8th Annual *ecopa* Workshop on "Cosmetics Directive, REACH legislation and novel Directive 86/609: realistic 2009/2013 – factual status?"

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Welcome

Prof. Vera Rogiers welcomed all attendants of this workshop and thanked the speakers for accepting the invitations. She said to be very glad to have some input from the regulatory side as well, since this is not always easy to get.

Opening address: The EU approach to deadlines regarding alternative methods: the Commission's support activities for 3Rs, by Mme. Dir. Georgette Lalis, DG ENT

This presentation focused on four points, being Regulatory incentives to the use of the 3Rs, Research, International Cooperation and EPAA.

The 3Rs concept was introduced in European law by Directive 86/609 of 24 November 1986 regarding the protection of animals used for experimental and other scientific purposes. Article 7 states that an experiment shall not be performed if another scientifically satisfactory method, not using animals, is available. A proposal is now adopted to adapt this directive (more information in Susanna Louhimies' presentation).
Recently, the 7th Amendment of the Cosmetic Products Directive introduced a ban on animal testing of final cosmetics products, with deadlines for 2009 and 2013. For the 2009 deadlines, 4 alternative methods are accepted and one more will be accepted soon. The Commission and Industry work hard to make alternative methods available for the remaining 2 safety tests, on eye irritation and acute toxicity.
The 2013 deadline deals with complex endpoints, for which the development of alternatives is difficult. In 2011, the Commission will judge whether the deadlines can be kept.
With REACH comes a temporary increase in animal testing, however regulations were taken to do animal testing only if no other methods are available.
From these examples it becomes clear that the 3Rs are promoted in all fields involving animal testing.

The EC also supports the 3Rs through research, mainly by different FP6 and FP7 projects. Replacement is most important in these projects, especially through the 'HEALTH' programme of FP7, however reduction and refinement are given a lot of attention as well. In these projects, research is also done for REACH, to find solutions for the massive testing that REACH will cause. Under FP7, a call will be launched together with US-EPA to develop screening methods.

The EC also launched IMI, which is a public private partnership between the industry, represented by EFPIA, and the EC itself. The goal of IMI is to promote the use of the 3Rs in different fields.
The EC was also approached by COLIPA, to make a new, joint effort between the industry and the EC, with the aim to intensify research on "repeated dose systemic toxicity". This is now under negotiation.
In the Institute for Health and Consumer Protection of the JRC in Ispra, knowledge about computer simulation methods and test batteries is being built, which will help the centre to validate more new technologies in the fields of toxicity testing. This will help to further reduce the use of animals.
The JRC will also launch a website, TSAR2, which will present information on the status of alternative methods, throughout the whole process of validation.

Promoting the 3Rs is only effective, when done on a global basis, therefore dialogue is held with the US, Japan, Canada and Australia.
In the area of pharmeceuticals, the EDQM is working to avoid duplication of testing for batch release.
In 2007, the EC launched, together with the USA, Canada, Japan and Australia, the ICCR, which has animal testing as a priority. The ICCR invited ICCVAM, ECVAM, JaCVAM and Canada to work on a collaborative approach on validation. In 2008, the ICCR created ICATM, to enhance cooperation on the field of the 3Rs. The main points of interest are: (1) validation studies, (2) independent peer review of the scientific validity of test methods, and (3) development of formal test method recommendations on alternative testing methods.

The EPAA is a voluntary, consensus based partnership launched in 2005 between the EC and the industry from seven large sectors, represented today by 37 companies. EPAA's activites range from research to validation. There also is a strong focus on dissemination, which will be the lead theme for 2009.
One of the successes of EPAA is promoting cross fertilisation between sectors. An example of this integrated approach is a project like acute toxicity, for which EPAA has initiated a project, which has the aim to verify the regulatory relevance of acute toxicity testing in the sectors of cosmetics and chemicals.
EPAA has two important databases on research. The first one is an inventory of alternative tests and approaches, employed in screening, related to product safety evaluation. The other one gives an overview of ongoing projects, centred around 3Rs in safety assessment.
An EPAA workshop organised in 2006 concluded that the extended one-generation study could be applicable to safety testing under REACH. However, the complex ACSA protocol would have to be modified in order to meet the current requirements for industrial chemical safety testing. Currently, the EPAA is involved in feasibility studies based on this modified protocol. This should lead to an OECD guideline for an extended one-generation study, which would lead to a more than 40% reduction in the number of animals used compared to the two-generation study.
Overall, EPAA has been instrumental in the implementation of the 3Rs, not only in Europe, but at the global level.

Session 1: The Theme of Deadlines

Realistic basis for implementation oflegislation and alternative methods?, by Dr. Bernward Garthoff, *ecopa*, DE

This lecture started with lining out some (historical) deadlines, such as the deadline to have 50% less animal experimentation in Europe, which was not made. Conclusions from this deadline were that it is important to always keep the evolving science in mind and be able to adapt in the course of events.
The fact that science has progressed, is not thanks to the goals/deadlines itself, but due to work in the academic and industrial scenes. World wide harmonization is also a very important process in reducing animal tests.
There are diverse timelines and legislations that have to be implemented, such as REACH, the 7th Amendment of the Cosmetics Directive, the Animal Welfare Directive 86/609…
For REACH the system of waiving was introduced, which means that animal safety testing experiments don't have to be done, if there already are data available, there are in vitro methods, substances can be grouped and read across… However, there should be world wide waiving and international acceptance. By now, the procedures are still unclear.
Examples for obstacles in the development and acceptance of tests was also given. For example, the EST was validated, but the experience of users was neglected.
Concerning working on an international scale, the ICH has done very good work.
The basis for the legislation must consist of good solid science, undisputable data, at best supplied by potential users.
For the international exchange of data, it is important to have a neutral institution, that could work as a "data trader" and assist in codifying and disseminating existing in vivo data. This institution should be non-for-profit and legally independent. A possible name for this could be InterXalt (International Institute for Exchange of Data for Alternative Method Development). The development of an institute such as this, should be followed up in an FP7 project.

