Optimisation of the Post-validation Process

The Report and Recommendations of ECVAM Workshop 67[a]

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Preface

This is the report on *Optimisation of the Post-validation Process*, one of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). ECVAM's main goal, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures that would enable it to become well-informed about the state-of-the-art of non-animal testing in regulatory procedures. It was decided that this would be best achieved by the organisation of ECVAM workshops on specific topics, at which small groups of invited experts would review the current status of *in vitro* tests and their potential uses, and make recommendations about the best ways forward (1).

The number of alternative test methods continues to increase. However, these new methods are not automatically accepted and implemented for use for regulatory purposes. From the experience acquired during the last decade, we know that, after the scientific process of validation, finalised by an ESAC statement, the process of acceptance by regulators often takes a long time. ECVAM therefore held a workshop on *Optimisation of the Post-Validation Process* at Ispra, on 19–22 June 2006. The workshop was chaired by Annamaria A. Bottini and Bas Blaauboer. The principal aim was to identify the factors and key players which influence the execution of the Three Rs in regulatory animal testing, and to provide, where possible, a critical appreciation of how scientists, regulators and legislators could interact to facilitate the promotion of alternative methods and their implementation into regulatory processes in the European Union and, ultimately, throughout the world. This report builds upon the expectations and recommendations of the ECVAM workshop participants on the feasibility of improving the communication among stakeholders and strengthening a Science–Policy Network in the post-validation process.

Introduction

Ensuring a high level of protection for human health and wildlife is the central feature of current and new regulation for substances (chemicals, cosmetics, food, pharmaceuticals, etc.) in the European Union (EU). To achieve this purpose, approximately 25–30% of the animal tests conducted in

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Europe annually are performed to meet regulatory requirements (2). The use of animals for testing has been highly controversial (see, for example, http://ecvam.jrc.cec.eu.int/ and http://www.peta.com/). Many people question the fundamental ethical and moral right to carry out experimental procedures on animals that (might) cause suffering and even death (3, 4). In 1986, with the intention of limiting the use of laboratory animals, the Council of Ministers adopted *Directive 86/609/EEC on the Protection of Laboratory Animals for Experimental and Other Scientific Purposes* (5). Article 23 of the Directive states that: *The Commission and Member States should encourage research into the development and validation of alternative techniques, which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field.*

In October 1991, the European Commission responded to this article by means of a Communication to the Council and the Parliament (6; see Annex 1), which established ECVAM. Taking into account that the Communication mentions the need "*to promote dialogue between the legislator…*" and the other stakeholders, this represents a mandate to involve legislators/regulators in the process. The involvement of regulators has been instrumental for many years, and ECVAM is a unique facilitator between regulatory needs and the development of alternative methods. However, these interactions were not structured, but developed on the basis of need or circumstances.

Interest at the European level in alternative tests is evident through the many efforts made to encourage the development of non-animal methods and techniques. Currently, the "validation" of these alternative toxicological tests is increasingly a prerequisite for regulatory acceptance, as is also now the case for new animal tests. To be accepted by regulatory agencies, a "validated method" should have undergone independent scientific peer review by experts in the field, to ensure that appropriate scientific information is provided for regulatory risk assessment. ECVAM is playing a key role in this area, by promoting the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals (7, 8).

The intention of this workshop was to identify and enumerate the principal factors which influence the acceptance of validated alternative methods, and to recognise the major factors and putative solutions that can facilitate the post-validation and acceptance process, and the potential for the incorporation of alternative tests into regulatory procedures, in order to establish an arena for dialogue and to speed-up the whole process of validation. Furthermore, the participants in the workshop wished to provide a critical view of how scientists, regulators and legislators are able to interact.

Description of the Problem

International public concern that animal testing is still required for registering a substance to be released onto the market is manifested at the social, scientific and political levels, despite the existing and increasing number of alternative tests (by November 2007, 34 alternative approaches had received validity endorsement statements by ECVAM's Scientific Advisory Committee [ESAC]). Nevertheless, these new alternative methods are not automatically included into the assessment procedures required for compliance with regulatory requirements — they have to overcome a number of obstacles before finally achieving regulatory acceptance and implementation.

One hindrance to the more extensive use of these methods is the question of recognition of validation, performed under the auspices of established international organisations such as ECVAM, the US Inter-Agency Coordinating Committee on the Validation of Alternative Methods (ICCVAM; http://iccvam.niehs.nih.gov/), or the recently-created Japanese Centre for the Validation of Alternative Methods (JaCVAM) at the Japanese National Institute for Health Science (http://www.nihs.go.jp/english/nihs/index.html). Once a new method has been validated, it usually takes many years before it is actually accepted by regulators and fully incorporated into regulatory procedures. Often, this recognition ultimately takes place in international bodies such as the Organisation for Economic Co-operation and Development (OECD; http://www.oecd.org/) or the International Conference on Harmonisation (ICH; www.ich.org/).

In order to reduce the number of animal tests that need to be carried out, it will be critical to identify any problems and difficulties, and to propose action and solutions for the post-validation process. The workshop was based on the background document produced by a Utrecht University study on *Regulatory Animal Testing* (9; http://www.bio.uu.nl/wetenschapswinkel/). This study showed that the slow pace of acceptance is caused by a combination of factors. To begin with, legislators and regulators are facing increasing demands for consumer safety and the minimisation of risk. In terms of policy development and implementation, it is the regulators, in particular, who are reluctant to implement the Three Rs in evaluating protocols, according to respondents. One main reason for this is that regulators are often unfamiliar with alternative test methods and they therefore prefer to adhere to existing animal models.

