

REVIEW

Making validated alternatives available – the strategies and work of the European Centre for the Validation of Alternative Methods (ECVAM)

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Abstract

ECVAM was created in 1991 further to Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes which prescribes to use alternative methods whenever possible. As a service of the EU Joint Research Centre, ECVAM has pioneered the validation process and became a proactive facilitator for effective animal protection, especially in the field of regulatory toxicology. The field of alternatives is currently driven by the expectations from both cosmetics and chemicals policies: The 7th amendment to the Cosmetics Directive published in 2003 foresees to phase out animal experiments completely within 10 years. A timetable for the phasing out of the individual animal tests published by the European Commission in September 2004 had been supported by a task-force of stakeholders chaired by ECVAM. The legislation is reinforced by an immediate testing ban for finished products and for all the human health effects, for which alternative methods have been validated by ECVAM. Furthermore, a testing ban and a marketing ban, which cannot be postponed, apply in 6 years for topical and acute systemic toxicity, while the animal tests for all the other human health effects should be phased out in 10 years with a possible postponement by codecision procedure.

The legislation for chemicals (REACH) is only emerging. It foresees data requirements for more than 30.000 substances produced at levels above 1 ton per year. Extensive in vivo data requirements are expected for a core of about 6.000 substances with highest production and concern. Alternative methods shall first be considered throughout the testing and be predominantly used for the largest group of chemicals, namely those produced between 1-10 tons per year. A Commission proposal as to the legislation has been handed to the European Parliament and Council, and is currently under consultation.

ECVAM has restructured its services directly targeting the animal tests to be replaced by in vitro and in silico approaches. Given the short time-lines to make available and implement validated methods, ECVAM is offering to steer the process of making validated alternatives available by bundling the inputs of stakeholders and by involving regulators in the process at an early stage. Steering groups composed of ECVAM's senior staff and complemented by external experts carry out the project management, which co-ordinates the various inputs. The collaboration with various stakeholders has also been reinforced. A series of activities including the initiation of three Integrated Projects have put the tailored development and validation of alternative methods on a new scale.

Key words: *toxicology, safety testing, nonanimal tests, validation, legislation*

Origin of ECVAM, legal basis, short history

ECVAM is an international reference centre for the development and validation of alternative testing methods aiming at the replacement, reduction or refinement of the use of laboratory animals in the biomedical sciences, with emphasis on toxicologi-

cal assessments. ECVAM was established by a communication of the European Commission (SEC 91/1794) to the European Parliament and Council referring to Article 7.2 and Article 23 of Directive 86/609/EC on the protection of animals used for experimental and other scientific purposes. The

Directive requires that the Commission and Member States should encourage research into the development and validation of alternative methods 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. ECVAM became operational as a Unit within the EU Joint Research Center in 1992. ECVAM's work is focused on the development and evaluation of in vitro methods (e.g. cell and tissue cultures) and of computer modelling based on structure-activity relationships; and on physiological and biokinetic modelling. Actual political needs for ECVAM's core activities are created by REACH and the 7th amendment to the Cosmetics Directive (Hartung et al.2003).

ECVAM objectives and strategy

ECVAM pursues its objective to pioneer the process of quality assurance in the life sciences and regulatory testing by:

- communicating the regulatory needs to test developers
- coaching the development and optimisation of methods
- tailoring the validation process and the good practices
- participating actively in R&D and validation projects
- development of test strategies
- promoting successfully validated tests.

From a strategical point of view, ECVAM has worked out a business plan for the next ten years. Overall costs of about 150 million \$ over these ten years were estimated, These costs will have to be shared with industry who now urgently needs validated alternatives. Structurally, a dense network with stakeholders is in progress of being established for the development of strategies, the provision of unpublished in vivo data and reference chemicals as well as in-house test methods from industry. Candidate tests for validation are selected either during taskforces/workshops or submitted by test developers, which is usually done via the ECVAM website, and then prioritised by taskforces.

Procedure for validation and regulatory acceptance

ECVAM operates as a coordinator of international validation studies, as a focal point for the exchange of information, as the provider of a central data-

base on alternative methods, as a centre of public dialogue and as a pre-normative research facility of the JRC. Participation in validation studies requires a running infrastructure and active research maintains a practical and realistic view on science & technology. Furthermore, high-quality research ensures credibility in the scientific community.

Due to the political sensitivity of its duties, ECVAM has its own Scientific Advisory Committee (ESAC) composed of members from all 25 European Member States, from relevant industrial associations, from academic toxicology, from the animal welfare movement, as well as from other Commission services with an interest in the area of alternative methods.

ECVAM has established a wide international network with OECD, with its American counterpart ICCVAM and with other Commission services, such as Directorate General (DG) Environment, DG Enterprise, DG Research and Development and DG Health and Consumer Protection. This network is used for reaching international expert consent, test implementation and emission of opinions.

