Comparing the Challenges in Developing and Implementing 3Rs Alternative Methods in Europe and the United States: Industrial and Academic Perspectives*

Chantra Eskes [1], Kristie Sullivan [2], Marilyn Aardema [3], Horst Spielmann [4], Erin Hill [5], Greet Schoeters [6], Rodger Curren [5], Mathieu Vinken [7], and Bas Blaauboer [8]

[1]Services & Consultation on Alternative Methods (SeCAM), Agno, Switzerland; [2]Physicians Committee for Responsible Medicine, Washington, DC, USA; [3]Marilyn Aardema Consulting, Cincinnati, OH, USA; [4]Free University of Berlin, Germany; [5]Institute for In Vitro Sciences, Gaithersburg, MD, USA; [6]VITO, Mol, Belgium; [7]Vrije Universiteit Brussel, Brussels, Belgium; [8]Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

Summary

The European Society of Toxicology In Vitro (ESTIV) and the American Society for Cellular and Computational Toxicology (ASCCT) held a combined session at the 8th World Congress on Alternatives and Animal Use in the Life Sciences to compare and contrast the challenges of developing and implementing new alternative (non-animal) methods in Europe and the United States (US) from both an industrial and academic point of view. The present paper summarizes the main discussions and overall conclusions of this session.

Keywords: 3Rs implementation, academia, industry, ASCCT, ESTIV, Europe, North America

1 Introduction

The European Society of Toxicology In Vitro (ESTIV), is a leading scientific organization in Europe that works to promote networking between *in vitro* toxicologists and to advance research, development and use of 3Rs alternative methods in toxicology. Founded in 1980, ESTIV currently has about 230 members from 30 different countries. Its activities involve organizing regular conferences, workshops, and meetings such as the next ESTIV Congress, scheduled for October 16-20, 2012 in Lisbon, Portugal. The goals of ESTIV Congresses are to promote exchanges on *in vitro* toxicology, encourage education and training, cooperate with relevant organizations and societies, and facilitate communication among regulators, industry, and academia.

The American Society for Cellular and Computational Toxicology (ASCCT) is the first US scientific society dedicated to the promotion of toxicology testing and research that reduces and replaces the use of animals. Founded in 2010, it aims to foster cooperation and dialog among North American scientists, regulators, and nongovernmental organizations from the pharmaceutical, chemical, pesticide, and consumer product sectors. Through its forums, meetings, and activities, the Society seeks to facilitate the development, acceptance, and routine use of cellular (*in vitro*) and computational methods.

During the 8th World Congress on Alternatives and Animal Use in the Life Sciences, ESTIV and ASCCT organized a combined session to compare and contrast the challenges of developing and implementing new alternative (non-animal) methods in Europe and the US from both an industrial and an academic point of view. To help discussions and comparison, the following questions were distributed to the speakers prior to the meeting:

1. What are the *driving factors* for using alternative 3Rs methods?
2. What *information sources* are used to retrieve suitable 3Rs methods?
3. What steps are taken for *alternative test methods development* (e.g., in-house, in partnership, which partners, funding sources)?
4. How involved do researchers become in the formal *validation* process? How do they gain access to ICCVAM[1]/ECVAM[2]? What funding sources are available for validation?
5. Once a test is validated, how do researchers *work with regulatory agencies* to ensure acceptance of data (e.g., challenges, communication channels used, acceptance of weight-of-evidence approaches)?
6. What are your proposed *recommendations* to make the implementation and use of alternative methods more efficient?

* The views expressed in this manuscript reflect the individuals' personal experience and/or opinions.

1 CCVAM: Interagency Coordinating Committee for the Validation of Alternative Methods (http://iccvam.niehs.nih.gov)

2 ECVAM: European Committee for the Validation of Alternative Methods (http://ecvam.jrc.it)

This paper represents a summary of the discussions and main conclusions regarding the challenges in developing and implementing 3Rs alternatives from industrial and academic points of view considering both the European and North American perspectives.

2 Industrial perspectives

To investigate the current challenges in development and implementation of the 3Rs by European industry, ESTIV members from the industrial sector were sent the questions listed above through an email survey. Answers were received from contract research organizations (CROs), pharmaceutical, biotechnology, consultancy, and cosmetics sectors, distributed across five European countries. Interestingly, no answers were received from the chemicals sector.