The main and most common obstacles found in relation to validation and regulatory acceptance are as follows:

1. *Data sharing and data protection* are not handled consistently by the various different laws and regulations. Industrial companies are seen as reluctant to make existing research data available, because that would harm their competitive positions. The availability of these data, according to respondents, would give strength to alternative methods, since much duplication could be avoided. The effective implementation of the Mutual Acceptance of Data (MAD) principle should ensure that much research does not need to be duplicated.

2. *Communication* between the different stakeholders was identified as in need of improvement, as a relevant tool for increasing the implementation of the Three Rs, and optimising the validation and acceptance process. Such communication should be actively promoted between: a) scientists and regulators, in order to define needs, criteria for test acceptance, and the follow-up of the test after acceptance; b) regulators and policy makers/legislators; and c) the different regulatory bodies or/and agencies.

3. *Fragmentation of information* within the national, European and other international institutions often results in difficulties in identifying the persons to be contacted for information.

4. *Variation in the implementation of the Three Rs* in different in areas, e.g. in relation to chemicals, cosmetics, food, and pharmaceuticals. The most prominent example is cosmetics, where, except in certain specific instances, only the use of non-animal methods will be acceptable after 2009. However, the use and interpretation of tests also differ between closely-related regulated sectors, e.g. chemicals and plant protection products.

5. *International mutual acceptance* needs to be recognised as indispensable. An alternative method which is accepted only in one market, such as the EU, will not be used, given that most products are marketed globally, and since the use of traditional tests is not explicitly forbidden after an alternative method becomes available (with the notable exception of the EU cosmetic legislation). An example of a lack of international harmonisation is the testing of veterinary vaccines, where major differences between the EU and the USA persist. The globalisation of alternative methods represents a major opportunity, as well as a challenge, for all concerned (10).

6. *Test strategies need to be validated*, as well as individual test methods, since only in rare cases can a single alternative test substitute for an animal test. However, the systematic development and validation of test strategies is still in its infancy, and needs to be addressed as a matter of great importance (11).

7. *Regulators tend to favour refinement and reduction approaches* over replacement alternatives, e.g. at the OECD. This is because they are more familiar with traditional tests, and thus find it more easy to change to modified animal procedures.

8. *Scientists are not sufficiently familiar with regulations or the opportunities for influencing them.* The targeted development of methods for application in the regulatory field is often not undertaken, because researchers in the basic biomedical sciences simply do not know enough about the available opportunities. Programmed research and the active search for novel methods by the validation bodies plays an important role here. At the same time, test developers often have unrealistic expectations with regard to the use of their methods for regulatory purposes.

Case study: the validation and acceptance of skin corrosion testing

The validation of alternatives to the skin corrosion test was discussed, as an example of delay in the acceptance of validated tests (Table 1). It took six years for the three tests to achieve final OECD acceptance, while EU acceptance took only two years. In comparison to the time taken for validation and peer review, this represented a considerable time from test development to actual regulatory use.

Opportunities for Optimising the Post-validation Process

The following major issues were identified as ways of speeding up the whole process of post-validation and acceptance of an alternative or animal method:

1. *Identification of the key players and responsible persons in each toxicological area.* Communication starts with identifying those who should be talked to. This is not easy, especially if several different sectors are involved. For example, valuable experience was gained in seeing how difficult it was to bring together different sectors of industry (and, even more difficult, competitors in one sector), when setting up the European Partnership for Alternative Approaches to animal testing (EPAA; see below).

Table 1: The history of the skin corrosion test

1995	Report on the ECVAM prevalidation study on TER, Corrositex™, Skin^2™
1998	Report on the ECVAM validation study on TER, EPISKIN™, Corrositex, Skin^2
2000	Report on the ECVAM catch-up study on the EpiDerm™ method
1998	ESAC statements on the scientific validity of the TER and EPISKIN methods
2000	ESAC statement on the scientific validity of the EpiDerm method
2001	ESAC statement on the Corrositex assay for acids, bases and their derivatives
2000	Acceptance of the TER and human skin equivalent methods into EU test guidelines
2002	Acceptance of these tests by the OECD
2004	OECD publication of TG 430 (*Transcutaneous Electrical Resistance Test*) and TG 431 (*Human Skin Model Test*)

2. *Developing networking in order to improve communication between stakeholders, including industry and animal welfare advocates*. The EC Communication correctly identified communication among stakeholders as a prime duty, to promote the availability of alternative methods. However, networking is more than communication, as it aims for collaboration, coordination and convergence. This requires both formal and informal ways of coming together and aligning different agendas.

3. *Taking advantage of the newly-established EPAA, in order to identify ways of optimising the post-validation process, legal acceptance and application of alternative methods in the relevant industrial sectors*. The EPAA (http://ec.europa.eu/enterprise/epaa/index_en.htm) is a joint initiative by the EC and a number of companies and trade federations active in various industrial sectors. It was launched on 7 November 2005 at a major conference, *Europe Goes Alternative*, by Commissioners Verheugen and Potocnik and industry representatives. Its purpose is to promote the development of new 'Three R' methods (to refine, reduce, and replace animal procedures), as modern alternative approaches to safety testing. The Partnership's work will focus on mapping existing research, developing new alternative approaches and strategies, and promoting communication, education, and the validation and acceptance of alternative approaches (Table 2). Activities and progress will be reviewed annually at a public Three Rs Conference. The EC will support the Secretariat of the Partnership.