A typical validation lasts 3 years (costs per test are about 300,000 \$) and the subsequent regulatory implementation lasts from 2 to 7 years. Main constraints in the process are the availability of reference substances and animal test data, the need for further optimisation of test methods, the duration of financial/administrative procedures and the long-lasting consensus process of regulatory implementation.

Changes in the political environment

The new European legislation on chemicals

The European Commission has proposed to harmonise the testing requirements for existing chemicals, for which there is a lack of safety assessment data (i.e. chemicals marketed before 1981) and new chemicals, by developing a new system for the Registration Evaluation and Authorisation of Chemicals (REACH). The new system will have less stringent testing requirements compared to those imposed by the current legislation on the new chemicals, but these will also apply to approximately 30,000 substances, that are currently marketed in volumes greater than 1 tonne per year. The extent of data requirements will depend on the tonnage of chemical produced or imported in the EU. Consequently, this will result in a substantial in-

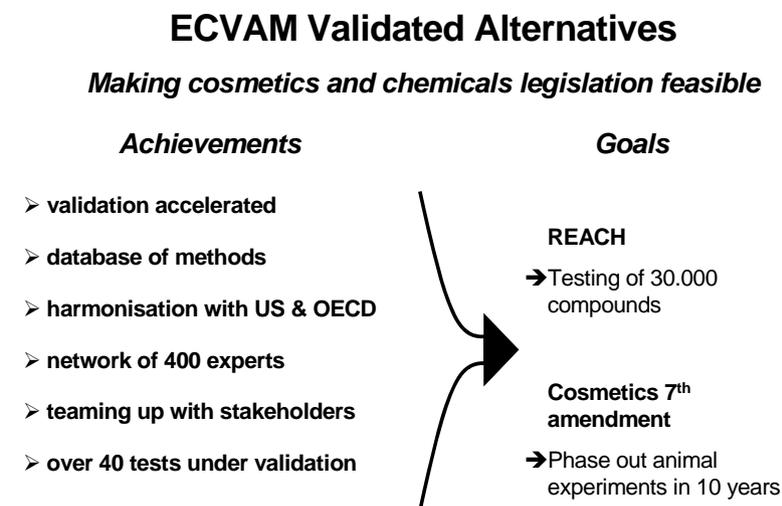


Figure 1

crease of animal use for the safety assessment of chemicals. Several estimates of the number of laboratory animals required for these assessments, and the costs for performing the tests, have been made. They indicate that several million of animals will be required, that testing costs will range in billions of \$ and that simply the availability of animal testing facilities will be a limiting factor. Beside the ethical aspects and the public concern, also economical considerations call for the timely development and validation of in vitro alternatives.

The Seventh Amendment to the Cosmetics Directive

Much of the scientific work on alternatives which has been conducted, coordinated and sponsored in the EU, was strongly pushed by the animal protection community and by the public opinion, who broadly do not support animal testing for cosmetic products. In the EU, the safety of cosmetic products is regulated by Council Directive 76/768/EEC (EC, 1976). Its 7th amendment was finally approved by the European Parliament and the Council in March 2003. It foresees the immediate ban on animal testing for finished products, and a complete ban on animal testing for cosmetic ingredients no later than six years from implementation of the Directive. Moreover, it requires the immediate marketing ban for new cosmetics (finished products and ingredients) tested on animals where alternative methods validated by ECVAM and ac-

cepted by the Community exist. It also foresees a complete marketing ban on cosmetics tested on animals within six years for some targeted human health effects and ten years for the repeated-dose toxicities, reproductive toxicity and toxicokinetics. This latter date can be postponed if by that date, no validated alternative methods will be available.

ECVAM Strategic Vision

From its beginning, ECVAM has been more than the administrator of alternative methods and their formal validation: The

field of alternatives requires a proactive role (Figure 1), where ECVAM:

- a) communicates the needs of the regulatory area to putative developers of new tests
- b) surveys opportunities for new technologies
- c) steers a strategic debate between stakeholders
- d) coaches the development and optimisation of methods
- e) develops and tailors the validation process
- f) participates in R&D as well as the validation process with its laboratories
- g) collects, compiles and provides the information on relevant methods
- h) integrates tests into test strategies
- I) promotes tests after validation
- j) pioneers the process of quality assurance in the life sciences

These roles have to be regularly revisited in light of the political needs. At this moment, two very obvious areas of concern are REACH and the 7th amendment to the Cosmetics Directive. Beside

Purposes of animal experiments in Europe in 2002

Total number	10,700,000	100%
Safety evaluations	1,060,000	10 %
Agricultural chemicals	123, 000	1 %
Industrial chemicals	136,000	1 %
Cosmetics	2,700	0.025%