Six main driving factors for European industry to use alternative 3Rs methods were reported: (1a) legislation, including the EU Cosmetics Directive and REACH (EC, 2009, 2006), was given the same weight as (1b) scientific relevance, including the use of relevant species and metabolic competences and (1c) ethical issues, followed in order of importance by (2) costs, (3) the efficiency and scientific validity/standardization of using 3Rs, and (4) other factors such as company image and the use of 3Rs for efficacy or screening purposes. To retrieve information on suitable 3Rs methods, (1) literature and publications appeared as the main consulted sources, followed by (2) official test guidelines, congresses/workshops, and (3) the ECVAM website, the ECVAM DB-ALM database[3], professional networking, and colleagues. The use of in-house databases was mentioned in only a few cases.

Very balanced answers were obtained regarding the development of alternative methods: 50% of the cases involved industrial funding through either in-house development (25%) or industrial partnership (25%) and the other 50% of the cases involved governmental funding from either national government (25%) or through EU research projects or in collaboration with academia (25%). It was reported that development of 3Rs alternatives may be initiated in-house and then pursued through collaborative efforts. Eight of the ten respondents indicated direct involvement in the validation process and multiple funding sources of validation. A balanced funding was reported, with ECVAM funding and industrial funding each representing 4 out of 10 answers. For the remaining two answers, funding from EU research projects and government was reported. Regrettably, half of the respondents had little or no contact with regulators to ensure acceptance of data from 3Rs methods. Among those who did have contact with regulators, the means of interaction included, in order of importance (1) direct communication, (2) publications, and (3) meetings and/or involvement in the validation management groups.

A number of recommendations were offered to help make implementation and use of alternative methods more efficient in European industry. Interestingly, 50% of the respondents concurred in suggesting "more and earlier involvement and communication with regulators," followed by 30% of the replies advocating "acceleration of the validation process and a decrease in its bureaucracy and/or the number of chemicals needed." Other single recommendations included:

- More ECVAM-industry interaction;
- More information on priority needs and data needed for validation;
- Easier access to a database of human toxicity;
- Promote exchanges of test results (including pre-clinical);
- Develop more focused/reduced Integrated Testing Strategies that allow reduction of costs;
- Develop a post-acceptance phase of validated methods to warn for misuse and adapt applicability domain;
- Make success and failures more visible, as it is expected that an increase in evidence will increase the acceptance of 3Rs methods;
- Identify the most suitable assays for tailored uses;
- Reduce emphasis on validation and increase emphasis on utility.

Regarding North America, it was discussed that a primary driving factor is the ethical consideration reflected by consumer interest in products that are developed with limited or no animal use. In addition, there are practical considerations that drive industry interest in the 3Rs, such as the ability to develop safe and effective products faster and more cheaply. This is especially important in meeting the needs of large chemical testing programs, such as REACH (EC, 2006), that have a global impact on industry. Regulatory guidelines that ban or reduce the use of animals are another important factor. Recent examples include the EU 7th amendment ban on cosmetic testing (EC, 2009) and the proposed ICH S2 Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use[4]. Lastly, but very importantly, within industry there is widespread interest in *in vitro* methods that further enhance our understanding of basic scientific processes and mechanisms involved in toxicity.

A variety of information sources are available to industry to identify suitable 3Rs methods; in fact, too much information is available. Here, authoritative groups such as ECVAM, ICCVAM, JACVAM[5], OECD[6], and others can prioritize suitable 3Rs methods and ensure proper validation and implementation into testing guidelines. Authoritative groups are guided by trade associations and other expert scientific groups, such as the ASCCT, ESTIV, COLIPA[7], and AltTox[8], which help in

[3] ECVAM DB-ALM Database Service on Alternative Methods to Animal Experimentation (http://ecvam-dbalm.jrc.ec.europa.eu

[4] ICH: International Conference on Harmonization (www.ich.org)

[5] JACVAM: Japanese Center for the Validation of Alternative Methods (http://jacvam.jp)

[6] OECD: Organisation for Economic Cooperation and Development (www.oecd.org)

[7] COLIPA: The European Cosmetics Association (www.colipa.eu)

[8] Website on Non-animal Methods for Toxicity Testing (www.AltTox.org)

the earlier stages when new 3Rs methods are being discussed and developed. CROs, such as the Institute for In Vitro Sciences (IIVS) and BioReliance, reflect the testing interests of a wide variety of industries and can be another source of information on suitable 3Rs methods.