The ECVAM Business Plan: realistic approach to deal with the upcoming dead-line challenge, by Dr. Elke Anklam, DG JRC, IT

As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, it functions however as an independent organ.
There are 7 centers in 5 Member states. The IHCP, located in Ispra, Italy, houses ECVAM (European Centre for the Validation of Alternative Methods).
A new business plan (the ECVAM Action Plan 2008-2013) is being made, due to the deadlines of the Cosmetics Directive, political pressure and budget complaints. There also was a too optimistic view on the situation of the available alternative methods. This new business plan deals with topics such as overviews on toxicological endpoints, prospects of validation, ECVAM's activities and methods validated so far, status of methods for deadlines as stated in Cosmetics Directive and bottlenecks to validation.
Reasons for the bottlenecks to validation are that there are not enough sound methods and testing strategies available, there is also a lack of information on reference methods (from animal tests) or reference data on human effects and too few test system materials (i.e. human tissue or cells) are available. There also are difficulties (of legal and ethical nature) in using human stem cells. A further problem is also that the process of validation takes long and the costs are high. There are also bottlenecks to the

acceptance of alternative methods. The regulatory acceptance process is slow as well and maybe there are too many organs with different approaches in this field. Another problem is that there is a lack of information on reference methods or reference data on human effects.
For Directive 2003/15/EC in Relation to Alternative Methods measures will be taken in the form of an immediate marketing ban (from 2009) on new cosmetics tested on animals if alternative test methods are available, after 6 years there will be a complete marketing ban on cosmetics tested on animals and on animal testing for cosmetic ingredients. For the 2013 deadline an immediate ban on animal testing for cosmetic finished products.and a marketing ban on cosmetics tested on animals for repeated-dose toxicity, reproductive toxicity and toxicokinetics 10 years after entry into force of the Directive are planned. This last deadline, however, could be postponed if necessary. Since this deals with more complex tests, there is a substantial chance that the deadline will have to be delayed.
To improve this situation more attention is given to international collaboration. There will be more collaboration between the international 'VAMs' in the future. Discussions are also being held between the EC and the OECD, as well as between the EC and the USA, Canada and Japan (ICATAM) in order to speed up the process and exchange knowledge. A new website (TSAR) showing the regulatory status of methods is also being established.
ECVAM takes measures through its earlier mentioned business plan and the annual work programme for 2009. The JRC-IHCP will also be restructured and ECVAM will be treated as a policy support action across the whole JRC-IHCP in stead of only in one unit. An Integrated Testing Approach will also be set up.

The JRC-IHCP, however, has expertise in a number of fields such as the validation of alternative methods (in ECVAM) and method validation in general, *in vitro* methods and *in silico* methods, automated systems for screening, nanosensors, omics techniques (however knowledge in this field still has to be increased) and databases.

The procedures of method validation will also be re-evaluated and restructured. Especially the following points are of concern: test method submission, evaluation and validation, confidentiality issues and the peer review process. A stakeholder experts group for the prioritisation of methods will also be established. The duties and structure of ECVAMs Scientific Advisory Board (ESAC) will be revised.
More focus will also be put to the post-validation process of available robust methods.
The IHCP laboratories will be restructured, attention will be given to GLP certification and ISO accredition of in-house validation. Laboratories will also be prepared for training purposes and a repository of chemical substances will be established.

Session 2: Are we prepared for deadlines and renewal?

The challenge of the Cosmetics Directive deadline of 2009: what do we have, by Dr. Odille de Silva, Colipa, FR

The EU 7th Amendment prohibits the performance of animal tests and the sale of cosmetics products in the EU, when the final formulation or ingredients have been tested on animals, to meet the requirements of the Cosmetics Directive.

In 2004 a testing ban on finished cosmetic products was introduced. In 2009 a testing ban on cosmetics ingredients and a marketing ban for products or ingredients tested on animals for local and acute effects evaluation will be put into process. The progress and difficulties of these bans will be reviewed in 2011. In 2013, a marketing ban for products or ingredients tested on animals for systemic and long term effects evaluation will be put into force.
Tests for phototoxicity, skin corrosion and irritation and percutaneous absorption for finished products are already replaced with alternative methods.
For 2009 there are three areas for which replacement methods are not yet validated or accepted: eye irritation, genotoxicity and acute toxicity. Work in these fields is ongoing and existing tests are being improved. The 2013 deadline covers areas such as: subacute, subchronic toxicity, skin sensitization, photosensitization, toxicokinetics… To have good alternative methods in these fields will pose huge challenges for the industry.

What is available for the 2009 eye irritation deadline?
Many *in vitro* methods are available, however, eye irritation is a complex field and more methods are needed. Validation will be done for 2 human corneal models in 2009 and 8 existing methods will be evaluated during 2009/2010. Colipa also sponsors research to include mechanistic endpoints which could improve the predictivity of existing methods.
For skin irritation validated replacement methods are available and are under discussion at SCCP and OECD levels.

Acute toxicity is a very complex test to replace and when it is needed, information is obtained from animal studies. More research is needed. Methods aiming at reduction are under pre-validation, results will become available from 2010 onwards.
For genotoxicity research, there are two important points: research to identify and resolve problems with current assays and developing new in vitro methods. In view of the first point Colipa established the “false positives” project, which is co-funded by ECVAM and NC3Rs, the project is extended to incorporate work with liver cells. For the second point, Colipa has the “3D skin project”, which goal is to evaluate genotoxicity in 3D human skin models. The first phase of the project (protocol optimization and transferability) has been finished successfully. The second phase (reproducibility between labs) started in May 08.
So, non animal tests exist and work is being done to improve their predictive capacity. COLIPA efforts are shared with scientists and regulators (SCCP).