Figure 2

The new dimension of development of alternative methods

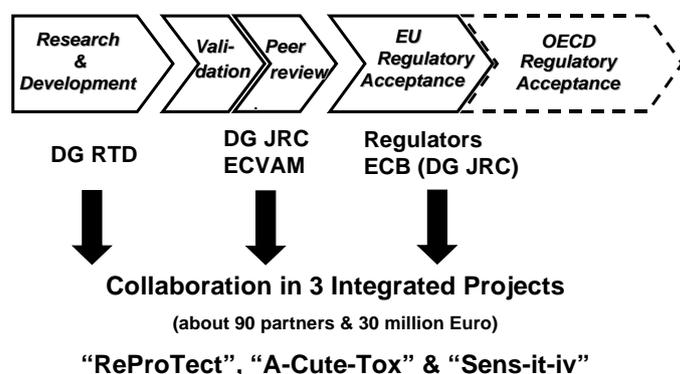


Figure 3

these, even more impressive with regard to animal consumption (Figure 2) are biologicals (16%) if compared to chemicals (1%) and cosmetics (0.025%). Areas like pharma articles (Hartung 2002) and basic research (Gruber and Hartung 2005) also deserve further attention.

Highlights of some of recent activities related to the ECVAM strategies include:

a) Communication of needs of the regulatory area to putative developers of new tests

The ECVAM workshop series has just celebrated the 50th workshop report (Gennari et al. 2004) published in the journal *Alternatives to Laboratory Animals* (ATLA). To acknowledge ECVAM's collaboration with FRAME, which is the publisher of ATLA, a joint workshop on 'Invalidation of test methods' is planned for September 2005.

b) Surveying opportunities for new technologies

Following a joint workshop with ICCVAM on validation of toxicogenomics in 2003, a taskforce was established in 2004 and a pilot study was carried out with Affymetrix and Bayer. At this moment a workshop on metabolomics in toxicology is in preparation. Very extensive efforts are spent on (Q)SAR (Worth et al. 2004 a & b). By purpose, this activity – a close collaboration between the European Chemical Bureau (ECB) and ECVAM – was put under the umbrella of OECD, where agreement about the principles of (Q)SAR validation and regulatory use are sought. In parallel, the first formal validation studies were initiated in 2004 addressing skin sensitisation, skin penetration, acute fish toxicity and endocrine disruption.

c) Strategic debate between stakeholders

ECVAM has established a network of about 400 experts regularly working in taskforces and workshops. A broad variety of collaborations with all relevant institutions in the field, aiming to bundle stakeholder activities, also exist. For the first time, an European opinion leader meeting was organised in 2004, convening the organisations acting on an European scale in order to discuss the perception of alternative methods in the (scientific) public and how to improve their image. In close partner-

ship, all ECVAM activities have been opened to the American counterpart ICCVAM. With a view to extend this partnership, the establishment of an International Council of Validation Bodies is under discussion.

d) Coaching of the development and optimisation of methods

The EU has already invested more than 200 million \$ into the development of alternative methods by funding respective research. Lately, by installing three large Integrated Projects a new dimension of tailored development of alternatives was reached (Figure 3). These projects which involve more than 90 institutions and a funding of about 40 million \$ aim to make available batteries of test plus the respective test strategies within five years each. ReProTect (Hareng et al. 2005), started in July 2004, and deals with the field of reproductive toxicology including endocrine disruption. Noteworthy, the different tests included into the project for optimisation and integration into a test strategy originate not only from the field of alternatives, but also from areas such as mechanistic biomedicine especially reproductive medicine, pharmaceutical agent discovery, clinical diagnostic, breeding of farm animals etc.. These models were never suggested as alternative methods, since they have different purposes and reflect only partial aspects of the reproductive cycle, but put together in a conceptual framework they might allow to build a predictive test strategy.

A-Cute-Tox was based on an ECVAM workshop in 2003 (Gennari et al. 2004). It started in January 2005 and aims to establish an animal-free classification of acute toxicity, substituting for the classical LD₅₀ test. Several studies have shown a

good correlation of in vitro cytotoxicity studies and the animal experiment. The project wants to improve this correlation to an acceptable level by outlier reduction.

Sens-it-iv will start towards the end of 2005 with a view to complete the development of animal-free test strategies for skin and lung sensitisation. It was based on an ECVAM workshop (Casati et al 2005).

e) Development and tailoring of the validation process

On the one hand, just now for the first time international consensus on the role and procedure of the validation process has been reached by creation of OECD guidance document 34 on the validation and international acceptance of new or updated test methods for hazard assessment. The guidance document mainly reflects the ECVAM principles. On the other hand, various challenges to the validation process as such exist. For example, the enormous efforts involved for a single test with regard to laboratory work and costs have often been questioned. In light of the need to validate a very high number of tests for the purposes of the chemicals and cosmetics legislations, this aspect had to be reviewed. So far, validation studies did not make use of existing data. However, in some instances such retrospective validation might be an appropriate shortcut to the assessment of validity. Further challenges to the current validation scheme originate from new technologies (pattern-based or 'omics' approaches, transgenic animals, in silico methods such as (Q)SAR or computer modelling). In response, ECVAM has proposed a modular approach (Hartung et al. 2004), which opens up ways to accommodate these needs. The discussion as to the optimisation of the validation procedure, however, continues.

