FRANCOPA - French platform for the development of alternative methods in animal experimentation

Waiving animal testing for regulatory purposes

Workshop in Paris (France),

September 1st, 2011

Thanks

Annick Pichard coordinated the redaction of this document.
She thanks the workshop speakers and the Waiving working group members* of FRANCOPA who contributed to this document.

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1. OPENING AND WELCOME OF PARTICIPANTS

This introduction is reported from the written speeches provided by the speakers.

- ***Ronan Stephan as the director for research and innovation at the French Ministry of Higher Education and Research congratulated the organizers for this event and thanked them for their invitation.***

Here it is the full text pronounced by Ronna Stefan.

"This is an honor and a pleasure to speak in front of an international audience of such quality. I congratulate the organizers for this event and thank them for their invitation.
As the director for research and innovation at the French Ministry of Higher Education and Research, I was asked to say a few words about our involvement on waiving animaltesting for regulatory purposes.
The French platform for the development of alternative methods in animal experiments,FRANCOPA, chaired by Alain NICOLAS, who I salute, was created in November 2007.
Since then FRANCOPA joined the ECOPA network and I am pleased to welcome its president Adela LOPEZ DE CERAIN.
As you know, the use of alternative methods in animal experiments when scientific objectives can be achieved without using laboratory animals is a legal requirement in France since 1976.
This obligation has also been introduced in the new European directive (2010/63/UE) that will come into effect on January 1st 2013.
However, this principle that seems simple is extremely difficult to implement. Although these methods can reduce the number of animals used and can optimize procedures for the obtention of maximum tangible results, to date, in pre-clinical research, in vivo experiments are still required in most cases.
This is certainly due to the need for physiological and integrated systems reproducing the organism as a whole.
So it is our responsibility to promote an optimal and rational development of alternative methods to animal testing. "Optimal", if through the use of the 3R concept (replace, reduce, refine), research is fostered and the safety of citizens is improved. "Rational", if the use of living animals is accepted as they can not always be replaced by alternative methods. Far from being antithetical these two solutions complete each other. Furthermore, alternative methods are not always developed to replace the use of laboratory animals but rather to analyse in detail a particular biological mechanism. As a result, researchers are not necessarily aware that they participate actively in the development of alternative methods.
It appears that sharing knowledge and data is necessary in order to get the maximal relevance of the chosen methods. This is the aim of this event organized by the FRANCOPA Platform to which the French Ministry of Higher Education and Research fully associates itself.
This conference's purpose is primarily scientific because of the possibility to increase the development and the scope of alternative methods. But it is also economic. Our recent reforms in France aim to unleash our scientists' potential for innovation and to create bridges between research and industry.
I attach a special importance to the fact that the excellence of our research can find practical applications. Technology transfer from basic research to applied research still needs to be more effective. In order to do this, it is necessary to identify the research carried on alternative methods and the needs for research and development.

This is one of the 18 recommendations in the report prepared by the FRANCOPA Platform. There is also the idea of creating an Information Center to publicize the actions and share the results across sectors. This report will soon be posted on the ministry's website.
In the future, the Directorate-General for Innovation and Research will pay particular attention that national and international regulations do promote the exemption rules whenever the waiving is relevant.
This past May 8, I renewed the agreement of the FRANCOPA Platform for a period of four years.
I have no doubt that its members will work efficiently both at the national and European level to seek the implementation of this conference's results.
I wish you a fruitful working session.

Thank you for your attention. "

- ***For Professor Adela Lopez de Cerain, President of ECOPA," the European Consensus Platform for Alternatives is a European non-for profit organization which was founded in 2001, according to the Belgian law.***

The aim of ECOPA is to advance in the development and implementation, or the putting into practice, of alternatives to animal experimentation.

There are many international organizations that share this aim but what makes ***ECOPA*** so original is the fact that there are four integrating parts to this organization, each one having a different point of view, with different interests. Academia or the scientific world, industry, regulatory bodies and animal welfare societies make up the four principal groups and their efforts to advance in the application of the 3Rs strategy by consensus are of vital importance. Consensus is the key word of ECOPA and of FRANCOPA. This four-pillar structure is reproduced in each country and all the NCPs members work together in order to advance in the 3Rs strategy at a national level. Networking among all the NCPs and with other organizations and stakeholders are improved thanks to the involvement of ***ECOPA***, permitting a more harmonized way of working together to reach a common goal"….

"There are 13 national consensus platforms (NCPs) currently represented in ECOPA"…

"The required criteria for becoming a member of **ECOPA** are as follows: 1) Accepting the principle of consensus regarding the delegation of the four parties and the 3R concept; 2) Establishing this consensus in an open and democratic way; 3) Functioning as a legally approved organization; 4) Having activities within the scope of the 3R Concept; and 5) Being open with regard to financial aspects. FRANCOPA, which joined us in 2007, is one example. It's evident that it is an active platform, capable of organizing a very interesting workshop which has brought all of us here together today."

- ***Professor Alain Nicolas as Director of Laboratories and Controls from AFSSAPS and President of FRANCOPA welcome the participants at the Ministry of Research for the first workshop of FRANCOPA, jointly organized by INERIS and AFSSAPS.***

"Today we will exchange ideas on the existing possibilities for waiving animal testing among the different sectors: human and veterinary medicines, cosmetics, chemicals, food additives, biocides, plant protection products.

We will try to answer the four following questions:

- What are the current uses of waiving?
- What efficiency in terms of reduction?
- Which are the barriers to overcome?

- What are the current scientific and technological needs?

Around **150 participants** will attend this workshop, representative of **12** European countries. I thank all of you for being here and I invite all of you to share your knowledge, your experience, from the different sectors that you represent, which can lead to avoiding unnecessary animal testing.

All your ideas will be collected and a report will be issued including your contributions."

2. SUMMARY OF THE CONFERENCES

2.1 HUMAN MEDICINE

2.1.1 Regulatory aspects

- **View of EMA - European Medicines Agency**

According to the sixth report on the statistics Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union, 530 000 laboratory animals are used each year in Europe to assess safety of products, substances or devices for human medicine and dentistry and for veterinary medicine.

For medicinal product, a comprehensive non clinical program is performed to support a marketing authorization of a new drug. It typically includes:

- Pharmacological studies to provide the proof of concept for therapeutic use, rational for dose selection in the human and safety issues at therapeutic dose level on vital functions;
- Pharmacokinetic in order to assess the relevance of animal species to be selected in toxicological program;
- Toxicological studies (single dose, repeated dose, developmental toxicity, genotoxicity, carcinogenicity).

For safety, hazard and target organ are firstly identified (hepatotoxicity, neurological effects, immunosuppression, etc.). Then, hazard is characterized, meaning dose response, critical duration and period of exposure is determined. Plasmatic level of drug at toxic level in animals is compared to plasmatic level of drug in the human at therapeutic exposure in order to assess the risk for the Human. There is thus a need for integrated systems that takes into account absorption and distribution of the drug, following unique or repeated exposure. In vitro models do not allow such integrated assessment.