4. *Ensuring that regulators are informed and strongly involved in the various European research programmes*. In order to understand and guide the requests for new or revised 'Three R' assays for potential application for regulatory needs, it is important that regulators are involved from the very beginning, in the conception and development of alternative methods. It is important that methods are optimised toward regulatory needs, and that the validation process challenges approaches in a manner relevant to decisions on regulatory use. This means that, especially for selection of test chemicals, but also with regard to definition of test (strategies), future regulatory use should be considered, and relevant regulators should be involved. At the same time, information concerning emerging new methods, possibly endorsed by validation studies, need to be communicated in a timely manner to regulators, to pave the way forward for validation and the acceptance process.

5. *Involving regulators at the various stages of the validation process*, and ideally asking them for: a) comments and opinions on the need for any proposed alternative from the beginning of the validation exercise; b) understanding and conformity with regard to the definition of the prediction model (PM) for specific chemical classes; and c) advice on the selection of reference chemicals and agreements on standards, as well as positive and negative controls.

6. *Identifying the intended regulatory use of the validated methods in ESAC statements*. Where applicable, the various uses of tests according to regulations should be considered in the design and peer review of validation studies. A reasonable assessment of the scientific validity of a method should be carried out in the context of the envisaged regulatory use.

7. *Speeding up the peer review process and the publication of ESAC statements*. An important step in speeding up the validation process was taken by introducing the ECVAM modular approach (12), which allows, for example, the combined use of existing (retrospective) information and

Table 2: The EPAA — A partnership between different industrial sectors and the European Commission

The partnership will encourage:

— the promotion of industry activities and investments in Three Rs research;

— a more-rational implementation of regulatory testing requirements;

— a more-streamlined process for the acceptance of scientifically-validated alternative testing approaches;

— the identification of needs for research on alternative safety testing methods and facilitation of relevant multi-stakeholder research projects;

— the sharing of knowledge and best practice between sectors in implementing the Three Rs Declaration agreed at the *Europe Goes Alternative* conference in 2005;

— the consistent communication on research and implementation of the Three Rs in relation to safety assessment.

The EPAA Action Programme is designed around five main themes:

— mapping and evaluating past and current Three R activities;

— prioritising and implementing research based on the Three Rs;

— implementation of best practice;

— implementation of the Three Rs in regulation and decision-making;

— validation and acceptance of new and alternative test methods and strategies.

The Action Programme, comprising short-, medium- and long-term activities, will be reviewed and updated every year. Implementation will be ensured through Working Groups in which stakeholders will be involved. Companies and stakeholders can participate by:

— providing expertise;

— joining research programmes;

— becoming involved in pilot programmes;

— providing project and/or financial support, where necessary and possible.

The structure of the partnership:

— an annual Three Rs Conference to review progress in the European and global contexts;

— the Partnership Steering Committee;

— the Executive (composed of European Commission services, industry associations and companies, to propose and review work plans, strategies and timelines);

— small Working Groups (individuals from European Commission services and industry, with support from appropriate experts), to work on specific topics;

— a Stakeholders' Mirror Group (chaired by Dagmar Roth-Behrendt), comprised of representatives from academia, animal welfare associations, patient groups, consumer protection groups, and other stakeholders, to advise the Steering Committee from a broader societal perspective.

The EPAA is a partnership of the following organisations:

— *Industry*: Abbot, AstraZeneca, Avon, BASF, Bayer, Beiersdorf, Chanel, Colgate-Palmolive, Dow, DSM, Elizabeth Arden, Estée Lauder, Euroderm, Glaxo SmithKline, Henkel/Phenion, Johnson & Johnson, Kanebo, Kimberley-Clark, L'Oréal, LVMH, Merck, Merck Sharp and Dohme, Novo Nordisk, Pfizer, Procter & Gamble, Reckitt Benckiser, Roche (F. Hoffmann-La Roche), Serono, Shiseido, Solvay, StratiCELL, Syngenta, Unilever;

— *Industry associations*: CEFIC, EFPIA, COLIPA, EuropaBio, IFAH-Europe, AISE, ECPA;

— *European Commission*: DG Enterprise, DG Research, DG Health and Consumer Protection, DG Environment, DG JRC/ECVAM.

prospectively gained results. The increasing use of weight-of-evidence approaches introduces further flexibility (13). However, the peer review process has not been streamlined so far. Current revisions of ESAC procedures aim to promote the process. Among these, the creation of an ESAC manager is a major contribution to make this independent evaluation more efficient.

International and Legislative Factors

The lessons to be learned from ICCVAM

Because of the similarity of their names, the respective roles of ECVAM and ICCVAM are often confused. In fact, the ESAC, as a peer review panel which meets at regular intervals, and advises and monitors progress, is much more similar to ICCVAM, while the active work on validation and creation of background review documents by NICEATM, is much more similar to the work of ECVAM. When comparing the two processes (Figure 1), it becomes evident that regulators play no major role in the European process, while they represent the majority of agencies in the US process. The US approach has the disadvantage of allowing regulators, who are often bound to the *status quo*, to dominate the process of evaluation and acceptance of new methods; however, this also safeguards that following acceptance, implementation of the new method is very much reinforced. For example, the agencies involved in ICCVAM have to state, within 180 days following a validation statement, whether and how the validated method will be implemented within their jurisdictions.