f) Participation of ECVAM in R&D as well as in the validation process with its laboratories

As part of the EU Joint Research Centre, ECVAM represents also a place of research and education. Repeatedly, ECVAM has made contributions to the development and optimisation of alternative methods. ECVAM's laboratories allow also to participate any time actively in validation studies. Details are given in a chapter below.

g) Collection, compilation and provision of information on relevant methods

ECVAM hosts a database on alternative methods

(dbAlm), which provides high-quality information related to alternative methods, including protocols for relevant in vitro tests. The major part of this scientific information service will be available online at the end of the year. A valuable resource of documents such as the ECVAM workshop reports is provided free of costs via the ECVAM website (www.ecvam.jrc.it).

Another major contribution to the field have been the compilation of inventories of alternative methods which are currently available. An inventory of 330 pages compiled with 75 experts in the context of the cosmetics legislation will soon be published as an ATLA supplement.

h) Integration of tests into test strategies

Many of the more complex toxicological endpoints will not be replaced by single alternative methods, but it will be necessary to develop testing strategies based on batteries of tests and their intelligent combination in test strategies. An important element of this is the concept of prevalence of health effects of chemicals (Hoffmann & Hartung 2005), i.e. the actual proportion of chemicals showing a certain toxic property. Furthermore, it is necessary in order to develop such strategies, to analyse the performance of the animal experiment, an effort which has just started for example in the field of skin irritation (Hoffmann et al. 2005). Methods of decision theory and evidence-based medicine will be employed in order to compose and validate finally testing strategies.

I) Promotion of tests after validation

Today, the regulatory implementation of validated tests often lasts longer than the validation process itself. This obvious bottle-neck can only be overcome by collaboration with regulators such as the National Coordinators of the Chemical Test Guideline Program in Europe or at the level of the OECD. However, for this purpose validation has to take the needs of regulators into account, i.e. a validity statement is less a scientific judgement but a proof that the method is fit for (regulatory) purpose.