Development of alternative test methods is best accomplished by conducting work collaboratively and globally with all relevant stakeholders. This helps ensure the widest acceptability of new alternative methods, since there is little use for a method that meets the needs of only one geographical region. It is especially important to include authoritative groups that have the responsibility for validation of new methods, such as ECVAM, ICCVAM, JACVAM, or the OECD. While this process is more complicated and time consuming upfront, it is more efficient in the long run since it helps ensure focus on methods that ultimately will be accepted. Development of alternative methods within industry often involves the conduct of studies in-house to gain "hands on experience" with the assay. Industry also supports the conduct of promising research at academic institutions, CROs, or other organizations. There are a number of funding sources for 3Rs research in North America, including:

- National Institutes of Health Small Business Innovation Research / Small Business Technology Transfer, which provides funding to small businesses/academic labs to conduct R&D that has the potential for commercialization;
- 3Rs groups, e.g. Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins University;
- Industry;
- Trade associations/scientific organizations, e.g. International Life Science Institute / Health and Environmental Sciences Institute (ILSI-HESI);
- EU sources, e.g. People for the Ethical Treatment of Animals (PETA) UK.

Once a new method has been developed, industry often plays a key role in the formal validation process. This varies with the interests and level of expertise within each industry, although it is common for companies with internal experts to be actively involved in helping "drive" the formal validation process. Involvement mechanisms include financial support for validation studies, either independently or through trade associations, or active involvement in conducting studies as part of formal ring trials. Authoritative groups such as ECVAM and ICCVAM often request involvement of industrial partners during formal validation and review of new methods.

Once a method is validated, researchers in industry continue to work with regulatory agencies to ensure acceptance of data and proper incorporation into testing guidelines, which is a natural outcome of a collaborative and multi-stakeholder effort. A recent example is the collaborative work on a non-animal ocular testing strategy for anti-microbial cleaning products that involved various industries and the US Environmental Protection Agency[9]. Another example is the development of new methods using 3D human reconstructed skin models for genotoxicity testing (Mun et al., 2009; Flamand et al., 2006; Hu et al., 2009). Based on promising industrial developments, COLIPA and ECVAM have undertaken a project to further develop/prevalidate these assays (Aardema et al., 2010; Dahl et al., 2011). Establishment of robust GLP assays adds further support to these important 3Rs methods.

Overall, 3Rs research and assay development/validation is increasing in North American industry. Further focus on and funding for the 3Rs will continue to drive this development and increase regulatory involvement. There is a clear need to establish more efficient processes for collaborative, multi-stakeholder validation studies, especially since it currently takes decades to develop and validate new methods and for the methods to gain widespread acceptance.

3 Academic perspectives

For European academia the driving factors relating to 3Rs methods are the EU Directive on the protection of animals used for experimental and other scientific purposes (Directive 86/609 updated as the new Directive 2010/63[10]; EC, 1986 and 2010) and the European Science Foundation (ESF) briefing on the "Use of animals in research"[11] (2000). In addition, the project evaluation by a governmental expert committee at the national level (Article 36 of Directive 2010/63) ensures that alternatives are being used.

EU Directive 2010/63 requests that (Article 4):

- Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used.
- Member States shall ensure that the number of animals used in projects is reduced to a minimum without compromising the objectives of the project.
- Member States shall ensure refinement of breeding, accommodation, and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals.

Moreover, it requests that the Commission and the Member States shall contribute to the development and validation of alternative methods to animal testing (Article 47).

The ESF policy briefing on the "Use of animals in research" strongly endorses the principles of the 3Rs and recommends that investigators and other personnel involved in the design and performance of animal-based experiments should be adequately educated and trained. EFS member organizations should encourage the development and organization of accredited courses on laboratory animal science, including information on animal alternatives, welfare, and ethics.