The effects of the 7th amendment and the REACH system in forcing collaboration between ECVAM and regulators

Two recent EU legislative moves, on cosmetics and chemicals, have significantly changed the political expectations of alternative methods and their roles as replacements for animal tests. The 7th amendment to the EU Cosmetics Directive requires the use of replacement alternative methods from 2009 onwards, irrespective of the availability of such tests. Thus, it requires a contribution to the availability of alternative methods from an industry which has, at the same time, the liability to ensure the safety of novel products put on the market, and market pressure for the continuous development of new products. As a result, the European cosmetic industry trade association, COLIPA, has markedly increased its research programme on alternatives and its collaboration with ECVAM. Similarly, Article 1 of the REACH system legislation has made the development of alternative methods a primary goal. The unprecedented dimension of testing demanded by the REACH system itself has created pressure to make available new alternative methods, in order to reduce animal use, reduce costs, and increase the throughput of testing. In both areas, not only are the industries challenged by legislation, but regulators must actively accommodate the need for changes toward alternative methods. Given the crucial role of ECVAM and its formal validation process, this has resulted in strongly increased interactions with regulators. In the case of cosmetics, this is mainly the Scientific Committee on Consumer Products (SCCP) at DG SANCO, and the respective unit at DG ENTR which is responsible for the legislation. The collaboration with both has flourished. For example, close collaboration with DG ENTR has resulted in a Commission proposal for timelines for phasing out individual tests, annual reports to the European Parliament, an inventory of alternative approaches, a stakeholder group which meets regularly, and the conference series, *Europe Goes Alternative*, which laid the ground for the EPAA.

In the field of chemical legislation, ECVAM acted on behalf of the Commission to coordinate the development of testing strategies in the REACH Implementation Project 3.3. Within 18 months, and involving more than 200 experts from regulatory authorities, industry, academia and NGOs, testing strategies for all the information requirements in REACH were developed. In fact, this represents not only a tremendous step ahead for the use of alternative methods, which have, in the spirit of REACH, to be fully incorporated; it is also a shortcut in the post-validation process, since many methods which have just been validated or in are the final stages of validation are already foreseen in the proposed testing strategies. They are already included in the test strategies produced to guide industry, before inclusion in the EU test guideline (TG) legislation or, in some cases, even before an ESAC statement (with a footnote: "validation pending"). This highlights the principle that direct interaction between validation authorities and regulators can facilitate the acceptance process, and will serve as a role model for further work.

Post-validation: Individual Regulatory Bodies and their Processes of Acceptance of Alternatives

The various regulated sectors differ strongly with regard to the acceptance process for alternative methods.

Until recently, the acceptance of methods for *chemicals* testing was by the National Coordinators of the Test Guideline Programme, for inclusion into Annex V of the Dangerous Substance Directive. As the EC service responsible for this, DG Environment (http://ec.europa.eu/dgs/environment/index_en.htm) was supported by the European Chemicals Bureau (ECB; http://ecb.jrc.it/) at the Joint Research Centre. Typically, draft method guideline proposals would be handed to the OECD in parallel, in order to initiate the development of an OECD TG, which after several consensus stages, would be accepted finally by the OECD Joint Meeting. With the coming into force of the REACH

system legislation, the Dangerous Substances Directive was revoked, and an EU Test Guideline Regulation is currently being developed. It is likely that the mechanism for amending the guidelines will remain. However, due to the REACH system, further opportunities for the use of non-guideline methods exist. First, the European Chemicals Agency (EChA; http://echa.europa.eu/), now established in Helsinki, will publish guidance for industry on test strategies. The blueprint for this guidance has been developed in a REACH Implementation Project, coordinated on behalf of the EC by ECVAM. The project, managed by the European Chemical Industry Council (CEFIC; http://www.cefic.be/), involved about 200 regulators and industry experts. However, the mechanism of updating this guidance is not yet clear. Second, the REACH legislation does encourage the use of all existing data, as well as the use of "suitable" methods, i.e. reliably documented methods which fulfil some minimum quality requirements. In the end, it is the liability of the manufacturer to propose and use adequate methods, in order to establish the safety of a chemical. Both the introduction of test strategies, and the deliberate use of (non-)validated methods, represent major changes to the use of alternatives.

Many other areas rely on the OECD TGs and former Annex V methods, including the regulations for cosmetic ingredients, foods, plant protection products, and, to some extent, drugs. However, there are differences in data requirements and in guidance for the application of the methods to different products. Again, EU regulations and other national or international regulations play a role.

In the *food* area, not all aspects of regulation are harmonised among the EU Member States, but, with the creation of the European Food Safety Authority (EFSA; http://www.efsa.europa.eu/), an independent advisor and means of collaboration with the national authorities was created. The work of the EFSA includes an animal welfare panel, which also makes recommendations concerning test methods.

The safety of *cosmetic products* is, first of all, the liability of the producer. The SCCP (http://ec.europa.eu/health/ph_risk/committees/04_sccp/04_sccp_en.htm) addresses questions in relation to the safety and allergenic properties of cosmetic products and ingredients, with respect to their impact on consumer health, as well as dealing with toys, textiles, clothing, personal care products, domestic products, such as detergents, and consumer services, such as tattooing. The SCCP regularly informs the EC and other stakeholders about the availability of alternative methods, and makes recommendations as to their use.

Pesticides (plant protection products) and *biocides* are the political responsibility of DG SANCO (http://ec.europa.eu/dgs/health_consumer/), on the basis, for example, of *Directive 91/414/EEC*, which is concerned with the placing of plant protection products on the market (currently being revised). Information requirements are regulated via an annex, which refers to the EU test guidelines. The EFSA advises on this field by carrying out risk assessments, while the EC takes the risk management decisions.