GLP and GCCP

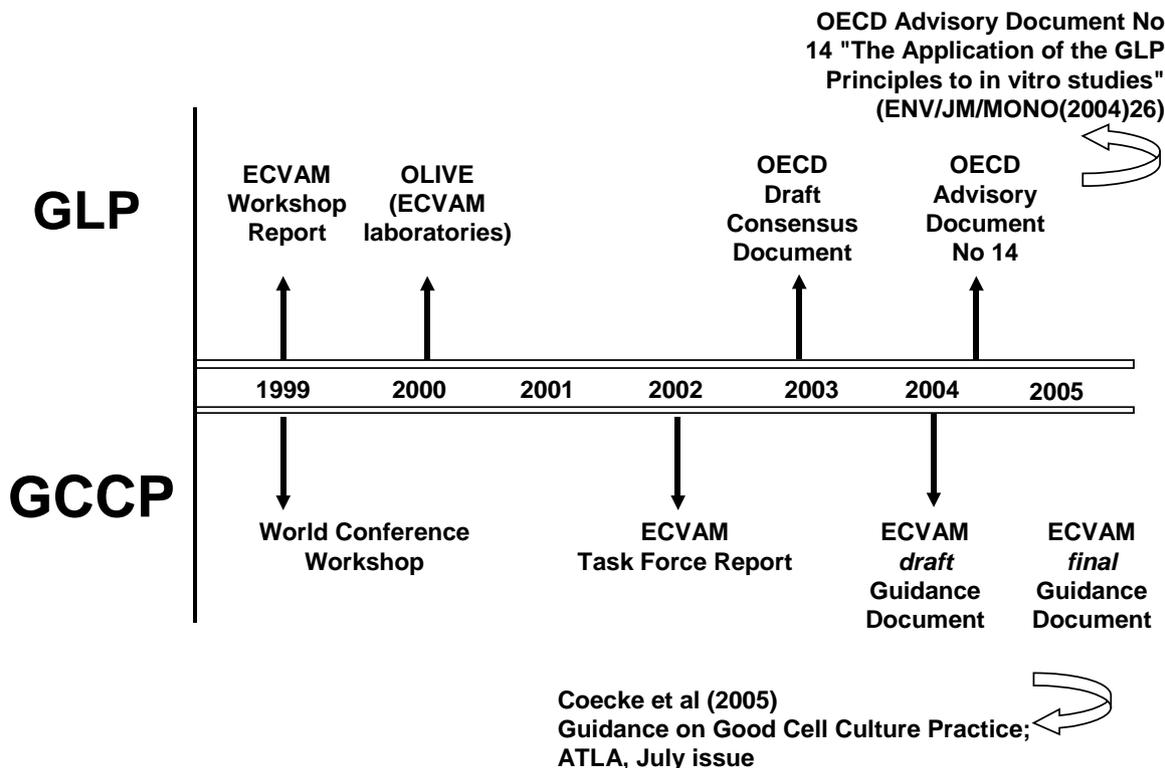


Figure 4

j) Pioneering the process of quality assurance in the life sciences

Further to an ECVAM workshop in 1999, an OECD guidance document on Good Laboratory Practice (GLP) for in vitro toxicological studies was accepted in 2004 (Figure 4). In parallel, a Good Cell Culture Practice (GCCP) guidance document (Hartung et al 2003) was completed recently (Coecke et al. 2005); it sets the minimal standards for quality control of in vitro work and will enable an international discussion over the next year. In the context of quality control, ECVAM is aiming for further adoption of principles on evidence-based medicine to the field of toxicology. The establishment of a taskforce on evidence-based toxicology has been agreed in 2004.

Validated and accepted alternative methods

Eight alternative methods for chemicals/cosmetics have been endorsed by ECVAM including skin corrosivity, skin sensitisation, phototoxicity as well as embryotoxicity testing of chemicals. Seven alternative methods reached scientific acceptance for

e.g. the safety evaluation of biologicals such as vaccines. Eleven alternative methods for myelotoxicity, pyrogen testing, acute fish toxicity, mutagenicity and chronic toxicity in dogs are currently under peer-review by ESAC.

Relevant methods were accepted by both the European Commission (Annex V to Council Directive 67/548/EEC on the classification, labelling and packaging of dangerous substances) as well as by the Test Guideline Programme of the OECD. Three potency tests of biologicals have been accepted by the European Directorate for the Quality of Medicines (European Pharmacopoeia).

Estimated impact of current activities

The impact of alternative methods can be very substantial: The most successful replacement method, the Limulus test for pyrogens, reaches an annual turnover of 200 million \$ and saved more than one million rabbits per year. The remaining 200.000 rabbits used per year in Europe can most probably be fully replaced by the six methods currently peer-reviewed (Hoffmann et al. 2005). For the time being a reduction method saving one third

of animals was proposed (Hoffmann et al. 2005). For chemicals and cosmetic ingredients the following animal experiments (% animal use in Europe in 1999) have to be considered:

- acute toxicity (35%): OECD-accepted alternative methods in 2000 reduced the number of animals used from 45 to 8 per chemical; alternative methods which have been currently validated (to be completed 2005) reduces this number again to estimated 3-6 animals per chemical. ECVAM set up an Integrated Project *A-Cute-Tox* (2005-2009, 11 million \$ funding, 37 partners) aiming for full replacement of the animal tests according to an ECVAM workshop (Gennari et al. 2004).

- Skin sensitisation (5%): An OECD-accepted refinement method has reduced the number of animals used per chemical and their suffering; ECVAM set up an application for an Integrated Project *Sens-it-iv* (2006-2010, 14 million \$, funding, 31 partners) following an ECVAM workshop (Casati et al. 2005).

- Chronic toxicities (27%): a strategy for chronic toxicity was developed at an ECVAM workshop held in 2004; currently a consortium and a work programme are being set up.

- Toxicokinetics (2%): a strategy on toxicokinetics has been developed at an ECVAM workshop in 2004. The OECD accepted a test for skin penetration in 2004. First validations on barrier models (blood brain barrier and gut absorption) and work on physiology-based pharmacokinetic modelling as well as metabolism start in 2005.

- Mutagenicity and carcinogenicity (8%, costs of an animal cancer study is up to 1 million \$ per substance): two validation studies started in 2005 in which variants of the cell transformation assay and the in vitro micronucleus test are being evaluated.

- Reproductive toxicity (13% animal use in toxicology in 1999; up to 55% of the testing costs of REACH): Three embryotoxicity tests were validated in 2002; ECVAM set up an Integrated Project *ReProTect* (2004-2008, 11 million \$, 27 partners) to develop an alternative test strategy.

- Endocrine disrupters (2%): Validation studies with US were agreed on for 2006 and will be parts of ReProTect.

- Skin-eye corrosion (3%): OECD accepted tests in 2004.

- Phototoxicity (3%): OECD accepted test in 2004.

- Skin irritation (1%): a validation study is currently running (2004-2005).

- Eye irritation (1%): Joint review of existing data with US ICCVAM from 2004-2005; depending on the outcome, joint validation studies will be organised in 2006-2007.