In restricted field, such as Genotoxicity assessment, in vitro data are valuable. Because considered toxic target is not an organ nor a tissue but DNA, in vitro models are providing relevant data. However, in case of positive in vitro result, an additional in vivo study is requested in order to confirm the hazard and assess the risk. In vitro methods are also suitable for local effects, screening tests, stepwise testing strategies and mechanistic studies. In vitro/in silico data allow hazard identification with extrapolation to human, which allows a level of risk management in area of low acceptable risk. For areas of high risk or uncertainty, we require in vivo studies to better define hazard characterization and risk assessment before conducting human studies.

Waive of animal testing is currently encouraged by the following ICH guidelines M3 (R2), S2 (R1), S9, S6 (R1), S10 that support the establishment of a non clinical strategy by laboratories as regards to the intended therapeutic use (including type of population, planned duration of the treatment, dose level). For example, developmental toxicity studies are not mandatory when a medicine is developed for prostate cancer treatment, nor are carcinogenicity studies when duration of treatment is less that 6 months, continuously. Acute toxicity studies are no longer a regulatory requirement. These studies could be requested on a case by case basis.

As a perspective, transcriptomic approaches are encouraged to allow increased release of data from in vivo studies. Production of database from in vivo data could be further performed to support further development of in silico methods. A new EMA "concept paper on the need for revision of the position on the replacement of animal studies by in vitro models" should help to promote 3Rs together with progress in science and use of non-clinical strategies by laboratories.

Key points

Waiving is encouraged by:

- The availability of tests as: genotoxicity assessment, in vitro tests for local effects, screening tests, stepwise testing strategies and mechanistic studies.
- The ICH guidelines that support the establishment of a non clinical strategy by laboratories as regard to intended therapeutic use.
- The transcriptomic approaches to allow increased release of data from in vivo studies and to support further development of in silico methods
- A new EMA concept paper on the need for revision of the position on the replacement of animal studies by in vitro mode

- **View of the European Directorate for the Quality of medicines - EDQM**

The European Directorate for the Quality of Medicines (EDQM) is part of the Council of Europe. The European Pharmacopoeia (Ph.Eur) as a quality standard for the Quality Control of medicinal products refers to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe 1986).

The Ph.Eur still requires animal testing for the routine Quality Control of biological products such as vaccines. For example, the potency control for several bacterial vaccines has to be performed on rodents according to the vaccine monographs.

However, in the General Notices of the Ph.Eur it is clearly indicated that "with the agreement of the competent authority, alternative methods of analysis may be used for control purpose, provided that the method used enable an unequivocal decision … as to whether compliance with the standards of the monograph would be achieved if the official methods were used".

The Ph.Eur thus cannot be used as excuse for performing tests on animal as it gives the general possibility to replace an animal test.

Furthermore, different strategies have been developed leading to a significant drop of animal testing for vaccines and blood derived products.

- In the context of batch release, only the final bulk is controlled on animals, whereas in vivo testing is not required on the subsequent filling lots.
- In cases where consistency of production is demonstrated, the official control laboratory may submit a proposal to the EDQM aiming at reducing the number of final bulks controlled in vivo.
- the monographs have been changed for some specific tests, allowing the omission of the animal testing when it can be demonstrated that the production process consistently ensures compliance with the specification: thus, the abnormal toxicity test have been moved from the final product test section upstream into the production section.

- The Biological Standardization Programme run by EDQM is actively involved in the purpose of waiving animal testing. The validation of alternative in vitro tests has been achieved through collaborative studies including Official Medicines Control Laboratories (OMCLs) and Industry. An example is the replacement of animal potency test by complete in vitro ELISA for hormones or certain viral vaccines. The replacement of extraneous agents testing in animals by cell culture is completely achieved for avian vaccines.

This approach is supported by the concept of Consistency of production leading to perform different in vitro tests for the Quality Control of medicines. This approach is also addressed in close collaboration with different bodies such as EPAA and WHO.

To summarize, The Ph. Eur continuously encourages the replacement of in vivo testing by reviewing monographs and searching for in vitro alternatives in collaboration with regulatory bodies and manufacturers.

Key points

The Ph. Eur still requires animal testing for the routine Quality Control of biological products such as vaccines.

However, the concept of consistency of production is being developed leading to a significant drop of animal testing for control of vaccines and blood derived products.

These new approaches need a close collaboration within regulatory bodies and manufacturers, this collaboration is mainly encouraged by the EDQM through the Biological Standardization Program.

2.1.2 Industry experience

▪ View of human medicine manufacturer - Sanofi

The greatest challenge for use of alternatives to animal studies models is validation (scientific) and regulatory acceptance (ICCVAM and ECVAM continue to have difficulties in this domain). Within the regulatory environment, for medicines, the biggest opportunity is for reduction and refinement and wherever possible replacement.

The screening approaches consist essentially of in vitro tests and can reduce the use of in vivo models by the earlier elimination of toxic compounds.

During the development of the drug and within the regulatory studies, reduction and/of replacement is also developed, for example, in the assessment of phototoxicity (3T3 test, photo LLNA), irritation and corrosivity (replacement of the rabbit by in vitro reconstituted skin), eye irritation (replacement of the Draize test by Het-Cam test), cardiovascular toxicity (HERG test, Purkinje fibre), ADME mostly performed in vitro models (cultured hepatocytes, CaCo2…).

Some tests are fully replaced (mainly local tolerance tests), others are optimized and screening was completed by additional tests to better select molecules to be tested in animals (specific strategy and final screen).

When waiving is not possible, industry makes continuous efforts to promote and develop the 3Rs via reduction and refinement:

- Reduction of animal number (no more supernumerary animals in non-rodent studies, reduced number of animals for toxicokinetic or clinical pathology evaluation with new micro-methods, use of one species only for teratology studies when applicable; help of statisticians for review of protocols; addition of

new end-points in general toxicity studies to avoid some studies (inclusion of safety pharmacology studies; genotoxicity or fertility end-points in general toxicity studies); schedule of rodent studies before non-rodent studies to optimize dose selection and re-use of animals after washing periods etc…);

- Refinement: technical improvement (new method for blood sampling or other examination), enrichment, special handling procedures, training, socialisation etc…

Within this process, ethic committees play a key role especially in the review of protocols.

Key points

The greatest challenge for use of alternatives to animal studies is scientific validation and regulatory acceptance.

Waiving approach is encouraged by:

- The in vitro screening approach

- The existence of regulatory in vitro tests for the assessment of phototoxicity, corrosion and irritation

When waiving is not possible, industry makes continuous effort to promote and develop the 3Rs via reduction and refinement based on use statistical tools, inclusion of endpoints in general toxicity studies, re-use of animal after washing periods, etc.

▪ View of a human vaccine manufacturer - Sanofi-Pasteur

Vaccine regulatory activities represent nearly 80 % of the total animal use in the vaccine industry. Most of this use is based on extensive required quality control testing. In the frame of the 3Rs particularly on the waiving of animal experiments are these methods needed and mandatory or whether they can be replaced by in vitro methods without compromising scientifically based assessment of their quality?

With the impulsion of authorities, animal methods has been waived either by deletion or by replacement recently mainly for the safety testing of vaccines but with proof of manufacturing consistency and after historical review.