For safety assessment multiple factors are taken into consideration, such as animal and *in vitro* data, data banks, analytical chemistry… Due to this variety of possibilities to gain information, business will be able to continue innovation. However, in some rare cases, there might be a lack of data to defend ingredients (new or existing), when no good alternative methods are available.

For the 2013 skin allergy deadline, Colipa aims to provide a first generation integrated testing strategy capable of providing skin allergy information, based on validated in vitro tests developed by COLIPA companies and research aimed at delivering more predictive second generation methods.
Colipa is also collaborating with the EC on the funding of systemic toxicity research.

To conclude it was said that it is very doubtful whether the 2013 deadlines will be made, but lots of efforts are done to do as much as possible. It is also important to remember

that not only research is important, but the applicability of test methods is very important as well.

Improved REACH implementation using new science based tools?, by Dr. Gernot Klotz, Cefic, BE

The implementation of REACH poses some challenges, such as the fact that REACH is not only about substances and testing, but also about participation of the industry and information. Therefore communication is very important.

There is an increase in the acceptance of high quality industry research as a basis for policy making. The complexity of testing has increased, which makes the results more diffuse. This leads to an increased precaution against new substances. There also is a bias in science nowadays towards identifying additional adverse effects of certain chemicals. This leads to a new challenge, being risk based decision making, which also has an impact on the innovation of the industry.
To this end, LRI focuses on three main points: Intelligent Testing and Assessment, Health Impact of Complex Environments and Societal Acceptance of New Technologies and Products.
In the light of intelligent testing and assessment Cefic LRI takes part in a number of projects, such as developing a new design for reproduction toxicty studies in mammals and delivering contributions within OECD to the Endocrine disrupter Testing and Assessment framework. Together with OECD, Cefic LRI also works on innovative technologies and computational tools to reduce the number of animals. Results from the ECB are used to build tools such as ToxTree and ToxMatch. Through Cefic LRI, REACH Implementation Projects (RIPs) have integrated the 3Rs principle.

Cefic LRI strategic themes for 2008 are focused on the acceptance of new technologies and products. Themes such as nano safety, male reproductive health and toxicogenomics, development of harmonised global standards for the assessment of NP in the environment and data from testing in toxicological framework and societal acceptance of risk assessments are treated.

The current REACH requirement for the assessment of reproductive toxicity in safety evaluation of e.g. tier 1 chemicals (>1000t/a) is the two-generation reproduction study, OECD Test Guideline (TG) 416. In cooperation with EPAA, OECD and government bodies, Cefic is working on an extended 1 generational study. This might safe approximately 1500 animals per compound.

Draft 5 must be discussed with WNT OECD, since there are some drawbacks, such as the fact that the inclusion of endpoints would result in a complex design for routine evaluation of industrial chemicals. This would have as a consequence that only a small number of laboratories would be capable of conducting such studies. It is also possible that more animals will be used and the costs for Annex X reproductive testing will double.

In risk assessment environmentally relevant doses must be understood by using new technologies. It is also important that the industry is aware of innovations in toxicology testing. Therefore academics, regulators, government and industry must be engaged to properly apply the innovative toxicology.

REACH challenge: can ECHA deal with it?, by Dr. Erwin Annys, Cefic, BE

REACH started with the pre-registration on June 1, 2008. The registration started with the chemicals produced in the highest quantities first. It was expected, by the EC and EChA, that about 30.000 substances would be registered, with an average of 5 manufacturers or importers per substance. This would result in about 180.000 to 200.000 pre-registrations.
Reality, however, showed quite different. On November 28, 2008 (near the end of the pre-registration period), there already were 2.000.000 pre-registrations, for 50.000 substances, done by 47.000 legal entities. This results in the fact that 3400 bulk files are in queue. On average there were 100.000 pre-registrations per day and 3600 legal entities signed up per day.

Obviously this raises concern with regard to animal testing. This, however, should not be a problem, since REACH tries to avoid animal testing wherever possible. If no test results are available, test proposals should be presented. Then these will be treated on a case by case situation.

Changes in the process of exposure based waiving are proposed. Initially it was stated that a DNEL derived from a screening test for reproductive/developmental toxicity would not be considered appropriate to omit a prenatal developmental toxicity study or a two-generation reproductive toxicity study. Also a DNEL derived form a 28 days repeated dose toxicity study would not be considered appropriate to omit 90 days repeated dose toxicity study.
However, a negative result can provide the basis for a DNEL in relation to reproductive toxicity derived from the highest dose level used in the study and using an assessment factor that takes into account of the limitations of this study.
For repeated dose toxicity studies, Guidance Document R8 defines default assessment factors for differences in exposure duration, including extrapolation from subacute to sub-chronic and subacute to chronic. Therefore it is generally accepted to perform such extrapolations.

Replacing Animal Tests – Do Other Continents do it Better?, by Emily McIvor, Humane Society International, BE

The Humane Society is one of the largest animal protection organizations in the world and is a member of Eurogroup for animals. HSI is active in the fields of REACH, Test Methods Regulation, Classification and Labelling, Plant Protection Products and the Revision of Directive 86/609.
Concerning animal and safety testing, some challenges are coming up. REACH may use up to 45 million of animals. In the pharmaceuticals sector, bringing a new medicine to the market can use up to 7.000 animals, while 92% of the drugs that pass the preclinical testing phase, fail the clinical tests. For nanomaterials validated risk assessment methods are not available yet and scientists doubt whether conventional animal tests are useful. *In vitro* methods must be developed.

Developping alternative methods is a process that costs a lot of time and effort. The whole process of research and development, validation and regulatory acceptance can easily take over 10 years.
An important question that has to be asked is whether replacing animal tests one endpoint at a time is a viable long-term strategy.

There is a growing agreement that new technologies are needed to be able to assess chemicals without using animals. In the report of the EPAA Workshop 'New Perspectives in Safety Testing' (April 28/29, 2009) it was stated that 'animal models do not provide the possibility of understanding fully how all the biological processes function individually and in concert.' The US National Research Council also recognizes the low predictive value of animal tests, recommends the use of *in vitro* assays, stresses the importance of using human-source material and puts effort in working towards an animal testing free future.