- Acute fish toxicity (up to 40% of animals used for REACH): Reduction alternative developed by ECVAM will reduce by 60% the number of fish used, The method will be peer-reviewed in 2005. The Fish egg test which is a full replacement to the current animal test will be validated in 2006-2007. The impact of QSAR (in silico methods) can not yet be anticipated since no experience with validation exists. Regulatory use so far was mainly in priority setting and not in substitution of the current animal tests. Furthermore, it is not clear which percentage of chemicals can be subjected to QSAR (lack of purity, mixtures, salts, metal compounds etc.). Calculations of the ECB suggest up to 60% reduction. Similarly, possible use of toxicogenomics is encouraged.

Beside the aspect of animal reduction, cost savings which in some cases can reach more than 90%, and a higher throughput which will be extremely relevant for the testing of 30.000 chemicals under REACH, will have to be considered.

ECVAM's own research activities

Participation in validation studies requires a running infrastructure. The active participation in validation studies which ranges from check of SOPs and transferability to full participation in blinded ring trials under GLP, helps to identify problems of methods and allows to flexibly fill gaps in studies. It also ensures neutrality of the study. Since GLP increasingly becomes a standard for validation studies, ECVAM currently establishes this quality assurance regimen.

Active research maintains a practical and realistic view on science & technology. ECVAM must not become an administrative body, because the validation process is interlinked with development aspects. The pipeline of methods to be validated has to be filled actively in interaction with the basic and applied research community. The own research safeguards that this dialogue is realistic and effective.

High-quality research ensures also credibility in the scientific community. This requires visibility and most importantly publications, favorably in higher impact factor journals. Currently, ECVAM researchers publish about 40 scientific papers per year, including prestigious journals such as Nature and Proceedings of the National Academy of Sci-

ence.

ECVAM's laboratories have high standards with regard to infrastructure, space and resources. A very unusual combination of technologies, and expertises (e.g. various in vitro technologies, metal toxicology, stem cells) is further amplified by the links to neighbouring units and the enormous network of external collaborators. This gives an excellent frame for about 20 Ph.D. students, post-docs and visiting scientists. All together a unique integrated approach spanning from basic to applied research and regulatory view is possible. ECVAM's research is uniquely positioned at the gap between basic research and validation. Given the difficult access to animal primary cells at ECVAM and the limitations of cell lines, clear priority is given to human (primary) cells. The emerging stem cell technologies (embryonic and adult) (Bremer & Hartung 2004, Pellizzer et al. 2005) offer new opportunities as do the classical accessible human cell sources blood and bone marrow. Cryo-preserved human blood (Schindler et al. 2004) represents a very promising source of standardized cell material without the problem of blood donations. Among the animal cell lines, Balb 3T3 deserve special attention since they are used both for the cell transformation assay and for the currently validated basic cytotoxicity test. Several projects have led to the identification of prototypic toxins, e.g. metal compounds or test substances from validation studies. In some instances, ECVAM possesses high-quality in vivo and human data. Many of these substances have already been characterized in several standardized in vitro systems. This allows synergy linking between projects by testing the same set of reference substances. This will be further increased by the planned establishment of repositories of substances. Currently, a high-throughput testing facility is established jointly as a JRC exploratory research project, which offers opportunities for several projects such as those on acute toxicity (A-Cute-Tox), acute fish toxicity, neurotoxicity and immunotoxicity. Work on human (stem) cells will always be limited by the number of cells available. Furthermore, organotypic (co)cultures require analyzing individual cells in mixtures, which is also the case for most stem cell derived differentiated cells. This calls for the establishment of technologies allowing single cell analysis, such as confocal microscopes, FACS, cell sorting (all existing at ECVAM), laser scanning microscopy, in situ PCR, laser microdissection, cell chips etc. Setting up an array of cut-

ting-edge methods should keep ECVAM also attractive for visiting scientists. As another aspect, the fate of test substances in vitro, e.g. its solubility, binding to plastic or serum albumin, is routinely not considered. Addressing this might improve the predictive capacity by reducing an uncertainty factor. Similarly, the effect of exposure patterns to test substances in vitro has been hardly studied. Several current projects aim to understand the effective exposure of cells in vitro and the consequences for toxic responses. Signature-based ("omics") and computational models promise new approaches in several fields. The full integration in all key areas will be important to leverage these technologies. Current projects make use of toxicogenomics and metabolomics (Nuclear Magnetic Resonance, NMR, and Mass Spectroscopy, MS).

Co-operation between ECVAM and USA

The close collaboration of ECVAM with the US is detailed here, in order to demonstrate the opportunities for such bilateral work, which would gain further from an extension to Japan and other major industrialised countries. Interactions between ECVAM and governmental bodies in the USA have started as early as 1993, shortly after the creation of ECVAM. Since 1995, ECVAM has a bilateral co-operation with the US Interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM). The aim is to have an early exchange of information on the validation of test methods as to facilitate mutual recognition, acceptance, and implementation of scientifically validated testing methods. Secondly, this co-operation serves to facilitate the OECD process in providing harmonised protocols to the scientific community and promoting international adoption of validated alternative methods.