Vaccines and *pharmaceuticals* are covered by European Pharmacopoeia (PhEur) and the European Directorate for the Quality of Medicines and Health Care (EDQM; http://www.edqm.eu/) under the auspices of the Council of Europe for quality control aspects, and the European Medicines Agency (EMEA; http://www.emea.europa.eu/) for efficacy and safety issues, as well as authorisation, together with the appropriate national authorities. International harmonisation, including recommendations on the methods to be used, is via the ICH, an unique project that brings together the regulatory authorities of Europe, Japan and the United States, with experts from the pharmaceutical industry in the three regions, to discuss the scientific and technical aspects of product registration (http://www.ich. org/). The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH; http:// www.vichsec.org/) is a similar, trilateral (EU, Japan, USA) programme, aimed at harmonising the technical requirements for veterinary product registration.

The Proposal for an ECVAM Regulatory Advisory Panel

The workshop participants discussed the opportunity to create a panel of regulators to support the interaction with ECVAM and the post-validation process. A possible set-up is outlined as follows, but does not preclude the input and advice from other Commission services and the ESAC:

1. *The suggested mission of ERAP*. The proposal is to establish an ECVAM Regulatory Advisory Panel (ERAP) as part of the activities of ECVAM, to facilitate the entire validation process. Consistent with the JRC's mission, ERAP will create a platform to consult and receive advice from regulatory agencies specific to each field, in order to: a) identify the different key players and responsible contact persons in each toxicological and substance/product area; b) to improve networking between stakeholders, industry, and animal welfare organisations; c) to inform the regulators and involve them in the various European research programmes (e.g. FP-7); and d) to include regulators at the various stages of the validation process. The main expected contributions will be: a) comments on

the regulatory needs and possible uses of any proposed alternative methods in the pre-validation process; b) agreements and conformity with regard to the definition of PMs appropriate for specific chemical classes; c) the selection of reference chemicals and agreement on standards and positive and negative controls; and d) participation in the post-validation process, in order to speed-up the implementation process.

2. *The structure of ERAP*. ERAP will help to implement the role of ECVAM (6 and Annex 1) in the validation process and will be linked to the new ECVAM expert Task Force on Animal Welfare Legislation. Thus, ERAP will: a) represent a platform for consultation and advice; b) contact experts in specific fields; c) select experts to be involved in management teams of validation studies; d) support the exchange and dissemination of information between ECVAM and regulatory authorities, and between regulatory authorities themselves; e) function as an intermediate forum in the discussion on coherence between the different testing requirements at the EU level; and f) define the possible need for alternative methods and their uses, the criteria for their acceptance, the need for new developments in regulatory requirements, and opportunities for networking and the transfer of information to end-users.

3. *Suggested ERAP membership*. A broad variety of regulatory bodies relevant to alternative methods exist in the various competent authorities of the 27 European Member States. It would not be feasible to involve all of them, or even a reasonable representation. However, many of them are organised at the European level via various European regulatory bodies. Key stakeholders related to ECVAM in the regulatory arena are: the EChA, which will be in service from June 2008, taking over many of the functions previously executed by the ECB; the PhEur/EDQM; the National Coordinators of the test guideline programme; the EMEA; the EFSA; the European Environmental Agency (EEA); and the SCCP. Whether the ESAC and the policy DGs should be involved as observers, needs to be considered.

Expectations on Validation and ECVAM's Responsibilities

ECVAM should continue to actively identify new and promising alternative tests, and establish and publicise harmonised protocols (INVITTOX) for the application of validated alternative methods. Scientists should understand that any method under validation will no doubt evolve. Increasingly, attention needs to be focused on the validation of testing strategies. Integrated Testing Strategies (ITS) are required for application in risk assessment. ECVAM needs to explore and improve strategic test approaches for this purpose. This includes defining the role of an alternative test in a testing strategy.

Figure 1: Comparison of the EU and US bodies involved in the development, validation and acceptance of alternative methods

	EU	USA
R&D	ECVAM + DG RTD + scientific committee	—
Validation	ECVAM	NICEATM
Peer review	ESAC	ICCVAM
Regulatory acceptance	Diverse	Agencies in ICCVAM

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Expanding the concept of validation as a quality assurance of tools in toxicology, to an evidence-based toxicology (EBT; 14, 15; www.ebtox.org), promises to introduce procedures for an objective assessment of toxicological tools and the revision of their use. A rigorous scientific review of current practices is suggested by EBT, to facilitate their revision and exchanges, following the role model of evidence-based medicine from clinical medicine. This will further aid regulators to modernise their risk assessment methodologies.

Before a validation exercise is started, the context in which the test method is intended to be used should be clearly identified, with sufficient emphasis on aspects related to legislation and regulation. For all alternative methods (whether they are non-animal or *in vivo* tests), ethical issues, safety needs and regulatory requirements should be considered for prioritisation. This will require establishing competence in-house and with collaborators, and, if necessary, the creation of new task forces. Where applicable, the different uses of tests in different regulations should be considered in the designing and peer review of validation studies. The involvement of National Authorities in the validation process should be increased. The ECVAM procedures should consider the inclusion of national validation institutions, including ICCVAM and JaCVAM as observers. ECVAM should explore the