There is a need for a global strategic partnership and the coordination of international efforts. This is important for the harmonization of methods and to avoid unnecessary replication of tests. The progress should reflect the input of all participating parties, such as regulators, industry, academia and animal welfare organizations.

Some efforts of the Humane Society include membership in the EPAA Mirror Group and the NRC Committee on Toxicity Testing (USA), being peer reviewer of the US EPA toxicity testing strategic plan. The Humane Society has also co-founded the AltTox.org website, which is an online platform for information exchange among stakeholders interested in non-animal methods of toxicity testing.
HSI calls to the EU, USA and other world governments and corporations to commit to a "big biology" initiative, the Human Toxicology Project, similar to the Human Genome Project, and fund this project for about $200M per year for the next decade. The goal is a multi-disciplinary, multi-national targeted research program, with an international, multi-stakeholder consortium.

In a number of fields there still is a need for better coordination, for example when it comes to animal housing and care. There also definitely is a need for better coordination and harmonization of the international acceptance of validated test methods. EU companies could also work together to promote animal welfare through application of EU standards in other countries, which are not applying these standards yet.

The conclusion is that the vision and technology are often already available, but lots of work still is to be done.

Revision of Directive 86/609 – as it regards 3R-alternatives, by Susanna Louhimies, DG ENV, BE

The EU legislation on the protection of animals used for experimental and scientific purposes has been in force since 1986. Since then national laws have changed, leading to a non-harmonised environment and new techniques have been developed. The attention given to ethics and animal welfare have also increased. For these reasons the EC concluded that it was time to updated the legislation.

There also are a number of other reasons why the Directive had to be revised, including the Protocol for Animal Welfare (1997), the Community Animal Welfare Action Plan (2006) and the new EU Chemicals Policy, REACH.
The ultimate goal of this new legislation is to totally replace the use of animals, however, it acknowledges that animals, including non-human primates, are still needed today. The principle of the 3Rs must be used for all use and care of animals for scientific purposes.

An Impact Assessment will be published simultaneously with the Commission proposal once adopted. This will include the qualification, quantification and monetization of impacts. However, quantification and monetization are not always possible, as in the case of the quality of life and ethical considerations.
Some of the options to increase the welfare for the animals were presented. The authorization and ethical evaluation of projects would have an impact on leveling the economical differences between Member States. Minimum housing and care standards would remove the current uneven competitive environment for users and breeding and supplying establishments, whilst improving animal welfare. Also standardised inspections would help to ensure the animal welfare and improve the confidence of the public.
A public consultation was held in two parts: a citizens and an experts questionnaire. The citizens questionnaire received over 42.000 responses. The experts questionnaire was answered by 283 experts, resulting in over 12.000 comments worldwide. The comments were used either to incorporate them in the Impact Assessment or to revise or fine tune certain of the chosen options.

The key objectives of the revision are a significant increase in animal welfare, to level the playing field for industry and academia, minimize any increase in bureaucracy and actively promote the 3Rs. There also should be transparency in the process, this would help with controls.
It is important to make sure that Refinement is not limited to scientific procedures only, but that it is also applied in relation to accommodation, care and breeding. An emphasis is made on a hierarchy between Replacement, Reduction and Refinement.
To ensure the implementation of projects, there must be an ethical evaluation looking at issues such as the use of available alternatives, the use of humane endpoints and the experimental design. Each establishment should also have an ethical review body of its own, to ensure that new developments in alternative methods are followed up. There also are provisions to make the legislation on the requirement to use alternative methods clearer. Re-using of animals is also looked at as a possible way of reduction. Minimum housing standards will be put into force. Finally, the competence to maintain animals must be demonstrated in companies, therefore education is very important.
Both the EC and the Member States must take part in the development and validation of alternative methods. To this end, a network of national reference laboratories must be formed. Each Member States must designate a national reference laboratory which will participate in validation studies. This way the work of validation can be shared, which is necessary to cope with the increasing amount of new alternative methods. The co-ordination of the pre-validation and validation will be done by ECVAM.

Session 3: Science Fair

Molecular imaging in vitro and in vivo, by Prof. Tony Lahoutte, Vrije Universiteit Brussel, BE

Molecular imaging is the visualization, characterization and measurement of biological processes at the molecular and cellular levels in living systems.
With these techniques it is possible to do imaging of the animals in the same way as is done with human patients.
Different techniques can be used, such as CT, PET, MRI, SPECT. The required information (anatomy, physiology, cell or molecular imaging) determines which technique has to be used. In the selection of the most suitable *in vivo* imaging biomarker for a given molecular target there are two important properties that need to adressed: affinity of the radiolabeled molecule for its target and background biodistribution of the radiolabeled molecule. Both issues are equaly important for the imaging process.
Bioluminescence, using luciferase, is a dynamic imaging technique. This is a 2D technique that can be used in cell tracking and oncology. The interesting fact is that the emitted light is proportional to the size of the tumor.
Fluorescence Imaging is a quantitative method with which 3D images can be acquired, when they are combined with MicroSPECT/CT and MicroPET/CT scans.
Another method is Nanobody Imaging, which uses molecular imaging probes for disease related cell surface biomarkers. The first step is to produce marked antibodies, which are injected in the animal. After 1h, the animal is anesthetized to make the image. This way you can measure quantitatively how much of the drug reaches the target. Nanobody Imaging can be used for organ biodistribution and targeting, pharmaco-kinetics, intra-individual comparison and serial intra-individual monitoring.
To conclude, imaging methods are non-invasive and allow repetitive measurements, disease related parameters can be measured at early stages, intra-individual comparison reduces the variability of the measurements. Thanks to this last point, a lower number of animals is needed for obtaining statistical relevant results.