The existing collaboration between ECVAM and ICCVAM in the field of alternative testing methods has been strengthened during the last three years and comprises the following activities: ICCVAM has an observer status on the ECVAM Scientific Advisory Committee. The Head of ECVAM became member of SACATM, the US Scientific Advisory Committee for Alternative Toxicological Methods. Both ESAC and ICCVAM have agreed on parallel peer-review and arbitration of results for the upcoming peer-reviews (pyrogen tests, haematotoxicity, chronic toxicity in dogs, micronucleus test). ICCVAM and ECVAM discussed on creating an International Council of Validation Bodies, to coordinate validation studies

at the level of OECD. Discussion about formal collaboration with OECD has been initiated. About 20 visits of ICCVAM members or ICCVAM-nominated experts to ECVAM taskforces, workshops and validation management groups take place per year. FDA has alloted a specific budget for parts of these travel costs. A sabbatical programme to exchange ECVAM and ICCVAM personnel was agreed upon. In 2003, the Head of ECVAM also became member of the Scientific Advisory Committee of CAAT, the Center for Alternatives to Animal Testing of the Johns Hopkins University, Baltimore, which has pioneered the field of alternative methods in the US for about 25 years. At the same time, he became member of the Scientific Advisory Committee of the Institute for In-Vitro Sciences (IIVS), Gaithersburg. Furthermore, a senior American manager from The Procter and Gamble Company is on secondment for two years at ECVAM.

Collaborative technical activities

Acute systemic toxicity

In 2002, NICEATM and ECVAM initiated the first joint validation study to evaluate the usefulness of two in vitro basal cytotoxicity assays for estimating acute systemic toxicity. The sponsors of this study are NIEHS/NTP, ECVAM and EPA. Seventy-two coded chemicals exhibiting a wide range of toxicity are being tested in mouse 3T3 fibroblasts and normal human epidermal keratinocytes (NHK) using neutral red uptake (NRU) to assess cytotoxicity. In vitro data will be used to predict the starting dose for acute rodent lethality testing. Preliminary analysis indicated a possible reduction in animal use of up to 30% (for the Up and Down Procedure), with fewer animals sacrificed when testing highly toxic substances.

Topical Toxicity

Skin irritation

Two members of ICCVAM have an observer status on the Management Team of the ECVAM validation study on acute skin irritation (2003-2005). Two US laboratories [Institute for In Vitro Science (IIVS) and DuPont] participate in the validation study. ECVAM collaborated with ICCVAM for the selection of appropriate chemicals to be tested in the validation study.

Eye irritation

The following activities where ECVAM and ICCVAM collaborate, were launched in 2004:

- An ECVAM representative was invited as liaison to the ICCVAM Ocular Toxicity Working Group
- ICCVAM and NICEATM are represented on the ECVAM Task Force on Eye Irritation
- An ECVAM / ICCVAM collaboration workplan was developed (February 2004) and the leadership for the evaluation of different in vitro assays was defined
- ICCVAM Federal Register Notices are regularly published on the ECVAM website
- Mutual sharing of non-confidential reports and data is in place
- ECVAM participation in the ICCVAM retrospective evaluation of four alternative test methods for severe eye irritants, recommended by EPA and EU National Competent Authorities, i.e. the Bovine Corneal Opacity and Permeability test (BCOP), the Isolated Chicken Eye test (ICE), the Isolated Rabbit Eye test (IRE) and the Hen's egg test on the Chorio-Allantoic Membrane assay (HET-CAM).

Three joint workshops were carried out in 2005, i.e. the ECVAM Expert Meeting to advance alternative methods for eye irritation, the ICCVAM Workshop on the mechanisms of chemically-induced injury & reversibility and the ICCVAM Workshop on humane approaches to ocular irritation.

Reproductive toxicology

In the framework of ReProTect, an Integrated Project, ICCVAM was offered an observer status on the supervising board. The project started in July 2004 and is planned to last for five years. The project is sponsored by DG RTD with 11 million \$: (see above).

Endocrine disrupters

Under the umbrella of OECD, ECVAM, the Chemical Evaluation and Research Institute (CERI, Japan), the US Environmental Protection Agency (EPA) and ICCVAM have agreed to collaborate on the formal validation of the estrogen receptor (ER) binding assay to evaluate the binding affinities of potential endocrine disrupting chemicals/substances. After several bilateral meetings, agreements were reached at an OECD meeting in November 2004. The prevalidation study should start soon.

Toxicogenomics

A joint ECVAM-ICCVAM workshop on the vali-

dation of toxicogenomic-based test systems was held in Ispra in December 2003. The workshop report is in press.