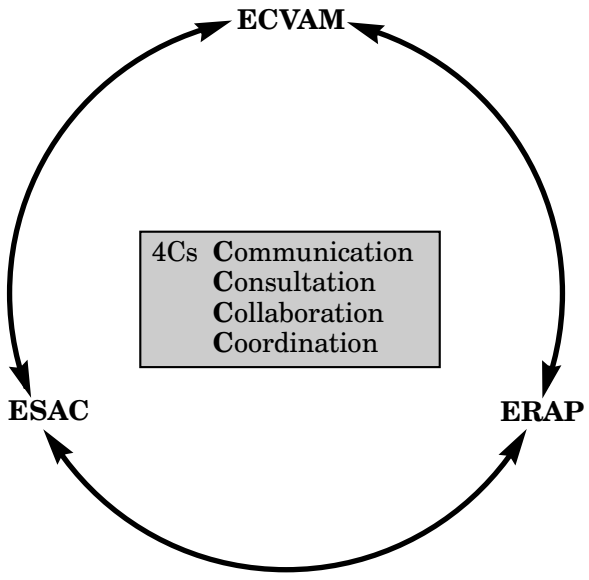
formation and establishment of an ERAP (as described above and in Figure 2), by inter-service consultation with other DGs and with the ESAC.

The peer review process toward ESAC statements needs to be speeded up, and validity assessments should focus on "fitness for purpose", not only on "scientific validity". ESAC statements should identify the intended regulatory use of the validation method. Guidance should be provided for the various groups (agencies and organisations related to different toxicological areas) on the possible use of the validated methods. The ESAC should discuss the incorporation of national authorities. Validity statements should be regularly reviewed or revised, and for this purpose have dated version numbers.

The preferential channels (e.g. EU National Coordinators, OECD Task Forces or the OECD Secretariat) for presenting methods under validation to the OECD need to be identified. As part of this, information on the tests should be sent to the National Coordinators before a validation study begins. One proposal could be that a new alternative method should be informally submitted to the OECD, prior to its validation, in order to evaluate whether it will meet the regulatory needs of countries as well as the prerequisites for MAD. ECVAM should contact the OECD National Coordinators concerning the selection of methods that are needed for regulatory purposes.

The dissemination of validation results needs to extend beyond the regulatory bodies, to include the scientific community, contract laboratories and industry. The implementation of validated tests should be facilitated by e-learning tools.

Figure 2: Proposal for an ECVAM Regulatory Advisory Panel (ERAP)



It is recommended that a further implementation workshop should take place, to bring together regulators and the users of validated tests, to explore their post-validation experiences. Another workshop, to deal with obstacles to the implementation of Three Rs methods at the local level, e.g. in individual laboratories, should be considered, in order to analyse the problem and make suggestions for improvement.

It is also necessary to follow up the "post marketing" of the alternative tests with the help of the different regulators involved, to identify new and/or different toxicological areas for their application, new modifications and uses, and new applicability domains with regard to chemical classes or products.

Conclusions

The EU is an active and dynamic political system, which requires ongoing interaction between the processes of governance, public opinion forming, and politics. Nowadays, legislators and regulators are facing, on the one hand, increasing demands for consumer safety and risk minimisation when developing regulations, directives and, broadly, policy implementation. On the other hand, animal welfare organisations and the general public are demanding clearer rules for the utilisation of animals for toxicological testing. Nevertheless, regulators are reluctant to implement the Three Rs in evaluating substances (2).

One reason for this is the serious responsibility regulators bear for the safety of the products they allow onto the market. In addition, they are not necessarily well informed about the existence and promising use of alternative tests, so they mainly consider the existing accepted (*in vivo*) models.

However, industry has been implementing Three Rs methods for some time, since some human health effects can be assessed by using *in vitro* methods, e.g. those for skin corrosion or skin absorption. Other health effects, such as systemic toxicity, can now be tested by using fewer animals and with less severe effects on them. The genuine aspirations of the participants at this workshop are to enhance the progress towards ultimately replacing animal testing, and to achieve reduction and refinement where replacement cannot yet be accomplished.

In order to achieve these changes, ECVAM has taken leadership in organising a workshop on *Optimisation of the Post-Validation Process*, and the participants in the workshop have made an extensive list of problems identified by the different authorities, as well as an exhaustive inventory of possible solutions, expressed as expectation and recommendation.

The ultimate goal would be coordination between scientists, regulators, policy makers and industry,

at the European level, or even at a broader level, including, for example, the other Member Countries of the OECD, and the harmonisation of legislation and regulations as a key requirement for reducing regulatory animal testing.

It is necessary to identify the different regulatory key players and responsible persons in each toxicological and substance/product area. This will serve as a basis for networking, and will improve communication among the stakeholders, including industry and animal welfare groups. Part of this should be the reactivation of the Panel of National Inspectorates to monitor the use of animal toxicological models and their alternatives. Advantage should be taken of the newly-established EPAA, for the identification of the ways and means to optimise the post-validation process, legal acceptance, and the application of alternative methods in the relevant industrial sectors. Regulators should provide information on the health effects for which *in vivo* tests are needed and for which chemical classes. They should explain their considerations when alternative tests represent an equivalent or better protection for humans and wild-life, to improve and clear them for regulatory acceptance. They should also clearly identify the health effects for which *in vivo* tests are unavoidable and still needed. The implementation of validated alternative methods should also be monitored by the National Competent Authorities of the Member States, and there should be clear incentives for the regulated community to use them.

Regulators should be informed and strongly involved in the various European research programmes, to understand and guide the requests for new or revised Three Rs assays for potential application for regulatory purposes (e.g. in the advisory committees for the current FP-7 projects).