MicroRNAs: novel players in skin research, by Dr. Andor Pivarcsi, Karolinska Institute, SE

MicroRNAs were first discovered in 1993, but now more than 8000 miRNAs are known. MiRNAs are short, non-protein-coding RNAs, of approximately 22 nucleotides, which regulate the expression of protein-coding genes. They inhibit gene expression at the post-transcriptional level and thus their function and importance can be compared to those of the transcription factors. They regulate basic biological processes such as apoptosis, morphogenesis, proliferation, metabolism…
MiRNA is encoded in the genome, transcribed from the DNA as pri-microRNA, then it is processed into pre-microRNA in the cell nucleus. In the cytoplasm it is processed into mature microRNA, this forms complexes with the 3 ´ UTR (untranslated region) of target mRNA and suppresses their translation or induces their degradation.
Abnormal miRNA expression can cause diseases, such as cancer and developmental and metabolic diseases.
Since the skin is a very important organ of the human body and an important number of diseases has effect on this, the relation with miRNAs is studied, for example whether miRNAs have a role in psoriasis.
Genome-wide analysis of miRNA expression using an array with LNA probes showed that a set of microRNAs is expressed in human skin and that healthy skin, atopic eczema lesion and psoriatic lesions display distinct microRNA expression profiles.

This research showed that miR-203 is a keratinocyte-specific miRNA and that it is overexpressed in psoriasis. The up-regulation of miR-203 in psoriasis is concurrent with the down-regulation of its target, SOCS-3, which may result in an increased inflammatory response.
Due to their role in these diseases, miRNAs are potential drug targets. MiRNA expression profiles can also be used to classify human cancers.

Human lymph node in vitro for immunotox, immunogenicity and pharmaceuticals antibody-production, by Dr. Christoph Giesse, ProBioGen AG, DE

Nowadays it becomes more and more clear that animal *in vivo* tests are not always adequate for safety testing. Therefore there is a need for more human tissue based models. To this end, ProBioGen has developed the Human Artificial Lymph Node model (Human ALN) for testing immune modulation potency of biologics and predictive testing of immunotoxicity and immunogenicity of compounds and drug candidates. Artificial organ models mimick human tissue or organ specificity and functionality, which are problems in either animal models or normal *in vitro* models.
Lymph nodes are biological cross flow filters between the blood and lymphatic systems. Human leucocytes are derived from healthy adult donors. With these, organoids are formed *in vitro* in a 3D - matrix assisted co-culture system. Continuous media perfusion makes this system mimic the *in vivo* draining of lymph node tissue.
Different parameters, for example process and immune related, can be used for monitoring. The formation of organoids can be analysed as an endpoint by histological methods. ProBioGen is also working on imaging for gene expression profiling and the monitoring of migration and cellular interaction.
This model can be used for predictive testing of immunofunction and immunotoxicity. Currently testing programmes for pharmaceutical customers are also being run, including programmes for interferons, superagonists, vaccines and adjuvant therapy. For the cosmetical industry cell based assays are performed.
The goal is to extend the collaborations and testing services using the human ALN model, so that the model can be used in a broader field. ProBioGen is also willing to apply the model in feasibility studies on pharmaceutical relevant drug candidates and compounds.

Session 4: The Interaction with Regulators

Case report of START-UP expert meeting, May 19, 2008 in the Ministry de Sanidad, Madrid, by Prof. José Castell, University of Valencia, ES

On the first expert meeting of the FP7 project START-UP, experts from different fields were present, from academia, companies and regulatory institutes.
Some of the points discussed there, were presented here.

Most animals are still used in toxicological research, especially in repeated-dose toxicity, teratogenicity and embryotoxicologicy studies. The problem with toxicology research, as opposed to pharmacology, is that the target is unknown. Therefore, the outcome of toxicological research is often not a good predictor for clinical phase testing.

Translating *in vitro* results to the *in vivo* situation is often not possible. An outcome for this is probably to describe processes and mechanisms first *in vivo* and identify appropriate biomarkers, then move to *in vitro* systems.
While a lot of money has been and is being invested in the development of alternative methods, the outcome is not as extensive as was to be expected. One of the problems is that the EU projects come together with a lot of bureaucracy, there is also little flexibility in the way projects are done. The result of this is that often pharmaceutical companies drop out during the projects. There is also a gap between the way academic institutions and the industry are doing their research. The goals of the projects are often not realistic enough for the industry.
It also was mentioned that it would be very helpful if negative results are published, as a lot can be learned from this as well.
There is a difference between the importance given to validation of tests between the industry and regulatory institutions. For industry, it is more important that a test works than whether it is validated or not.
In vitro tests are often oversensitive, a problem which might be reduced by the use of 3D models.
Since *in vitro* tests lack the heterogeneity of human populations, there still is a need to do animal experiments in pre-clinical research. It is then also important to select the species which has the highest predictive value for humans. Also certain studies, like volume distribution, are not possible *in vivo*.
In some exceptional cases clinical trials could be performed without prior animal testing. For example, if dealing with life threatening diseases and in cases when there really are no good animal models available. In some cases, for example with anticancer drugs, vaccines, mABs…, reduced animal testing can be accepted.

Lessons learned: use of questionnaires and workshops in checking regulator's response within carcinoGENOMICS, by Dr. Coenraad Hendriksen, NVI, NL

In test method development, different steps are to be ran through, starting with R&D, then pre-validation, validation and peer review. These first steps are performed by the industry and the academic world. After this, regulatory bodies are involved in getting regulatory acceptance for tests and finally implementation. Often test methods do not get to implementation, due a lack of regulatory acceptance. Reasons for this include the unavailability of conclusive data, poor design of validation studies and the fact that the application domain is often not well defined. Due to this, it became clear that there should be more exchange of information between regulators, the research community and the industry.
Objectives for regulatory input include a focus on the most promising methods (priorities depending on the needs of the 3Rs and tests), to fine tune validation to specific regulatory needs and to speed up regulatory acceptance and implementation. This last point can be reached by engaging regulatory bodies, the availability of a clear and efficient information structure and reviewing whether regulatory needs are met.