Neurotoxicology

The interest of ECVAM in the field of neurotoxicology (Coecke et al. 2005) focuses on the development of in vitro test methods to screen chemicals for their ability to cause neurotoxicity and developmental neurotoxicity. More recently, the development of alternative test methods for neurodevelopmental toxicity has also become an important issue for the US EPA. The EU (ECVAM) and the USA [The Johns Hopkins Center for Alternatives to Animal Testing (CAAT)] are currently setting up joint efforts in this field, also with a view to satisfy European legislations, such as REACH. A list of workshops were held jointly by ECVAM, CAAT and CEFIC.

Good Laboratory Practice – Good Cell Culture Practice

The requirement for carrying out validation studies under standardised conditions, i.e. apply GLP and GCCP rules, has been recognised by national and international validation bodies. ECVAM plays a leading role in this process and actively contributes to the drafting of advisory and guidance documents. Both ECVAM, DG Enterprise and ICCVAM were part of a GLP working group in order to draft an OECD Guidance document on GLP and in vitro toxicology finalised in May 2004.

ECVAM is also playing a leading role in drafting a new Guidance Document on Good Cell Culture Practice (GCCP). The aim of this GCCP document is to reduce uncertainty in the development and application of animal and human cell and tissue culture procedures and products, by encouraging the greater international harmonisation, rationalisation and standardisation of laboratory practices, quality control systems, safety procedures, recording, reporting, and compliance with regulations and ethical principles. In order to give this document an international dimension ECVAM invited ICCVAM as part of the steering group who drafted this document (Coecke et al. 2005).

Databases

As an outcome of a project of the ECVAM Task Force on Alternatives Databases in collaboration with the Head of the thesaurus section of the US National Library of Medicine (NLM), a first version of a thesaurus on animal alternatives has been

developed by using the novel 'bottom-up' approach. The thesaurus has been generated in a semi-automatic manner, by selecting actual phrases that occurred in 2000 documents, and should therefore reflect the preferred terminology used by the authors of the articles. This first version focuses on toxicity testing and the first 11 main sectors identified. Following two consultation rounds the thesaurus will be made available throughout the new Internet version of the ECVAM Database for Alternative Methods (formerly ECVAM Scientific Information Service) for practical application by the end-users to be expected in fall 2005.

ECVAM has been invited to participate in a newly formed ad hoc group of the NLM to address the following main subjects:

- Journals on Alternatives and MEDLINE
- Keywords on alternatives in MEDLINE
- Adding Search Filters to PubMed

The following significant outcomes have been reached: Based on the advice given by the participants during the expert meeting, the Directorate of the NLM decided to add 9 Journals on alternatives to MEDLINE, changed the index terms for MeSH to better identify papers related to the alternatives concept and to sponsor a study on the feasibility of adding query filters for animal alternatives searches.

Twenty-two percent of subscribers to the ECVAM Database for Alternative Methods are from the USA.

(Q) SAR

A (Q)SAR Working Group on Endocrine Disruptors was created under the umbrella of OECD [Validation Management Group – Non-animals (VMG-NA)]. The EU is represented by the JRC, and the USA by EPA and ICCVAM.

Collaboration with OECD

The ultimate goal of all efforts towards alternative methods must be an international acceptance. In terms of tests for chemicals, this typically means acceptance at the OECD level. In order to improve this collaboration, already two ECVAM staff members have been seconded to OECD for several months, and since this year OECD has an observer status on the ECVAM Scientific Advisory Committee. Several alternative methods were included as OECD test guidelines (tiered testing strategies for acute toxicity, local lymph node assay, skin penetration, skin corrosion, phototoxicity), the lat-

ter two represent in fact the first two full replacement methods which were finally accepted, in 2004, at an international level. Via the collaboration on a guidance document for Good Laboratory Practice for In Vitro Toxicology Studies (OECD Advisory Document No 14 "The Application of the GLP Principles to in vitro studies" (ENV/JM/MONO(2004)26)) and one on Validation (guidance document 34, 2005), the basis for a full integration of alternative methods was created. Noteworthy, close collaboration of the ECB, ECVAM and OECD expand this currently to (Q)SAR (Worth et al. 2004 a&b). The early involvement and parallel work on test guidelines and method validation is crucial to speed up the regulatory implementation process. Notably, at this moment several such parallel and coordinated activities exist (cell transformation assays, in vitro micronucleus test, endocrine disrupters).

Towards an International approach in the field of alternatives

Over the last three years, an enormous effort towards collaboration and harmonisation with the US and OECD took place. The extension especially to Japan is a desire of all partners and the plans to create a Japanese center for the validation of alternative methods represents an important step towards this. Already now, ICCVAM and ECVAM are discussing the creation of an International Council of Validation Bodies, in order to harmonise procedures and collaborate strategically with OECD. At this moment, several individual involvements of Japanese institutions in the validation of both cell transformation assays and those for endocrine disrupters exist, which make these the first real world-wide validation studies for alternatives. We hope to further this approach, because we believe that there are few things for which there is no alternative, but there is no alternative to international collaboration.

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