To provide information on the 3Rs, Article 47 of EU Directive 2010/63 recommends that Member States shall, at the na-

9 http://www.epa.gov/oppad001/eye-irritation.pdf

10 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF

11 http://www.esf.org/nc/publications/science-policy-briefings.html?tx_ccdamdl_cart%5Badd%5D=4755

tional level, ensure the promotion of alternative approaches and the dissemination of information thereon. The EU Commission provides information on alternatives to animal use in the area of regulatory testing via the DB-ALM ECVAM database[5]. A similar service is provided by the ZEBET AnimAlt database[12]. In contrast, the "Go3R" search engine allows searching for 3Rs alternatives in all areas of the life sciences[13].

Since public pressure in Europe is focused on replacing animal use for regulatory purposes, e.g. for safety testing of cosmetics, drugs, and chemicals, funding programs for academia to develop 3Rs models for replacing animal methods used in basic research remain limited.

Non-regulatory alternatives, which are used by academia, generally do not require formal validation by ECVAM or ICCVAM. However, if a new non-animal method has been published in a peer reviewed journal and the results have been confirmed in a second, independent laboratory, the non-animal method must be used by scientists in Europe, according to EU Directive 2010/63. As a consequence, public funding for a study will only be provided in Europe if the investigator begins using non-animal methods as soon as they are available, and this needs to be proven by a literature search.

For researchers in academia, the only regulatory agency that has to approve animal experiments is the national/state government evaluation committee. In Germany, experienced independent experts nominated by animal welfare are members of the evaluation committees and ensure the use of non-animal methods as soon as they are available.

It is urgently recommended that the following three areas be addressed to reduce animal testing in academia in Europe and beyond, since animal numbers have been increasing during the last decade:

– alternatives to the use of transgenic animals;
– alternatives to human disease models in animals;
– alternatives to transgenic human disease models in animals.

Regarding North American academia, ASCCT members and others in the US who work to develop non-animal methods for basic research and regulatory use were surveyed with the same questions as the other sectors, and literature references were consulted as well (e.g., Gruber and Hartung, 2004).

The drivers that respondents identified for the use of alternative methods related primarily to external rather than internal factors. These included grant money from foundations, federal agencies, or other institutions targeted specifically at non-animal methods, the 2007 National Academy of Sciences Report on Toxicity Testing in the 21st Century[14] (National Academies, 2007), the efficiency of non-animal methods and, conversely, costs and other practical issues associated with animals, and the applicability of (human) cell-based models to human populations. In contrast to European researchers, US respondents did not consider legislation and regulation related to alternatives to animals a primary driver.

Information on available 3Rs methods is obtained, for the most part, from general biomedical search sources such as PubMed. A few respondents mentioned "alternatives"-specific sites such as DB-ALM and AltWeb, and some mentioned specific scientific journals such as *Nature Medicine, Toxicology In Vitro, ALTEX* and *Toxicological Sciences*. Overall, it is difficult to match methods to research interests because of terminology differences (i.e., heart disease and cardiomyocytes) or lack of information regarding experience with methods.

The process for development and dissemination of new models is less standardized than in industry and government. The development of new models is led by the research questions to be answered in a particular laboratory, and thus the use of new models is often restricted to individual labs; further dissemination to others depends on peer-to-peer contact and the maintenance of funding streams. Many models are reported in the literature for certain research purposes that are "valid" for that purpose but not for use by others. Unless the method is to be used for regulatory purposes, formal validation is very rarely done. It is usually dependent upon companies or regulatory bodies to take up the standardization and validation of the test method. Respondents identified information transfer as a key challenge: how does a researcher get others to take up his or her method? What is the best way to communicate and train other scientists? What determines whether other basic researchers use a new model?

Respondents offered several recommendations to make the implementation and use of 3Rs methods more efficient in academia. These include increasing legislative and government incentives and requirements, as well as making non-funding resources for cellular, tissue, and computational research more available, for example through the NIH Office of Research Resources. For methods intended to become part of regulatory test schemes, increased knowledge of regulatory testing requirements and standards is needed, and developers of *in vitro* models should aim to address mechanisms or toxicity pathways and not to expect methods to address all chemicals, mechanisms, or disease states.

One major theme was support for the 3Rs within the scientific community. Unlike in the EU, many scientific societies do not explicitly promote or support the 3Rs as a worthy pursuit. Respondents felt that this could be addressed by communicating the ethical and scientific shortfalls of answering research questions with animals and by fostering better information dissemination among scientists regarding the utility of non-animal approaches and the process of developing new methods.