The gold standard remains animal tests for the potency evaluation of vaccine lots.

In vivo potency tests are used and relied upon for decades but they behave as the first bottleneck in release lead-time because of the following:

- High variability (C.V: ~15-50% versus 2-10%)
- Invalidity requiring investigations & re-tests
- Animal strain selection-Genetic backgrounds
- Animals availability and health status
- Animal house capacity
- Retesting by National Control Authorities…

However recently, new concepts in vaccine quality control have been brought to the forefront: the consistency approach is that each vaccine batch produced at the vaccine production facility is developed in the frame of a design space allowing limits of manufacturing.

Consequently, the new batch shares many of the characteristics of the previous batches produced. This approach allows for a new strategy of quality control, demonstrating

consistency in production, giving emphasis to aspects such as in-process testing, Process Analytical Technology, the implementation of Good Manufacturing Practice and to Quality Assurance.

This holistic strategy particularly focuses on a set of non-animal test models including cell based assays, physicochemical and immunochemical methods to monitor key aspects of vaccine quality. Physicochemical methods are particularly useful for showing deviations of antigen conformation and structure and therefore potency of the vaccine. The presence and functional expression of specific B cell epitopes on the antigen can be tested by interaction between the vaccine antigen and a panel of monoclonal antibodies.

Some key elements in immune responses are still not fully understood, such as interaction between vaccine antigen and adjuvant product and major research efforts are still needed. In the near future in vitro artificial immune systems could boost our understanding of these elements or even replace in vivo methods.

However, it is not sufficient to have validated alternative methods if these are not accepted by authorities in charge of implementation and enforcement. Ways should be sought to increase involvement of authorities at all stages in order to ensure that alternative methods are effectively being used in regulatory compliance testing. Isolated initiative has limited chance of success and regional initiative limited impact if not accepted worldwide.

EPAA has launched a project aiming at the integration and synchronisation of initiatives, prioritisation and transversal collaboration leading to a strong worldwide alignment on 3Rs alternative programme and execution of the consistency approach as a strategy to implement 3R's.

In any case, the implementation of the consistency approach should contribute significantly towards the waiving of the use of animals in regulatory required vaccine quality control.

The successful implementation of waiving for regulatory testing of vaccine depends on high quality science and the understanding, recognition and implementation of the change by all stakeholders.

Key points

The waiving approach is encouraged in the vaccine quality control by:

- New concept in vaccine quality control as the consistency approach. This approach gives emphasis to aspects such as a design space allowing limits of manufacturing.

- This strategy particularly focuses on a set of non animal testing models including cell based assays, physicochemical and immunochemical methods to monitor key aspects of vaccine quality

But

There is a need of acceptation of this holistic approach by authorities and all stakeholders in charge of implementation and enforcement Chemicals

2.1.3 Regulatory aspects – REACH

- **View of ECHA – European Chemicals Agency**

REACH Regulation is a product of 10 years of legislative process. One of the main reasons for developing and adapting this regulation was that a large number of substances have been manufactured and placed on the market in Europe while only very limited information on their hazards were available. In the past, Authorities had to prove chemicals posed risk before they were regulated. Nowadays The REACH Regulation places greater responsibility on industry to manage the risks from chemicals and to provide safety information on the substances. Thus REACH ensures a high level of protection of human health & the environment.

Another aim of REACH is to promote alternative methods for assessment and testing of chemicals and this is clearly states in article 1-1 of the REACH Regulation.

Manufacturers and importers are required to gather information on the properties of their chemical substances to demonstrate using scientifically reliable their safe handling. The REACH Regulation requires EU companies to document such information in registration dossiers for chemical substances manufactured or imported in quantities of one tonne or more per year. The higher the tonnage, the more information submitted needs to be important. For substances manufactured or imported in quantities of 10 tonnes per annum (tonnes p.a.) or above, the registration dossier must include a chemical safety report. For dangerous substances, i.e. substances which are classified or substances considered as persistent, bioaccumulative and toxic (PBT substances), an exposure assessment must be included in the chemical safety report.

The standard information required for registration comprises a number of hazard endpoints which are usually based on information from standard experimental studies with vertebrate animals. The standard data requirements are linked to the tonnage of the substance and are listed in Annexes VII to X of the legislation. Where there is insufficient to meet REACH.

Requirements information gaps are identified, the registrant must generate new information or, for tests at higher tonnage levels (100 tonnes p.a. or above), prepare a testing proposal. Core data are those specified in Annexes VII and VII and higher-tier data, as specified in Annexes IX and X. The increase of animals used and/or costs rise with the number of the Annex.

For each endpoint listed in Annexes VII – X (column 1) an integrated testing strategy (ITS) has been generated to provide an endpoint specific guidance on how to gather and assess available information and consider new data and testing strategies. The ITS approach has for goal to obtain the right information, to limit the use of animals, to reduce the cost for industry and to speed up the assessment process. An extensive guidance developed with the stakeholders has been issued in August 2011.

On a legal basis the use of alternative methods for the REACH registration is clearly stipulated in the regulation (see Article 13 (1), 25(1) or Annex VII-X). The annex XI gives the general rules for adaptation of the standard testing regime with the use of existing data, historical human data, QSAR, grouping of substances and read-across approach, in vitro methods or weight of evidence approach.

However, it is reminded that adaptation is not un-conditioned and that their acceptation by authorities will be depending on:

- the adequacy/reliability to cover the key parameters,
- the information used for the (non)classification and labeling,

- the scientific validity of the methods,
- the adequacy and reliability of the documentation provided.

In order to promote the use of alternative methods ECHA provides a number of guides as well as a web page on information toolkit dedicated to strategies and available tools. In particularly, ECHA provides information on the QSAR toolbox, software to help registrants and authorities. This project co-managed by ECHA and OECD should facilitate the practical application of grouping and read across approaches for data gap filling by read across, trend analysis or (Q)SARs assessment. The Toolbox software is available for download free of charge along with additional information materials and guidance for installation and use.

The experience so far of ECHA is that the reduction of animals testing was essentially due to the data sharing. In addition, information was provided by using mainly existing animal studies but also via read-across and weight of evidence approaches. The computer modeling was rarely used and mostly used for the environmental endpoints.

Through the compliance checks performed in accordance with Article 41, deficiencies have been discovered and one of the key problems in the registered dossiers is the missing or inadequate justifications used for adaptations made. This was found for read-across approaches as well as options to waive studies.

This is the reason why ECHA asks for a best practice of these adaptation rules. Registrants should present convincing cases to provide transparency and allow for independent evaluation. A fully and adequate justification must also be provided.

In conclusion, REACH sets the standard information requirements as baseline. However, opportunities exist for the use of alternative methods (see ITS information for each end-point) when they are fully justified. Collaboration should continue between regulators, researchers and industry to achieve progress and consensus on the use of alternative methods in a regulatory context. Particularly, it is asked to Industry to find collaboration for the creation of efficient databases.

Key points

REACH is the most accurate regulation to promote the use of alternative methods by limitation of animal testing or using waiving. (see articles 13 and 25).