Necessity of regulators and authorities' input into FP-projects such as carcinoGENOMICS, by Jos Kleinjans, University of Maastricht, NL

The demands on chemical risk assessment are increasing.

One of the risks in chemical risk assessment are false negatives. Among new drug candidates, there is a high failure rate due to unmanageable toxicity. This accounts for about 30% of the attrition.
On the other hand, there is also the risk of false positives. According to the EU REACH policy program on industrial chemicals, existing and new substances should be subject to the same procedure under a single system, in the future. Due to this, large amounts of additional tests are required before 2018. This will cost about 2.5 to 6.5 billion Euro and use about 8 to 20 million animals.
Finally the EU's 7th Amendment has a ban on animal experimentation for developing new cosmetics.
For these reasons there is a need for new assays, which are cheaper, less time-consuming and non-animal based. Omics based *in vitro* techniques offer potential in this field.
Toxicogenomics has an interesting potential and in the USA regulatory authorities are working on the implementation of these techniques, for example by the ToxCast project of EPA. The goal of this project is to develop a reference base from a wide range of *in vitro* endpoints generated from 320 pesticides, for the purpose of evaluating safety of new industrial chemicals.
In Europe, initiatives taken by the regulatory authorities to possibly implement new technologies are slow. However, to achieve the reduction of animals used for testing, it is important that all parties (the EC, member states, industry…) continue to contribute to new alternative testing methods, such as toxicogenomics. To this end, the EC should make certain that initiatives are taken and that all stakeholders and all interested parties participate in these initiatives.
Microarray technology for gene expression analysis is being tested. Different articles show that the reproducibility is good, even between laboratories, especially when a common set of procedures is used.
In a REACH testing strategy, 'omics can be used in conjunction with other alternative methods in a weight of evidence evaluation.
To successfully incorporate new methods into risk assessment strategies, risk assessors must become accustomed by the new 'omics techniques. A good collaboration between regulators and toxicogenomicists is also necessary for the further incorporation of this techniques.

What can be done from the regulatory side?, by Sonja Beken, Federal Agency of Medicine and Health Products, BE

Developing new medical products is a process which requires a lot of time and labor and bears a high risk of early termination. Therefore, strict regulations are needed to ensure the quality and safety of the new products.
For non-clinical studies recommendations are made by various institutions, for example in Europe by EMEA and Eudralex, in the USA by the FDA and in Japan by the Japanese Ministry of Health and Welfare. But regulatory acceptance of *in vitro* methods passes mostly via the ICH (International Conference on Harmonisation). Tasks of the ICH include monitoring and updating harmonised technical requirements leading to a greater mutual acceptance of research and development data, facilitating the adoption of new or improved technical research and development approaches…
The CPMP Position Paper on Replacement of Animal Studies by *In Vitro* Models (CPMP/SWP/728/95 - adopted 1997) gives recommendations on a number of topics, such as the feasibility of replacing *in vivo* animal studies, the procedure for validating *in*

vitro tests, the procedure for incorporating *in vitro* tests into the regulatory requirements and areas for which the acceptance of *in vitro* tests can be considered.
The criteria of acceptance of *in vitro* methods depends on the goal of the test. For example early toxicity, compound screening tests, can go by in-house validation by companies, there is no regulatory involvement. Whereas for exploratory, mechanistic studies for regulatory decision-making, they have to be based upon demonstrated "scientific validity".

In May 2007 ICH had a first meeting with ICCVAM, ECVAM and JaCVAM to discuss about the possibility of future collaboration and input of the CVAMs on future guidelines and criteria. Current topics include the revision and updating of the S2, S6 and M3 guidelines and the new S9 guideline. One of the focuses here was (better) inclusion of the 3R principles.

From the regulatory side there are different ways to give input and feedback, such as involvement in EPAA, EU initiatives in collaboration with pharmaceutical industry, input in ICH related activities, interaction with EFPIA on 3R-related issues…
To this end there is a need for a structural approach to align early method development with (future) regulatory requirements and interact with ECVAM-driven validation exercises, among other reasons. To this end there is a need for a specific 3R regulatory "task force" to serve as a single contact point/watch dog to ensure continuous input/feedback with stakeholders in the field of the 3Rs, increase communication and interaction strategies with 3R stakeholders and other involved sectors and continuous and up-to-date 3R input in the regulatory process in balance with maximal protection of human health.

Round Table: Lessons learned?

The first question to the different discussants was whether they thought whether there had been fundamental changes in animal testing and research.
A first comment (by Sonja Beken) was that there mainly has been a change of focus. By now, more and more focus is being put on involving regulators, this in a worldwide context. This is important, because only when regulators work closely together with other stakeholders and there is clear communications of the wishes and the needs of the different groups, real progress can be made.
Another opinion (from Jos Kleinjans) was that there mainly has been process-wise change, but not so much content-wise. One problem related to this is that the time taken for each process is too long, so that it's getting contra-productive. An analysis should be made of why the process is so time consuming. It probably is also better to concentrate now on getting things done in the EU and only afterwards handle the problems worldwide.
Another lesson learned, according to another discussant (Gernot Klotz), is that it is important to be more careful when testing new products. In the OECD, it is often not known where a certain test is in the test battery, when it comes to risk assessment or screening tests.

According to Roman Kolar there is a need for more interaction, also, these cooperations should be formalized for clarity and good organization.
New demands for safety are coming up. If you want to have 100% safety, products should be tested on humans. This is not done, because the price is too high, which is

why animal experiments are performed. Nowadays, however, there is a large part of society that thinks that performing tests on animals is also a too high price.
Another reason, why it is important to start with changing in Europe and not worldwide, is because other parts of the world have other ethical views, which is why it should be easier to make a change in Europe than on a global scale.
Finally, it often seems that the results of the European projects end up in a drawer, instead of being followed up.