Some respondents suggested increased promotion of and access to 3Rs information at both general alternatives conferences and field-specific conferences. One commonly-identified problem was that it is difficult to publish "negative" results in the literature, but this and other information about the process of developing a new model is essential for other scientists to take up new models in their own labs. Scientific journals can have a

12 AnimAlt-ZEBET database on alternatives to animal experiments (http://www.dimdi.de/static/en/db/dbinfo/zt00.htm)

13 Go3R Semantic Search to avoid animal experiments (http://www.go3r.org)

14 http://www.nap.edu/catalog.php?record_id=11970

role here, by encouraging scientists to share negative data and mistakes along the way, and also by requiring authors to detail explicitly how they pursued the 3Rs in their research. Other platforms could also be envisioned to share this information and to document a model's applicability domain, relevance, and feasibility, such as online databases or Wiki-type platforms that would allow scientists to upload information not necessarily fit for publication but essential for model circulation and uptake.

4 Concluding remarks

Based on the surveys carried out, it was found that the industrial drivers in Europe and in North America are similar: the ethical, scientific, and economic need to reduce and finally replace the use of animal models in toxicity testing. This is reflected in the change in a number of legal requirements, such as the European policies on cosmetics and chemicals testing and also in the upcoming change in chemicals legislation in the US.

From an academic point of view, however, differences seem to exist between Europe and the United States. Whereas research in Europe needs to comply with the Directive on the protection of animals used for scientific purposes and requires approval by national government evaluation committees, in the United States legislation and regulation related to alternatives to animals was not considered a primary driver for the implementation of 3Rs alternative methods.

Differences also exist in the practical attitudes on both sides of the Atlantic. Whereas the European Commission and also a number of EU Member States have an active policy in financing programs directed towards the 3Rs application in toxicology, in the US this is currently confined to a number of NIH activities, while the sources for financing academic research in this area are limited. This also reflects the different approaches in research management – either top-down or bottom-up.

Finally, ther was a strong consensus on the need to involve all stakeholders, especially the regulatory bodies, in the process of developing new strategies for toxicity testing. This will need to be done on an international level, with involvement of the "VAM's," the OECD, industrial organizations, and academia. It is essential, therefore, to follow the example set by the AXLR8[15] program, bringing together the manifold initiatives and comparing approaches and good practices.

References

Aardema, M. J., Barnett, B., Khambatta, Z., et al. (2010). International validation of the EpiDerm™ 3D Human Reconstructed Skin Micronucleus (RSMN) Assay: Transferability and Reproducibility. *Mut. Res. 701*, 123-131.

Dahl, E. R., Curren, B., Barnett, Z., et al. (2011). The Reconstructed Skin Micronucleus Assay (RSMN) in EpiDerm™: detailed protocol and harmonized scoring atlas. *Mut. Res. 720*, 42-52.

EC – European Commission (1986). Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Union L358*, 1-28.

EC (2006). Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Official Journal of the European Union L 396*, 1-849.

EC (2009). Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. *Official Journal of the European Union L342*, 59-209.

EC (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union L276*, 33-79.

Flamand, N., Marrot, L., Belaidi, J.-P., et al. (2006). Development of genotoxicity test procedures with Episkin™ , a reconstructed human skin model: Towards new tools for in vitro risk assessment of dermally applied compounds? *Mut. Res. 606*, 39-51.

Gruber, F. P. and Hartung, T. (2004). Alternatives to animal experimentation in basic research. *ALTEX 21, Suppl. 1*, 3-31.

Hu, T., Kaluzhny, Y., Mun, G. C., et al. (2009). Intralaboratory and interlaboratory evaluation of the EpiDerm 3D Human Reconstructed Skin Micronucleus (RSMN) Assay. Mut. *Res. 673*, 100-108.

Mun, G. C., Aardema, M. J., Hu, T., et al. (2009). Further development of the EpiDerm™ 3D Reconstructed Human Skin Micronucleus (RSMN) Assay. *Mut. Res. 673*, 92-99.

National Academies, Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council (2007). Toxicity testing in the 21st century: A vision and strategy. Washington, USA: National Academies Press.

Correspondence to

Chantra Eskes
Via Fontanone 27
6982 Agno
Switzerland
e-mail: chantra.eskes@secam-ce.eu

15 http://axlr8.eu