validation activities for skin sensitisation, eye irritation, skin absorption and reprotoxicity. The importance of integrated testing strategies (ITS) was highlighted as the sole means to realise replacement for complex endpoints. This will include the integration of technologies such as “-omics”. Given the likely importance, ECVAM will support both the development and validation of the constituent building blocks and integrated testing strategies *per se*.

Speaker: Watze De Wolf (Dupont)

Title: Industry efforts and perspective on alternatives in REACH

The role and contribution of the chemical industry in the development and implementation of the 3Rs was discussed. The idea of the six Rs was advanced. These include the traditional 3Rs of reduction, refinement and replacement, together with reliability, relevance and regulatory acceptance. The latter three are of particular relevance to industry when attempting to employ alternative methods for risk assessment purposes. From industry’s perspective, the drivers for alternatives are

societal expectation, community strategy, legislation and scientific advancements. Whilst REACH is an obvious source of significant animal use, the potential for replacement was deemed limited. In contrast, the potential of impact on REACH animal use from reduction - in particular as part of intelligent testing strategies – was emphasized.

This understanding of the importance of reduction has informed the research investment strategy of the Cefic Long-Range Research Initiative (LRI) programme. Various examples of relevant projects were provided. A particular focus of the LRI has been the development of computational tools for use as part of ITS. These includes QSARs and databases to support read-across. Such initiatives have often been undertaken in collaboration with the OECD, ECB, JRC etc. To facilitate the development of such tools, Cefic LRI has supported the development of AMBIT. This is a software package for chemo-informatic data management i.e., a searchable relational database that allows the storage of chemical structure and property information including data for toxicity endpoints from various sources. It can be employed to development and applicability domain assessment for QSARs, read-across/categorisation or the establishment of TTC (thresholds of toxicological concern).

Speaker: Andrea Tilche (DG Research, European Commission)

Title: Research support for 3Rs alternatives in Europe

An overview of EU FP6 and FP7 projects on alternative methods supported under the Environment and Health Programmes was provided. A significant number of FP6 projects are currently underway and include;

- Environment Programme: NoMiracle, INTARESE, CAESAR, OSIRIS, ERAPHARM
- Health Programme: ACUTETOX, EXERA, PREDICTOMICS, RAINBOW, SENS-IT-IV, Carcinogenomics, ARTEMIS, RETHINK, ReProTect, COMICS, LINTOP, MEMTRANS, TOXDROP, VitroCellomics, InVitroHeart, BBMO, ForinVitox and InVIToPharma

Allocated support for FP7 projects include €30 million under the Health Programme and €13.5 million under the Environment Programme. Scheduled projects include;

- Environment Programme: MIDTAL (€2.2 million), CADASTER (€2.7 million), RISKCYCLE (€1.0 million), ORCHESTRA (€0.9 million), ENFIRO (€3.2 million), CHEMSCREEN (€3.4 million)
- Health Programme: NANOTEST (€3.9 million), OPENTOX (€3.0 million), ESNAT (€11.9 million), PREDICT-IV (€11.3 million) and START-UP ((€0.3 million)

One particular example was highlighted. The CAESAR project aims to produce QSAR models to predict BCF, skin sensitisation, carcinogenicity, mutagenicity and developmental toxicity.

Speaker: Deborah Declercq (Straticell)

Title: SMEs experiences and needs in Europe

The experiences of Straticell as a contract research organisation (CRO) in the area of *in vitro* toxicology were shared. Demand for any given test is essentially driven by legislative demand, although opportunities for cost reduction in regulatory compliance are also an important driver. Key to the implementation of a method is the likely rate of return on the investment required to establish the capacity necessary to be able to offer that test on a commercial basis. The complexity required to be able to do so is often underestimated. It necessitates the availability of highly qualified staff, the provision of appropriate training, investment in equipment and sufficient lead time (~1 year). There is also a general requirement to both achieve and maintain GLP. Many CROs are also SMEs and this carries additional implications regarding resources (especially financial investment). The specific needs of SMEs active in alternative methods were highlighted. Greater success in technology transfer would result from (public) financial support for method optimisation, broader stakeholder involvement in test development and greater transparency in the validation process. Test quality in terms of reproducibility, robustness and simplicity are also important factors. The ability of SMEs to promote novel alternative methods they have developed is greatly limited by the magnitude of the investment required for validation. This is likely outside the scope of most SMEs.