Susanna Louhimies explained that in the reviewed Directive 86/609 there are also clues on how to engage the different stakeholders. To this end, sometimes one has to take steps back.

Odile De Silva told that for the last validated alternative method, being a test for skin irritation, research was started in 1999. It was finally validated in 2007. This method was developed long before the deadlines were being talked about and thus done independent of any deadlines. This is just an example of how long it can take before an alternative method is tested and validated. It also makes clear that science is not really helped by deadlines, because it can't be commanded. Safety of products is more important, than deadlines. Legislation also has to take care not to become sectorial, since science isn't either. A transversal approach is important. If the different stakeholders and sectors work together, good progress can be made. To this end the timelines and activities of the different partners must also be harmonized to each other.
The process of validation should also be reworked, so that it becomes more cost-effective and less time-consuming.

A reaction to this, by Roman Kolar, was that progress can also go beyond legislation and do more than the regulators ask.

Horst Spielmann voiced his opinion, that despite the economical difficulties, the research to alternative methods should continue, because otherwise a lot of work would be lost.
A setback is that the regulators always seem to ask for more and more data, which makes it difficult for companies to keep up with this, because then safety testing costs increasingly more time and resources.

Testing on non-human primates is important, but as long as no important scientific breakthroughs are available, it does not make much sense to only do a lot of tests on primates, according to Vera Rogiers.

The EU is doing it's best for the development and use of alternative methods, but Bernward Garthoff has the feeling that still more could be done. Therefore, it is also important, not to only support the initiatives taken, but also be critical where needed.
It is time to start thinking what we have achieved in the EU unto now, said Susanna Louhimies.

Horst Spielmann mentioned differences between the US and the EU, as an example with stand alone tests, which are accepted in the EU, but not in the US.

Finally, Susanna Louhimies explained that good and clear communication to the public is very important and should be taken care of. Sometimes great expectations are wrongfully created, which is dangerous, because people will be disappointed. This works contraproductive.

Sunday, 30/11/08

ecopa Business Matters: National Consensus Platform, chaired by Prof. Peter Maier

Peter Maier gave an overview of the planning for Sunday morning. He listed the current board composition, the names of the NCP representatives and stressed the importance of ecopa in networking. He asked the present representatives again to share information with the other
countries and to hand over the annual reports of the platforms in order to be able to coordinate activities.

Prof. Rogiers asked everyone to think about the structure and future of *ecopa* since next year Board elections will take place. She referred to the statutes and explained that all platforms will have to designate new young people for the follow-up since all Board members shall be appointed for a period of office of two-years; they may be re-elected twice for the same period of office and this for a maximum of 6 years. According to statutes only 3 current Board members could stay (Roman Kolar, Odile de Silva and Isabella de Angelis), the other Board members will have to be replaced.

Furthermore, Prof. Rogiers mentioned that a part of directive 86/609 was about reference laboratories in different countries. The way how it will be organized was not yet defined, so *ecopa* and national reference laboratories should work together. ECVAM coordinates the validation of alternative approaches but there is an increased need for new methods and validation of alternative methods therefore, reference laboratories for validation should be designated by each Member State.
Vera Rogiers gave an overview of the initiatives taken in 2008 and the planned further activities of *ecopa*; She listed the EU projects in the 6th FP and 7th FP where the *ecopa* Board is involved in for the dissemination part:

She informed the public about the meetings that were and are going to be held in the context of the START-UP project; the Kick off meeting was held in Leverkusen, Expert's Meeting 1 ("3Rs needed in the Pharmaceutical Research and Development", Madrid, May 19 – 20, 2008), Expert's Meeting 2 ("3Rs and Animal Disease Models in Pharmaceutical Research and Development", Basel, September 5, 2008) and 3rd eSI meeting, October 17 – 19, 2008, in Alicante, sponsored by epaa.
The richness of ideas, produced out of the Madrid meeting, still needs to be worked out in a scientific way and put out in recommendations for the Commission. Preparations are being made for the Workshop on Refinement: February 26 – 27, 09, Rome, IT (organised by IPAM, Polcopa, Fincopa). The next workshops that are scheduled are Workshop Reduction: July 3 – 4, 2009, NL (organised by ZonMW, ZET) and Germany took the organisation up of Workshop Replacement: October 2 – 3, 09, Budapest, HU (organised by SET, Hucopa).
Preparations for all workshops have been initiated!

ecopa is present in the ReProTect board and there were a lot of discussions about the applicability of the EST. Therefore, *ecopa* initiated and organised the discussion about

this topic in an EST Workshop on May 5, 08, in Frankfurt, Germany. There was also a General Assembly Meeting, which took place on June 11, 08, Uppsala, Sweden and a Supervisory Board Meeting on July 8, 08, Dresden, Germany. As a result of these meetings, the ReProTect ESt applicability is much better defined.

*ecop*a applied as a dissemination partner in 2 projects: ALTERNET and ROAD ; These are both support actions, with the aim of trying to define research ideas and giving advice to the Commission (see the presentation of Jose Castell "Scientific Plans"). Prof. Rogiers applied for both projects in order to maximise the probability that *ecopa* could be involved in one project. The proposed budget over 4 years was € 120.000 and board member Isabella de Angelis was introduced as *ecopa* representative in these projects.

Finally, the website and the newsletter, get constant attention as important dissemination tools. Four newsletters which can be retrieved on the *ecopa* website (www.ecopa.eu) under Newsletter/ archive, appeared in 2008.
Next to this, Prof Rogiers mentioned the Article published in Altex 25, 3/08:"Research Expenditure for 3R Alternatives: A Review of National Public Funding Programmes in European Countries", Tonia Devolder, Kirsty Reid, Vera Rogiers, Simon Webb and David Wilkins.