For each endpoint listed in Annexes VII – X an integrated testing strategy has been generated in order to obtain the right information, to limit the uses of animals, to reduce the cost for industry and to speed up the assessment process.

The Annex XI gives the general rules for adaptation of the standard testing regime with the use of existing data, historical human data, QSAR, grouping of substances and read across approach, in vitro method or weight of evidence.

Adaptation is not un-conditioned and their acceptation will be depending of various factors.

Experience of ECHA is that the reduction of animals was essentially due to the data sharing. In addition, information was provided by using mainly existing animal studies but also via read across and weight of evidence approaches. The computer modeling was rarely used and mostly for environmental endpoints.

2.1.4 Industry experience

- **View of a chemicals manufacturer - Arkema**

As a global chemical company, Arkema had registered around 140 chemicals for the initial phase in the REACH regulation process. Throughout this phase which had required producers coming together within consortia, some strategies have been developed to reduce the use of animals for ethical and/or financial aspects as described in annex XI and further exemplified in the Technical Guidance Document R6.

Under REACH, the grouping approach is foreseen as a possibility for filling data gaps in the absence of relevant, reliable and sufficient experimental data for a chemical.

In general, 2 possible strategies can be adopted to carry out the grouping approach: the analogue approach and the chemical category approach. In both cases are driven by the principle of read-across. Read-across is a technique used to predict endpoint information for one chemical by using data from the same endpoint from another chemical which is considered to be similar.

According to the Technical Guidance, a chemical category is a group of chemicals whose physico-chemical, human health and/or environmental properties are likely to be similar or follow a regular pattern as a result of structural similarity like common functional group(s)/constituents, the likelihood of common precursors or an incremental and constant change.

On the other hand, the analogue approach is a less formalised method by which 2 or more source chemicals are used to qualitatively or quantitatively estimate the unknown properties of 2 or more target chemicals. In general, the chosen “source” chemicals have to be data-rich substances sharing similarities with the “target” chemicals. Similarly to the category approach, the robustness of the analogue approach can be improved by comparing all the available properties of the target chemicals to those of the respective source chemicals.

Concerning the toxicological endpoints, a grouping approach has been performed for hydrofluorocarbons molecules (HFCs) whose similar physicochemical properties (state, solubility, chemical reactivity…) and toxicological profiles (TK, acute and repeated toxicity, local tolerance, genotoxicity, reprotoxicity and carcinogenicity…) have been demonstrated. Indeed regarding the REACH registration some datagaps have been identified for several substances in mutagenicity in mammalian cells, fertility and carcinogenicity.

Although it was not possible to identify clear structure-related trends along the series, the common features present in the profile of these substances demonstrated that analogue approach was justified. Thus the available data of the “sources” were used to extrapolate the data of the “targets” within the group. In some cases, the available data within the group allowed also to omit the information or the performance of the study.

During the elaborating of the grouping approach attempt was made to use the OECD toolbox to build a larger chemical category however the proposal elaborated by the tool was not robust due to important differences among the proposed members in terms of structure or TK behaviour. In addition, despite their great similarity, it was not possible to obtain all the HFCs of interest in the same category. At last, the data of the selected chemicals were often inaccurate or incomplete.

Concerning the ecotoxicological endpoints QSARs and read-across approach were performed also in the framework of a category.

Data gaps were identified concerning the aquatic toxicity. However aquatic toxicity testing with gases often lead to invalid results because of the technical difficulties associated with the physical state of the substances. Besides, the exposition of the aquatic compartment will be very low based upon the volatility of the considered substances. Thus, the aquatic compartment is not the final environmental compartment of the concerned substances. That is the reason why it was proposed to use category approach and fill the missing data by using QSARs data and/or read-across. As HFCs share common physicochemical and environmental fate, category approach was justified.

The Stepwise approach followed the recommendations of the Technical Guidance Document R6 as presented below:

- Step 0 and 1: Check whether the chemical is a member of an existing category and (if necessary) develop category hypothesis and definition and identify category members
    - Use of OECD Toolbox
- Step 2 - 4: Gather data for each category member, Evaluate available data for adequacy, Construct a matrix of data availability
    - Estimation with free software recommended in the Technical Guidance Documents.
    - Grouping HFC substances according to their carbon number
- Step 5: Perform a preliminary evaluation of the category and fill data gaps

In this case, even if the use of OECD toolbox has not been possible for a QSAR estimation, the profiling step and the finalization of the data matrix was performed with the help of this tool. Then the EPISUITE modelisation was used to fill the data gap.

In this approach we use each time the most conservative approach in order to fill the missing data with data that would not underestimate the hazard for the aquatic compartment.

In conclusion the avoidance of additional tests was particularly relevant for toxicology in vue of the available data and for ecotoxicology since aquatic toxicity testing with gases often lead to invalid results. However, difficulties have been encountered regards to the use and availability of relevant validated QSARS and the use of OECD Toolbox in order to help for the use of prediction tools. Besides, the most important remaining question is what will be the acceptability of such approach by the European and national authorities.

Key points

The grouping approach is foreseen as a solution in absence of reliable and sufficient experimental data for (eco)toxicology. Grouping approach covers analog approach (use of data from one substance to another) and chemical category approach (use of data from one or more substances to extrapolate the results of an other substance).

Grouping approach can be made by using the OECD tool box in order to build a larger chemical category.

These approaches are particularly relevant when experimental data can lead to invalid results (i.e. for ecotoxicology when aquatic testing is performed with gases).

However, difficulties have been encountered regards to the use and availability of relevant validated QSARS and the use of OECD Toolbox.

The most important issue is the acceptability of such approaches by European and national authorities.

2.2 COSMETICS

2.2.1 Regulatory aspects

- **View of the Scientific Committee on Consumer Safety - SCCS**

The 7th Amendment of Council Directive 76/768/EEC of 27 July 1976 provided the regulatory basis for the elimination of animal testing of cosmetic products and ingredients. This has been recast as Regulation (EC) No 1223/2009 on Cosmetic Products, which applies from July 2013. No substantial changes with respect to animal testing were introduced in the recast, and the testing and marketing bans remain.

Testing Ban in the EU

- Finished cosmetic products since 2004.
- Ingredients or combination of ingredients (in order to provide data pursuant to the particular safety evaluation requirements of cosmetic ingredients) has applied since March 2009, irrespective of the availability of alternative non-animal tests.

Marketing Ban in the EU

- March 2009 (irrespective of origin of products).
- Applies for all human health effects except repeated-dose toxicity, skin sensitisation, carcinogenicity, reproductive toxicity and toxicokinetics. For these specific effects, there is a deadline of March 2013, irrespective of the availability of alternative non-animal tests.