Regulators should be involved at the various stages of the validation process, and should be asked for comments and opinions on the need of any proposed alternative from the beginning of the validation exercise. Their understanding and conformity with regard to the definition of the PM for the alternative method, for specific chemical classes, should be confirmed. They should be involved in the selection of reference chemicals, as well as of standards and positive and negative controls, and last, but not least, in the development of the performance standards for subsequent use in the validation of me-too tests. Due to their key role in the post-validation process, they should participate where possible in the peer review process, and in the origination of proposed ESAC statements and in ESAC's recommendation for the use and acceptance of a Three R method. Regulators and other peer reviewers, in general, should be asked to comment on the extent to which the validation process has identified the proposed uses and limitations of the validated alternative method.

Regulators need to make sure that the implementation of alternative methods replaces the use of outdated animal tests. The costs of conducting animal tests must be transparent, whenever industry is making use of them and/or when regulators require them. Legal instruments (at the EU level) should be explored to enforce the use of validated alternatives. For example, the implementation of Three R methods for a product already on the market is sometimes discouraged by charging fees, as is the case for pharmaceuticals. Such fees should be avoided.

It will also be necessary to harmonise the regulatory use of alternatives at the international level. This should start with communication, e.g. with the US Food and Drug Administration and the World Health Organisation, recognising that industry and academia will not necessarily use alternatives, if is still legal to use *in vivo* tests and the need necessarily to use alternatives is not clearly enforced.

Recommendations

1. ECVAM should give far greater emphasis to aspects of legislation and regulation by establishing competence in-house and with its collaborators (e.g. via task forces).

2. Before undertaking a validation exercise, ECVAM should identify in which context the test methods would be used. A new alternative method should be informally submitted to the OECD prior to validation, in order to evaluate whether it would meet regulatory needs of Member Countries, as well as MAD requirements. Where applicable, the different uses of tests in various regulations should be considered when designing and peer reviewing validation studies. The regulatory bodies should be informed and invited to participate in the management teams of studies. An ECVAM observer should always participate at the OECD National Coordinators Meetings, as part of the EC delegation.

3. ESAC statements should identify the intended regulatory use of the validated method. Validity assessments should focus on "fitness for purpose", and not only on "scientific validity". Validity statements should be regularly reviewed, and for this purpose have dated version numbers.

4. A workshop should bring together the (regulatory) users of validated tests, to explore post-validation experiences. Post-validation review should be considered, in order to ensure consistency across sectors of use (chemicals, cosmet-

ics, plant protection products, pharmaceuticals, medicinal products, etc.).

5. The implementation of validated tests should be facilitated by e-learning tools. It should be monitored by the national competent authorities, and there should be clear incentives to use the tests. The costs of conducting animal tests should be transparent.

6. Annual animal-use reports should include an indication of the implementation of new methods. When the expected changes in animal-use do not occur, there should be a confirmation that alternatives were considered, but not deemed appropriate for use.

7. Communication between regulators and other stakeholders, such as industry and animal welfare protagonists, should be improved.

8. An ECVAM Regulatory Advisory Panel (ERAP) should be established, to create a network of "contact people". This would help to define the need for new methods and their possible uses, the criteria for their acceptance, and the need for new developments in regulatory requirements, by creating opportunities for networking and the transfer of information to end-users.

9. The implementation of a validated test requires that the regulatory authorities are consulted, to confirm the potential uses of the method. That the implementation of alternatives replaces the use of outdated animal tests should be ensured. It should not be discouraged by the charging of fees, which should be avoided.

10. Industrial companies should be encouraged to use alternative methods and to include the results in the dossiers they submit, in order to increase the regulatory acceptance of such methods.

11. The outcomes of validation studies should be widely disseminated, to include the scientific community, contract laboratories, industry, and animal welfare organisations. Scientists should be encouraged to increase their knowledge and understanding of regulatory requirements. A training programme and/or proficiency testing for a newly-validated method (not necessarily organised by ECVAM) would be necessary to improve its implementation.

12. The legal instruments at the EU level should be explored, on how to enforce the use of validated alternatives. The Panel of National Inspectorates should be reactivated.

13. The EPAA should identify ways of optimising the post-validation process, and the legal acceptance and application of alternative methods in the relevant industrial sectors.

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References

1. Anon. (1994). ECVAM News & Views. *ATLA* **22**, 7–11.
2. Anon. (2007). Fifth Report from the Commission to the Council and the European Parliament on the Statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union COM/2007/675 final. Brussels, Belgium: EC. Available at: http://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm (Accessed 11.06.08).
3. Russell, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique*, 238pp. London, UK: Methuen.
4. Hartung, T., Bremer, S., Casati, S., Coecke, S., Corvi, R., Fortaner, S., Gribaldo, L., Halder, M., Roi, A.J., Prieto, P., Sabbioni, E., Worth, A. & Zuang, V. (2003). ECVAM's response to the changing political environment for alternatives: Consequences of the European Union chemicals and cosmetics policy. *ATLA* **31**, 473–481.
5. Anon. (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 Communities* **L358**, 1–28.
6. Anon. (1991). *Establishment of a European Centre for the Validation of Alternative Methods (CEVMA)*. Communication from the Commission to the Council and the European Parliament, SEC(91) 1794 final, 6pp. Brussels, Belgium: EC. (See Annex 1.)
7. Hartung, T. (2006). ECVAM's progress in implementing the 3Rs in Europe. *ALTEX* **23**, Suppl., 21–28.
8. Lahl, U. (2006). *Strategy for Minimising the Use of Animal Testing as Part of REACH*, 12pp. Berlin, German: Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Available at: http://www.bmu.de/English/chemical safety/doc/35887.php (Accessed 11.06.08).
9. Schiffelers, M-J., Hagelstein, G., Harreman, A., & van der Spek, M. (2005). *Regulatory Animal Testing — A Survey of the Factors Influencing the Use of Animal Testing to Meet Regulatory Requirements*, 80pp. Utrecht, The Netherlands: University of Utrecht. Available at: http://www.bio.uu.nl/wetenschapswinkel/content/publicaties/pub-dierenwelz.html (Accessed 11.06.08).