Next Board Meetings that are scheduled are on February 25, 2009, in Rome, Italy and October 1, 2009, in Budapest, Hungary.

Emily Mc Ivor asked if scientific experts could join in the close meetings in the START UP project as an expert in the absence of a UK national platform member. Prof Rogiers replied that this was to be taken up with the responsible organisers.
At this point, the UK is not present anymore in *ecopa*; Jon Richmond, of the UK home office, asked to be involved even though not all the four parties are equally represented.
P Maier sent them upfront the questionnaire with the criteria on how to apply for membership as a platform. R Kolar referred to the criteria of the eligibility of a platform, written down in the statutes. The proposed UK composition by Mr. Richmond would only fit as an associated platform and not as a full platform. Mrs. De Silva said first to clarify the unclear specific configuration of the UK, before any further steps were taken.
Netherlands; Jan Van der Valk resigned from the board, ZonMW is taking over from NCA as a temporary Dutch NCP member until the reorganisation of the Dutch platform is done. Therefore, Prof Rogiers asked Prof Pfaller to coordinate the organisation of the START-UP workshop. According to Dr. Garthoff, an addendum to the START-UP report should be sent to the European Commission

Mr Philippe Hubert, the director of the French platform, presented its structure, whose head office is situated with Afssaps, in Paris. It consists of 12 partners; Government (4), Academia (2), Industry (3), Animal Welfare (2) and the Scientific Society (SPTC). The steering committee consists of a President, Marie-Hélène Tissier (Afssaps) and a Director, Philippe Hubert (Inéris), appointed March 2008. Two technical scientific committees (15 members each) were appointed and the 1st meeting will be held in January 2009 .The committee consists of a health products section with as secretary, Isabelle Fabre (Afssaps) and a chemicals section with as secretary, Emmanuel Lemazurier (Inéris). Mr Hubert gave an overview of the meetings and events held in 2008. Mr Hubert remarked that the area of medical research is less worked out in toxicology and considers this as an important issue.

Rema gave an overview of the activities of the Spanish Platform in 2008; they focused on 4 areas; The first area is education; the organisation of official courses for category B or C of experimental research with animals: alternative methods (theory and practice) Madrid Bilbao, Salamanca, Zaragoza, Leon.
Second focus is maintaining the website (www.remanet.net), thirdly the organisation of scientific activities: participation in European projects such as START-UP, and ForInvitox, organising the 3rd REMA Workshop: "Bottlenecks in the application of 3Rs in the R&D process in the pharmaceutical industry", participation in the eSI –meeting Pueblo Acantilado, by Eugenio Vilanova at the EchA.
And a fourth focus is on the opinion making regarding new legislation and other affairs: REMA gave advice on the profile of professional qualifications needed for those working in the field of animal experimentation. Proposal of the new directive relating to the protection of animals used for scientific purposes.

Prof Pfaller informed about the held LINZ congress, where after Prof. Rogiers asked whether a timeslot could be reserved for *ecopa* or national platforms in the next LINZ meeting, end of June 2010.

Scientific plans (6/7th EU Framework Program), by José Castell

Prof. José Castell talked about the FP6 projects *ecopa* is involved in, being ReProTect, AcuteTox, Carcinogenomics, Sens-it-iv, Liintop, for the dissemination part.
He also summarized what has been done so far and will be done for the START-Up project.
He talked about the IMI call, HERCULES (HEpatic and Renal Cellular models for the Use of assessing Long-term adverse Effects on Safety) by Jos Kleinjans.
Prof Castell said that *ecopa* has to screen future calls very carefully since the *in vitro* part is not always so clearly mentioned, e.g.: HEALTH-2009-2.3.2-3: Discovery and/or development of new and promising anti-HIV microbicides. FP7-HEALTH-2009-single-stage. "The successful projects should also include studies on new and improved tools for *in vitro* research and for testing toxicity and efficacy in preclinical as well as in human studies.
HEALTH-2009-2.4.4-1: Rare neurological diseases. FP7-HEALTH-2009-single-stage. Pathophysiology, diagnostic and therapeutic approaches of (a) non-infectious, non-malignant rare disease(s) affecting primarily the nervous system. Attention should be given to the development / use of adequate models *(in vitro*) in identifying / testing new targets for diagnostic, therapeutic and potentially preventive approaches.
Next to that, Prof Castell explained that *ecopa* applied to participate in the coordination and support actions such as Road and was asked to apply in Alternet project.

Mrs Stammati talked about the IPAM activities . A successful annual workshop was held in January.
Mrs. Tähti, of Fincopa, mentioned that last May 2008 an annual meeting was held, next meeting will be in Tampere with the 30 anniversary Finnish Society of Toxicology. Fincopa has an active website, gave an opinion to government about the new law of experimental animal care and is involved in planning the START-UP workshop with Annalaura Stammati.

Financials, by Dr. Bernward Garthoff

Dr. Bernward Garthoff gave the budget overview and listed the NCP's that paid their annual contribution fee.
The budget was overall approved by an external auditor (Belgian accountant), and the auditors Mrs. Annalaura Stammati (It) and Mrs Lisbeth E. Knudsen (Dk) approved the financial books.
Castell suggested to have 2 NCP's look to the accounts instead of a single person: Italy and Finland volunteered for next year, represented by Annalaura Stammati and Hannah Tähti.

Furthermore, there was a discussion how to prepare for the next elections. It was said to form a nomination committee, the structure will be set up during the next Board meeting in Rome**.**
There was also a discussion on the organization of the next annual meeting and it was suggested to have a general assembly on Friday, to have the science and political lectures on Saturday and the elections on Sunday.

Closing of the Meeting, by Vera Rogiers (chair of ecopa)

The 9th annual meeting was closed by the chair of *ecopa*, Prof. Dr. Vera Rogiers. She thanked everyone for having attended the meeting and for the constructive collaboration, The next annual meeting of *ecopa* will be held on 28-29 November 2009.

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