Extracted from Regulation (EC) No 1223/2009

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

- Cosmetic products should be safe under normal or reasonably foreseeable conditions of use. In particular, a risk-benefit reasoning should not justify a risk to human health.
- At present, there is inadequate information on the risks associated with nanomaterials.
- The safety of cosmetic products and their ingredients may be ensured through the use of alternative methods which are not necessarily applicable to all uses of chemical ingredients.
- It will gradually become possible to ensure the safety of ingredients used in cosmetic products by using non-animal alternative (validated) methods.
- The Commission shall study possible technical difficulties in complying with the ban in relation to tests, in particular those concerning repeated-dose toxicity, reproductive toxicity and toxicokinetics, for which there are no alternatives yet under consideration. Information about the provisional and final results of these studies forms part (of) yearly reports… On the basis of these annual reports, the timetables established ….may be adapted up to March 2013 …… if these studies conclude.... that for technical reasons one or more tests …… will not be developed and validated …… it shall inform the European Parliament and the Council and shall put forward a legislative proposal in accordance with Article 251 of the Treaty.

In exceptional circumstances, where serious concerns arise as regards the safety of an existing cosmetic ingredient, a Member State may request the Commission to grant a derogation ……. only where:

- (a) the ingredient is in wide use and cannot be replaced by another ingredient capable of performing a similar function;
- (b) the specific human health problem is substantiated and the need to conduct animal tests is justified and is supported by a detailed research protocol proposed as the basis for the evaluation.

JRC Evaluation

Recently, a report was made by JRC to combine scientific argumentation that a postponement of the deadline of 2013 is necessary because of lacking essential in vitro tests for risk assessment e.g. repeated dose toxicity, developmental toxicity, carcinogenicity, sensitisation and toxicokinetics.

A summary is available:

http://ihcp.jrc.ec.europa.eu/our_activities/alt-animal-testing/report_2010/findings_ecavm_2011.pdf

The full text is available:

http://ihcp.jrc.ec.europa.eu/our_activities/alt-animal-testing/report_2010/fulltext.pdf

Notwithstanding the substantial progress made over the past years, for five specific areas full replacement alternative testing methods will not be available by 2013.

- Due to the underlying scientific challenges, no specific timeline in the areas of toxicokinetics, repeated dose toxicity, carcinogenicity and reproductive toxicity.
- The timelines estimated for skin sensitisation point to 2017-2019, including the possibility to differentiate weaker from stronger sensitisers. Methods discriminating between skin sensitisers and non-sensitisers might become available earlier.

The European Commission will now review the situation regarding the technical difficulties in complying with the 2013 ban and inform the European Parliament and the Council, proposing any measures to be taken.

Key points

Testing ban and marketing ban are scheduled since 2004 and will finish in 2013.

Cosmetic products should be safe under normal or reasonably foreseeable conditions of use.

At present, there is inadequate information on the risks associated with nanomaterials.

The safety of cosmetic products and their ingredient may be ensured through the use of alternative methods which are not applicable to all uses of chemical ingredient.

The European cosmetics legislation foresees a review in 2011 and possible postponement of the 2013 marketing ban to enforce the testing ban for systemic and repeated-dose animal tests.

The current lack of availability of a full replacement was underlined in the report commissioned to JRC by the European Commission.

2.2.2 Industry experience

- **View of a cosmetics manufacturer – L'OREAL**

Cosmetic products present on the European market must be safe for the consumer. The tools to determine the toxicological profile of cosmetic ingredients, consisted of animal experiments, have now been narrowed down substantially by the legally imposed animal testing ban on cosmetic ingredients, taken up in the Cosmetic Products Directive (76/768/EEC). The waiving is/will not be possible dependent of the toxicological endpoints. Cosmetics industry makes continuous efforts to promote and develop the "1R" via replacement of animal testing.

For ingredients, the first milestone was March 2009 for acute toxicity, skin and eye irritation, percutaneous absorption, photo-induced toxicity, mutagenicity and genotoxicity endpoints. Various *in vitro* test systems are currently used that include both formally validated tests (e.g. skin irritation, 3T3-NRU phototoxicity test, *in vitro* micronucleus test) and *in vitro* methods that have been 'in-house' introduced based on accumulated experiences (e.g. eye irritation). As presented, L'Oreal has participated to ECVAM validations and regulatory OECD acceptance processes of reconstructed human epidermis EpiSkin and SkinEthic RHE for use as a full replacement (1R) for the Draize test. Therefore through the illustrated approaches for photo irritation and genotoxicity, *in silico* approaches, read across, and physical-chemical characterization as part of a tiered non-clinical testing strategy contribute to the decision-making process.

For the second milestone of March 2013, no animal testing will been permitted in the EU, whether or not an alternative method is available. Cosmetic point of view was illustrated with the skin sensitization endpoint since the biological process of skin sensitization is complex but well known in comparison to other 2013 repeated dose endpoints. Today, the murine local lymph node assay (LLNA) is the most used. The current *in vitro* tests, allowing to distinguish Sensitizers versus Non Sensitizers (hazard classification), have not yet demonstrated their validity and therefore will not be available for risk assessment for 2013. There is general agreement that replacement of all animal experiments to evaluate all relevant toxicological endpoints will be impossible by March 2013. International efforts of research lean on a shift of paradigm and rely on human cells as well as on adverse outcomes pathways are still needed.

Key points

For ingredients, the first milestone was March 2009 for acute toxicity, skin and eye irritation, percutaneous absorption, photo-induced toxicity, mutagenicity and genotoxicity endpoints. Various *in vitro* test systems are currently used.

Through the illustrated approaches for photo-irritation and genotoxicity, *in silico* approaches, read across, and physical-chemical characterization as part of a tiered non-clinical testing strategy contribute to the decision-making process.

There is general agreement that the lack of full replacement for the areas of repeated dose toxicity, carcinogenicity testing, and reproductive toxicity by March 2013 is not a reflection of the efforts spent but rather the size of the challenge and the time science needs to develop new approach and undergo transition.

2.3 PLANT PROTECTION PRODUCTS

2.3.1 Regulatory aspects

- **View of ANSES – Agence Nationale de Sécurité sanitaire, de l'Alimentation, de l'environnement et du travail**

The Regulation (EC) n°1107/2009 will update the EC's regulatory framework, Council Directive 91/414/EEC, for placing plant protection products on the market.

The main points in the new regulation are set out below.

Approval conditions are specified, including new hazard criteria based on substances' intrinsic properties. These are for health effects: no category 1a or 1b mutagens*; no category 1a or 1b carcinogens*, unless exposure is negligible; no category 1a or 1b reproductive toxins*, unless exposure is negligible; no endocrine disrupters which may cause adverse effects in humans, unless exposure is negligible (interim provisions will apply until the Commission develops definitive measures); no persistent organic pollutants; no persistent, bioaccumulative and toxic substances.

New data requirements for active substances and products are listed in the annexes of the regulation which will be applicable by 2013-2014. The goals of the new requirements are the following:

- to increase quality and relevance evaluation
- to converge to OECD requirements
- to reduce animal testing (3Rs principle)
- to address new criteria of Regulation (EC) n°1107/2009.

For the active substance major changes are the following:

Acute dermal toxicity study will be conditional to the oral acute toxicity, and to the dermal absorption %.

The 90 days dog study will remain mandatory, on the contrary the one year dog study will not be required.

A long term rat oral toxicity and carcinogenicity study shall be conducted, a second carcinogenity using the mouse as a test species shall be conducted, unless it can be justified scientifically that this is not necessary.