Speaker: Syed Ahmed Mustafa (Ceetox Inc.)

Title: SMEs experiences and needs in the USA

An analysis of the key challenges facing SMEs offering *in vitro* toxicology services was presented. One set of challenges relate to conflicting and inconsistent regulations across industry sector and between regulators. Regulatory acceptance is key to the wide scale adoption and usage of alternatives. Whilst alternatives may offer benefits to industry in terms of cost or time, they will not employ alternatives unless their respective regulators will accept the resulting data. Other significant challenges are concerned with “equivalence” to animal models and the availability of *in vivo* data against which to benchmark new alternative models. The protection of intellectual property rights (IPR) can also be an issue for SMEs actively engaged in the development of novel alternatives. The need to protect such rights is at odds with the needs for transparency in the validation in order to ensure acceptance. Indeed, validation as a whole presents numerous hurdles to SMEs. The process is often slow, cumbersome, expensive, does not necessarily directly translate in to regulatory acceptance as well as not encouraging the creation of IPRs. This is inconsistent with

the particular needs of SMEs which are often limited in capital, have aspirations for growth, susceptible to competition and sensitive to the demands of their customers.

Speaker: Dick Phillips (Exxon Mobil Chemicals)

Title: Panel Discussion

The main points highlighted during the panel discussion pertained to the following;

1. Regulatory Acceptance: The extent of the acceptance of alternative methods by regulators was a topic of some discussion. A level of "comfort" and "experience" appears necessary for regulators. This is understandable given their central role related to the policing of consumer or environmental safety. Without acceptance, the performance of any *in vitro* testing is effectively rendered redundant as *in vivo* confirmation will always be necessary. Some general differences between US and EU regulators in terms of their respective approaches to alternatives were noted. The EU regulatory framework is predicated on the formal validation of and subsequently acceptance of methods as a whole. In contrast, some US regulators tend to approach the issue with a different perspective and employ a case-by-case data based approach whereby all relevant evidence is considered and evaluated – whether from *in vivo* or *in vitro* studies. The question is one of data quality and relevance to the evaluation of the substance in hand rather than of validation of any given method *per se*. Within the context of REACH, the limitations of timings are such that the integration of *in vitro* methods will be particularly challenging. Especially when the problem of extrapolation from *in vitro* effects to dose at the *in vivo* level, remains unresolved.

2. Negative Results: Regulatory acceptance appears to be a particular problem when *in vitro* test results are "negative". This presents industry with a dilemma as there is then no longer any incentive to employ alternatives. There was an explicit call from industrial participants for regulators to accept "negative" findings from *in vitro* test systems. A debate amongst regulators is necessary.

3. Evidence Based Approaches: The potential confounding effects of false positives were highlighted. This will be exacerbated by the testing of large numbers of compounds under the likes of REACH etc. This will necessitate a new emphasis on understanding the quality of the underlying data. The question of the predictability of animal models versus human responses was also raised. Stand alone direct replacement *in vitro* assays are increasingly unlikely. Alternatives approaches will entail *in vitro* methods supplemented by other models or data sources.

4. Different Standards: There were comments related to the different and inconsistent expectations placed on *in vitro* alternatives relative to established *in vivo* assays. Many such assays have not been validated with the same

degree of rigour now expected for alternative methods. The bar is consequently set higher for alternatives than traditional methods.

5. The problems of SMEs: Intellectual property rights are problematic for SMEs. The need to protect novel ideas is at odds with the desire for transparency necessary in the validation process. This would tend to preclude a role of SMEs in the innovation process. Confidence on the part of CROs to invest in the capacity to offer alternatives is related to the prospect for return on the necessary investment. This is ultimately dependent upon industry demand as driven by regulatory acceptance. Hesitant regulators correspond to hesitant companies which corresponds to hesitant CROs.

6. Research Funding Effectiveness: Whilst the extent of support for alternatives in the EU relative to the USA is very evident, questions were raised regarding the success to date in translating framework programme research into practicable methods that can be employed by industry. This is a "disconnect" that needs to be addressed. Part of the problem lies in understanding industry's needs and more effectively accessing industry knowledge in this respect.

7. International Cooperation: The issues faced by industry and regulators are the same on both sides of the Atlantic. Better and more effective collaboration between the EU and US – for all stakeholders - is undoubtedly the way forward.

List of Participants

[Insert list of participants]