10. Bottini, A.A., Amcoff, P. & Hartung, T. (2007). Food for thought… on globalization of alternative methods. *ALTEX* **24**, 255–261.
11. Hartung, T. (2007). Food for thought… on validation. *ALTEX* **24**, 67–72.
12. Hartung, T., Bremer, S., Casati, S., Coecke, S., Corvi, R., Fortaner, S., Gribaldo, L., Halder, M., Hoffmann, S., Roi, A.J., Prieto, P., Sabbioni, E., Scott, L., Worth, A. & Zuang, V. (2004). A modular approach to the ECVAM principles on test validity. *ATLA* **32**, 467–472.
13. Balls, M., Amcoff, P., Bremer, S., Casati, S., Coecke, S., Clothier, R., Combes, R., Corvi, R., Curren, R., Eskes, C., Fentem, J., Gribaldo, L., Halder, M., Hartung, T., Hoffmann, S., Schechtman, L., Scott, L., Spielmann, H., Stokes, W., Tice, R., Wagner, D. & Zuang, V. (2006). The principles of weight of evidence validation of test methods and testing strategies. The report and recommendations of ECVAM Workshop 58. *ATLA* **34**, 603–620.
14. Hoffmann, S. & Hartung, T. (2005). Diagnosis: Toxic! — Trying to apply approaches of clinical diagnostics and prevalence in toxicology considerations. *Toxicological Sciences* **85**, 422–428.
15. Hoffmann, S. & Hartung, T. (2006). Towards an evidence-based toxicology. *Human & Experimental Toxicology* **25**, 497–513.

Annex 1: The EC Communication of October 1991

COMMISSION OF THE EUROPEAN COMMUNITIES

SEC(91) 1794 final Brussels, 29 October 1991

COMMUNICATION FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

ESTABLISHMENT OF A EUROPEAN CENTRE FOR THE VALIDATION OF ALTERNATIVE METHODS (CEVMA)

The objective of the present Communication is to inform the Council and the Parliament on the establishment of a European Centre for the Validation of Alternative Methods (CEVMA).

In November 1986 the Council of the European Communities adopted *Directive 86/609/EEC* on the approximation of the laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.[1]

Article 23 of this Directive calls on the Commission and Member States to encourage the development and validation of alternative techniques which could involve fewer animals or entail painful procedures.

The European Parliament called on the Commission to provide a mechanism for the validation of alternative testing methods including, where necessary, the provision of financial support to the participating laboratories.

However, a recent Commission report to the Council states that "the Commission recognises that a critical stage in the development of an alternative method is the transition from that of a potentially useful procedure to that of a method accepted as part of a regulatory testing system. The Commission therefore proposes to provide a framework for the evaluation (validation) of alternative test procedures.[2]

The Commission has therefore examined the possibility of establishing an appropriate European body primarily to coordinate the validation of alternative methods, a European Centre to cater for the needs identified in this area by the Council, the European Parliament and the Commission.

After assessing all possible options, the Commission has decided to set up a Centre of this kind within the Joint Research Centre's Institute for the Environment in Ispra, Italy.

The fact that it would be part of the JRC in Ispra and therefore in an environment which is neutral, multilingual, and has long been active in international scientific collaboration would enable it:

a) to use the technical infrastructure of the JRC which already has toxicological laboratories with facilities for conducting experiments *in vitro* and at molecular level;

b) to benefit from multidisciplinary scientific support of the JRC which has lengthy experience in database management and the validation of protocols and methods of analysis in various fields;

c) to help to expand the JRC's role in pre-normative research.

Clearly, however, a Centre of this kind will be unable to perform its tasks to the full unless it can rely on the cooperation and expertise of all parties concerned, namely the Member States, industry, the academic world and animal welfare organisations. It is therefore proposed that a Scientific Advisory Committee be set up, on which all those parties and the Commission would be represented and which would have the task of advising the Centre and helping it to set priorities when drawing up its annual work programme.

In practical terms, this means that a special administrative unit, to be know as the European Centre for the Validation of Alternative Methods (CEVMA), will be set up within the Institute, and will report directly to the Director of the Institute. Suitably qualified staff will be recruited for the Centre. Its primary task will be to coordinate the validation of alternative test methods at Community level. This will involve the specification of test protocols, the organisation of ring-test exercises, the choice of chemicals to be used in these tests, and the analysis and evaluation of the results, etc.

In addition to this main activity, the Centre will also have the following functions:

— to act as a focal point for the exchange of information on the development of alternative test methods;

— to set up, maintain and manage a database on alternative procedures, with associated user services (help line, advice service, etc);

— to promote dialogue between the legislator, industry, biomedical scientists, consumer organ-

isations and animal welfare groups with a view to the development, validation and international recognition of test methods.

Establishing the European Centre for the Validation of Alternative methods is an initiative which will have very favourable repercussions for the development, acceptance and use of such methods.

[1]OJ L n. 358, 18/12/1986.

[2]Report on the possibility of modifying tests and guidelines laid down in existing Comunity legislation in compliance with the Article 23 of *Council Directive 86/609/EEC* 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 (COM(88)243 final, paragraph 23).