Toxicokinetic data will be integrated in short and long term studies.

The F1-extended one generation studies should be considered as an alternative of the multi-generation studies when validated and adopted as UE/OECD.

Where necessary parameters to address neurotoxic, immunotoxic effects, change in hormonal system should be included in 90-day, or reproductive toxicity studies.

For the plant protection products, the major changes are the following:

Directive 99/45/EC and Regulation (EC) n°1272/2008 where relevant can be invoked for waiving studies, classification by calculation will be based on the properties of the chemicals components of the products for acute oral, dermal, inhalation toxicity.

To address dermal and eye irritation, a tier approach including in vitro studies is proposed.

Key points

The Regulation (EC) n°1107/2009 will update the EC's regulatory framework,

New data requirements for active substances and products are listed in the annexes of the regulation which will be applicable by 2013-2014

One of the goal of the new requirements is to reduce animal testing (3Rs principle)

For the active substance there are major changes in the data requirements and it can be considered as Waiving.

For the plant protection products the changes to avoid animal testing are in the acute toxicity and dermal and eye irritation.

2.3.2 Industry experience

- **View of a plant protection products: BASF**

Plant protection products (PPP) are tightly regulated and require a large set of data to address their safety with respect to human health and the environment. Generally data requirements are rather similar across most of those worlds regions with extensive PPP legislation (EU, NAFTA, Brazil, Japan). Taking into account the aforementioned tight regulation and global nature of data requirements it is not surprising that this system in rather rigid and leaves little possibilities for targeted research or for waiving of studies deemed unnecessary for a particular compound. In the EU there are some options in area of acute toxicity and local tolerance which can be used to apply waiving (Creton et al CRC, 40, 2010). A well known example is the waiving of skin and eye irritation testing for substances with very high/low pH. For studies with repeated administration there are very few possibilities. If there is scientific evidence that a substance is not absorbed than in principle a number of these studies can be waived. For oral studies, in practice, this only plays a role with polymers and very few PPPs are polymers. Chances for waiving repeated dermal toxicity testing are better if it can be shown that the substance is not bioavailable following dermal application. For inhalation, no testing is required if it is technically not possible to generate a test atmosphere or if inhalation is not considered to be a relevant route of exposure for human risk assessment.

With the availability of new, in vitro, methods the possibilities for waiving in vivo studies increase. In the EU in vivo irritation testing can be waived if the appropriate in vitro study (OECD 430, 431 or 435) demonstrates that the substance is corrosive. It is also reasonable to waive in vivo mutagenicity testing (e.g. mouse micronucleus test) if the in vitro tests (Ames, HPRT and micronucleus in vitro) are negative.

Occasionally there are also reviews on the usefulness of standard data requirements. In the EU and USA the need for a 12-month dog study has been debated for some time, and it was concluded, that this study type provides very little, if any, new information that is actually used for risk assessment purposes, over and above information obtained from other standard studies (particularly data from the 3-month dog study and the long-term rat studies). Therefore, this study is no longer a standard data requirement in the EU (and USA)

The UK health authorities have extensively investigated the usefulness of the cancer mouse study. Their data analysis showed that in only 3 out of 202 studies (1.5%) there was a contribution of the mouse cancer study, over the data available from the rat cancer study.

With the extended 1-generation study, with its branches for the assessment of developmental neurotoxicity, immunotoxicity and endocrine effects, a new type of study

has been introduced, which should allow for a more directed, compound specific, research in toxicology. Within the guidance for this study type, possibilities for waiving the in depth assessment branches can be defined. If accepted by regulatory authorities, this study would be the first significant study which would include the concept of targeted research (waiving).

It should be noted, however, that the development of new active ingredients for PPPs is nearly always performed for the global marked. Therefore, even for the few above mentioned possibilities for waiving, the chances of actually doing so are minimal, because of lack of global acceptance of waiving. The highest change for successful waiving is currently for the 12-month dog study. This, however, would require that health authorities in Brazil and Japan would also accept this concept.

Occasionally there are also reviews on the usefulness of standard data requirements: in the EU and USA the 12-month dog study is no longer a standard data requirement. The UK health authorities have extensively investigated the usefulness of the cancer mouse study. The extended 1-generation study if accepted by regulatory authorities would be the first significant study which would include the concept of targeted research (waiving).

Key points

Plant protection products (PPP) are all over the world tightly regulated and require a large set of data to address their safety with respect to human health and the environment.

The data requirements are very similar.

The system in rather rigid and leaves little possibilities for targeted research or for waiving of studies deemed unnecessary for a particular compound.

There is a lack of global acceptance of waiving of data requirements by Authorities.

2.4 OECD ACTIVITIES TO AVOID UNNECESSARY ANIMAL TESTING

Thirty four OECD countries worldwise work together, to combine their skills and knowledge, to discuss issues of mutual concern, to avoid duplication by harmonization of policies and free-access tools and to share the burden of testing. In the context of chemicals testing and assessment, OECD has developed several approaches leading to avoid unnecessary animal testing:

2.4.1 Available tools

- **The use of existing data & non-test information**

- For existing data, the eChemPortal offers free public access to information on properties, hazards and risks of chemicals found in the environment, home and workplaces and in products used daily. Users can simultaneously search data from multiple data sources.
- Non-test information can be provides by different ways: - Grouping chemicals into categories using read-across, trend analysis or (Q)SAR models, or based on molecular similarity and reactivity analysis or based on common metabolites, identify chemicals with specific metabolic pathways or toxicity mechanisms. The (Q)SAR Application Toolbox allows user to built his own predictive model using computational methods to estimate physical-chemical and biological properties of chemicals based on characteristics of molecular structure.

▪ Test Guidelines (TGs) and Mutual Acceptance of Data

- *In vitro* Test Guidelines replacing animal testing are available to assess, genotoxicity (TG 471, 473, 476, 479, 480, 481, 482, 487), skin absorption (TG 428), Skin corrosion (TG 430, 431, 435), skin irritation (TG 439), phototoxicity (TG 432), ocular corrosion/irritation (TG437, TG438), endocrine disruption (TG 455, TG 456). Number of others test guidelines are currently under evaluation in the same areas. It is also important to mention numbers of adopted test guideline using a reduced number of animals, the most recent under investigation being the extended one generation reproductive toxicity study (TG 443) that is expected to be published in 2011.
- The most efficient tool to avoid unnecessary testing is the System of Mutual Acceptance of Data (MAD) which avoids duplication of testing by guaranteeing that data generated in the testing of chemicals in an OECD country or adhering non-member country, in accordance with OECD test guidelines and OECD principle of Good Laboratory Practice. All sectors are concerned by the MAD, industrial chemical, plant protection products, cosmetics, substances (and mixtures). Non clinical health safety studies (for pharmaceuticals, ICH methods are more currently used).

2.4.2 Tools under investigation

▪ In vitro testing, High Throughput screening and toxicogenomics

An extend Advisory Group on Molecular screening and toxicogenomics, exchange information on projects for new approaches to testing, in particular high throughput in vitro assays (based on US Toxcast program), subgroups work on pathways/mechanisms of action.

▪ Testing strategies, conceptual framework and integrated approaches, based on the tools

Integrated approaches taking into account the tools outlines above for testing and assessment of chemicals, will probably significantly reduce or avoid animal testing in the future.

Key points

OCDE has developed several approaches to avoid unnecessary animal testing: uses of the available tools as existing data and non-test information or use of test guidelines and the development of new in vitro tests.

Mutual acceptance of data and development of testing strategies are the basis to reduce or avoid animal testing.

3. SYNTHESIS OF THE ROUND TABLE CONFERENCE

3.1 CHEMICAL INDUSTRY - RHODIA

Antoine LEPLAY, explains the strategy of its company regarding animal testing and waiving:

- Due to the worldwide organization of the company, testing is optimized and tests duplication for different regulations in different countries is avoided,
- Waiving is a priority: for example, internal experts have done their best to use read-across and other adaptations in the context of the first registration phase of REACH, so that testing proposals are minimized in the REACH dossiers. It has also been a challenge for Rhodia experts to convince other consortia members to use waiving (with a relevant justification), but they actually succeeded in promoting their views,
- As a company committed in the Responsible Care® initiative, there is a political ambition to avoid testing on vertebrate animals, especially if there is no improvement in the protection of human health and environment. Rhodia aims to promote this position towards authorities and the last example that illustrates this, is the industry position on the strictly controlled conditions for intermediate dossiers.

Antoine Leplay ends his presentation by thanking FRANCOPA for having organized such an interesting workshop with representatives from regulatory bodies, academia and industry

3.2 HUMAN MEDICINE - EDQM

Karl-Heinz Buchheit talked about the possibilities for the introduction of 3R methods in the field of quality control of medicines and in particular of biologicals. According to a recent survey by the EDQM, manufacturers hesitate to establish 3R methods mainly because of costs for introduction of the methods and for the difficulties to get them accepted by the licensing authorities (e.g. need to submit variation of the marketing authorization dossier). K.H. Buchheit proposed that licensing authorities should consider rewarding manufacturers which implement 3R methods, e.g. through waiving fees or by accelerated evaluation of the variation application.

K.H. Buchheit also mentioned that licensing authorities play a critical role in accepting animal-based quality control tests upon the first submission of a licensing dossier for a new medicine. The likelihood that methods that are accepted at this phase will later one become the “standard method” which will even enter into the European Pharmacopoeia are very high. Regulators should thus encourage manufacturers at the earliest possibility (e.g. during pre-submission meetings) to consider alternative methods.

K.H. Buchheit also mentioned that in the global market in which the manufacturers act today require that 3R methods are accepted in other regions of the world and not only in Europe. If this is not the case, manufacturers are obliged to continue with animal tests for products going to such regions where alternative tests are not acknowledged. Thus, for the ease of the procedures, manufacturers might be discouraged to introduce the 3R methods for the European market products, as this will not oblige them to run two different methods for the same purpose. It is thus of utmost importance to involve WHO as early as possible into the process of establishing 3R methods, as to allow uptake of such methods as early as possible into the WHO guidelines. This will encourage the non-European authorities to accept such alternative methods.

3.3 PHYTOPHARMACEUTICALS PRODUCTS - ANSES

Thierry Mercier explained that the incentives for waiving are related to a reduction in resources necessary to provide safety information. Waiving may be related to the lack of (significant) exposure, knowledge about intrinsic (e.g. physico-chemical) properties of the compound or to the availability of relevant data of compounds which are considered to be substantially similar. There are, however, also hurdles that will reduce the willingness to apply this concept. They are in principal related to uncertainty. For regulators the quality of the waiving statements will be essential for acceptance. As there are no fixed rules for waiving (or predefined quality standards), there is inherent uncertainty if the waiving argument will be accepted. For those in industry responsible for project development, which if involving new active ingredients may cost > 100 mio €, this uncertainty is a significant hurdle in advancing the waiving concept. Individual waiving of studies will reduce the resources needed for active ingredient development only to a very minor extent relative to the overall project cost. Non acceptance of a waiving argument may result in an increased time to market with huge financial consequences.

Therefore, in order to reduce the uncertainty hurdle, waiving should not be based on bright but particular arguments for individual studies, but should rather be part of a general concept which is based on targeted testing.

For the development of a new active ingredient up to 40 different toxicological studies may be required. Out of these only a few will be finally used for regulatory purposes. However, at the start of the toxicological testing program, it is not known which of these studies will end up to be the (regulatory) relevant ones. If we were to perform more and better short-term studies (which could include analysis using'omics sciences (transcriptomics, proteomics, metabolomics) and receptor (de)activation), I believe that toxicologists will be able to target their testing to those endpoints which are relevant for the compound in question, and consequently waive those studies which do not address the toxicological profile of the compound. Provided that we can agree on the types of short-term studies needed, waiving could then be based on targeted testing, and this will make waiving arguments comprehensible and scientifically sound.

I applaud FRANCOPA's intention to further use waiving in toxicology and urge them to advocate and cooperate with other organizations to ensure that this concept is placed in an overall framework.

3.4 COSMETICS - SCCS

Ian White explained that the 7th Amendment to the Cosmetics Directive introduced the prohibition of testing finished cosmetic products on animals and the stepwise prohibition in testing cosmetic ingredients on animals in order to provide safety data required specifically for the particular requirements of cosmetic safety evaluation.

The inclusion of the prohibition in the Amendment was forced by the European Parliament through a process of conciliation despite concerns from other parties.

There is general agreement that replacement of all animal experiments to evaluate all relevant toxicological endpoints will be impossible by March 2013. This is the date when it will not be possible to sell on the European market cosmetic products containing ingredients recently tested on animals to provide data to support safe use in cosmetic products.

Despite the above, the Amendment has provided the catalyst for efforts to find appropriate non-animal replacements. These efforts should be recognized and the enormous progress applauded.

Nanomaterials are becoming increasingly important but validated alternatives to animal testing of these materials is lacking.

Cosmetic products do provide improvements to quality of life to the European citizen and some ingredients are used to help reduce the burden of disease in the population. An important example of the latter is the case of UV filters. Novel filters will not be permitted for use in cosmetic products as it will be impossible to provide the necessary data to ensure that they may be safely used. This also stifles research in Europe.

In 2013, the Cosmetics Regulation will have succeeded the Directive. To 'ensure' safe use of cosmetic products, some animals testing of new ingredients will be required for the foreseeable future. Recognition of this and 'loosening the noose' around innovation and development of potentially important ingredients for society is essential.

"The health of the people is the highest law":

- Industry, academia have been achieved a lot of work within the last decade,
- Ian refers to the manuscript from 2011: Adler et al. Review, Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010 Arch Toxicol 2011 → "we need to be careful because we talked about safety",
- From 11 July 2013, Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products will replace the "Cosmetics Directive" which until now ensured that products circulate freely whilst guaranteeing a high level of protection for consumers. The provisions of the Regulation aim at ensuring that consumers' health is protected and that they are well informed by monitoring the composition and labeling of products. The Regulation also provides for the assessment of product safety and the prohibition of animal testing.

3.5 OECD

Laurence Musset focused on two points for OECD program:

- The improvement of the mechanism of action comprehension and the knowledge,
- The need to push forward the integration approach and safety testing evaluation (as illustrated by 3 workshop organized in 2011).

And she concluded that "The more we see, the more complex Alternatives Methods are".

3.6 Ministry in charge of Ecology and Sustainable Development – General Direction of Risk Prevention

Catherine Mir explained: As representative of a competent authority in charge of Reach implementation, I can say that we are downstream users of toxicological tests and of any method which assess hazard of chemicals. Then these scientific assessments of chemicals are used by scientific organisms in charge of risk assessment which give us advice to better manage the risks of chemicals in their different uses.

Toxicology and toxicological studies have a huge importance to help the authorities for that, and it is sure that we always will need these tools for risk assessment of chemicals and reduce their impact on environment and health. Moreover the scientific community has improved all over these last decades the methods and today once more we have seen examples of the progress made.

From the point of view of downstream users of toxicology, what are our challenges for the next decade? These challenges appear to us very clearly, especially after the large discussions we had during “le Grenelle de l'environnement” about environment and health, discussions that led us to adopt in France our second National Environment and Health Action Plan.

One action mentioned in this action plan is to better assess the risks linked to reprotoxic chemicals and endocrine disruptors and particularly to contribute to the development and adoption of tests at European level to better define endocrine disruptors.

Another is to strengthen regulation, expertise and prevention of risks linked to nanomaterials and particularly to develop relevant tests.

Last but not least, it is necessary to identify and to manage the risks in the geographical areas where populations are exposed to over exposition of different substances, through research to better assess synergies between different pollutions. This enforcement of research is also expected to better assess the risks of food contaminants and their cumulative effects or low dose effect.

So, endocrine disruptors, nanomaterials, cocktail effect of chemicals are our clearly identified challenges for the next decade.

On another hand, we all know the limits of toxicological tests on animals to face these challenges: these limits are not only ethic limits expressed in the animal welfare regulation. They are technical and intrinsic limits as well. We perfectly know the question of interpretation results observed from one species to another, the question of window exposition for endocrine disruptors, the fact that human and animal populations in nature are not as homogenous as animal populations used for experiments and that they are exposed to different pollutants at the same time and some time through different exposure pathways.

So in fact we have different tools to face these challenges, not only toxicological studies, but toxicology in general, studies about mechanisms of action of substances, alternatives methods, and also environmental and epidemiological survey, which have their own limits as well. The task is not easy and we have to use these different tools in the most efficient way. This is one of the aim of this workshop.

We have had exchanges about assessment of endocrine disruptors today, scientifically we are in progress and authorities in charge of risk management will have to decide how to manage the risks linked to these substances, we are strongly encouraged by NGO and parliamentarians.

For nanomaterials the challenge is quite different, because of the large possibilities to create new nanomaterials, the question to adapt the tests and the impossibility to use epidemiological studies.

Clearly for cocktail effects, toxicology can help but the combination of substances exposures is unlimited and the challenge for authorities is also, with help of a scientific approaches, to imagine how to manage the risks.

With the system of waiving animal testing for regulatory purposes, Reach has open a new way to better assess in a more efficient and more ethic way chemicals. This has been widened to assessment of other products as phytopharmaceuticals or biocides.

Nevertheless we must be conscious of the limits of the different methodologies that we can use.

4. CONCLUSION OF THE WORKSHOP

Laurent MICHEL, General Director of Risk Prevention at the French Ministry in charge of ecology and sustainable development was invited by FRANCOPA to close the workshop and that was a great pleasure for him.

- Waiving animal testing for regulatory purposes is a transversal deal which concerns human and veterinary medicines, chemicals, plant protection products, cosmetics. The aim is to get good quality data for the safety and limit or avoid animal testing.
- The regulatory context is more and more accurate :
    - The revised European Directive 86/809/EEC has been in place in European union since 1986 and was revised in 2010 (Directive 2010/63/UE) This directive also calls for the application of the Three Rs (Reduction, Refinement, and Replacement),
    - REACH is the most accurate regulation and recommends the use of alternative methods in order to limit the animal testing. Some tests are submitted to an ECHA authorisation,
    - In the Biocides project regulation is closed to the REACH process and is under negociations with the European Parliament.

The common principles to these regulations are:

- Data sharing,
- Gathering the existing data,
- Grouping or read across,
- Qualitative or quantitative structure-activity relationship models,
- In vitro models.

Considering REACH regulation, some signals of improvement and some margins.

For example, in REACH registration process: among the 24 560 registered dossiers, 90 % are joint submission with data sharing between industry companies. Industry used in majority alternative methods and there are only 1175tests required among 574 dossiers. However, there is a margin of progress in the justification of the uses of alternative methods.

How to go further?

- Communicate on the existing regulations and the use of the alternative methods,
- Encourage industry to the data sharing,
- For industry : take care to make a good justification of the alternative methods use,
- For research : develop new alternative methods which could be supported by the INERIS platform ANIMEX,
- And speed the validation between scientists-industry- managers at international level,
- More extensively promote the dialogue between all the actors, inter sectors, international (WHO…).

In Conclusion

Animals testing will be always necessary but can be reduced.

Regulation shows the way to do but it is not so simple.

We are improving collectively and it is necessary to go on.

Thank you to FRANCOPA to have organised this workshop with presentations of public actions (regulations and science) and concrete experience of industry.

It is time to inform and modify the habits and go through all the barriers.

5. GENERAL COMMENTS ON THE WAIVING IN THE DIFFERENT PRODUCTS – PERSPECTIVES

- Waiving has been introduced in the European regulations for human medicine, human vaccine, chemicals, biocides and phytopharmaceutical products. For cosmetics, waiving is not an option because animal testing will be definitely forbidden for products and ingredients in 2013. Return from experience is nevertheless useful to others operators.
  Waiving is an issue that is at the crossroad of regulatory acceptance, research, and data management both on hazards end exposures. Indeed it must be grounded, on bases that are recognized as solid by all partners.

  Waiving applies when animal testing is not necessary. As a concept, it is basically different from replacement. In some cases such as exposure board waiving this difference is obvious. The distinction can vanish when waiving and sound science need to match. Nevertheless, the philosophy of waiving is practical, and to be practical in toxicology and ecotoxicology is a goal that must be shared.
- Through the various presentations, the use of the alternative methods, the substitution, reduction of animal testing were arisen. A clear definition of the Waiving should be proposed and discussed.
- Industry is very active to promote the development and the use of waiving. Specific actions were presented: use of immunological methods for vaccine control, use of in vitro or read across, in silico methods for chemicals. For phytopharmaceutical products the way is to act on the regulatory data requirements and to suppress some irrelevant animal testing.
- Nowadays, local specific tests are available and repeated toxicity tests are desperately missing. In the cosmetics field no solution will be ready for the 2013 deadline.
- The question of the acceptability of the use of Waiving or alternative method by the Authorities is a question for the whole industry.
- The role of the European (ECHA) and International (OECD) organizations is important: the data sharing, the mutual acceptance of the data and the development of the integrated strategy are the best tools to decrease the animal testing.