

JSAAE & JaCVAM Joint Workshop
“International Trends on 3Rs in Animal Experiments”

- Program -

- AM
10:00 Chair: Masaharu Akita (Kamakura Women's University)
Opening Address
- 10:05 **Tightened control on laboratory animals and animal tests both in Japan and other countries**
Takuya Ikeda (CHARLES RIVER LABORATORIES JAPAN)
- 10:45 **International trends in alternatives to animal experiments**
Hajime Kojima (JaCVAM/NIHS)
- 11:25 **The FP7 Project AXLR8 – Accelerating the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally coordinated research and technology development**
Horst Spielmann (FU Berlin, Pharmazie, Berlin)
- Lunch -
- PM
1:30 Chair: Hajime Kojima (JaCVAM/NIHS)
Approach for OECD Test guideline of alternative methods developed by Japan
Hitoshi Sakaguchi (Kao Corporation)
- 2:10 **Approach of pharmaceutical companies on 3Rs for the animal experiment**
Fumio Sagami (Eisai Co., Ltd)
- Coffee break -
- 3:10 Chair: Yasuo Ohno (NIHS)
Japan Chemical Industry Association's Activities on 3Rs
Shinji Kotachi (Japan Chemical Industry Association; JCIA)
- 3:50 **Contribution to 3Rs by Japan Cosmetic Industry Association**
Hiroshi Itagaki (Japan Cosmetic Industry Association; JCIA)
- 4:30 **Introduction of WC8**
Japanese Society for Alternatives to Animal Experiments
- 4:55 **Closing Remarks**

Tightened control on laboratory animals and animal tests both in Japan and other countries

Takuya Ikeda (CHARLES RIVER LABORATORIES JAPAN)

Our society has become increasingly strict about laboratory animals and animal tests. Under such circumstances, those who are involved with animal experiments need to understand and comply with relevant laws and regulations more than ever. Therefore, they have shown strong interest not only in domestic trend and regulations but also in those of Europe and the U.S. This is a welcome trend and we can expect actual improvement of laboratory animal welfare. However, many of them show low level of attention in moves of OIE or ILAR and news reports on bans on animal experiments. They only know those facts.

On the other hand, for reasons of language difference and limited information, some people reacted sensitively and sometimes excessively without deep understanding of the fact and backgrounds. In addition, some people with fragmentary knowledge and information on situations of Europe and the U.S. tried to apply them in their facilities without knowledge of different social systems and backgrounds. In Japan, we are currently in the process of revising the Law for the Humane Treatment and Management of Animals. When this law was last revised in 2005, basic policies were presented by three ministries, Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, Ministry of Agriculture, Forestry and Fisheries, and a detailed guideline “Guidelines for Proper Conduct of Animal Experiments” was also presented by the Science Council of Japan. With a series of these movements, the concepts of 3Rs, five freedoms, and other concepts have been widely spread. It facilitated further understanding of laboratory animal welfare among animal experiment personnel. Ministry of the Environment set up Animal Welfare Subcommittee under Animal Welfare Group of Central Environment Council with the objective of amending the law. The subcommittee started the discussions from July, 2010, aiming at submission of the bill during regular Diet session in 2012.

However, after the last revision, have people involved with animal experiments fully responded to expectations and requests from our society or the public? It’s an open question. Therefore, in preparation for the next revision, we need to understand not only the situation of other countries such as European countries and the U.S., but also background information of the last revision as well as the situation after revision to analyze them. Having those in our minds, we need to fully understand what is discussed and what is required in Japan and other countries in order to conduct proper animal experiments and to comply with the next revision.

International trends in alternatives to animal experiments

Hajime Kojima

JaCVAM (Japanese Center for the Validation of Alternative Methods)

NIHS (National Institute of Health Sciences)

Japan is not a central participant in the world in implementing the 3Rs of animal experiments, namely Reduction (reduce animal experiments), Refinement (relief of pain in laboratory animals), and Replacement (replacement of laboratory animals). In part due to Japan's geographic distance from Europe and the U.S., Japanese researchers have been swayed by political forces and have not kept pace with developments in animal experimentation practices. Although Japan has an extensive information network, the information that needs to be distributed must be updated to meet international practices in nonclinical research.

The Japanese government and researchers have an advantage of high quality technology as well as a commitment to performing validation studies for the developed of test methods. However, they do not have a strategy for adapting to the needs of the world and for becoming world leaders by utilizing this advantage. In light of recent discussions on revising the animal welfare law in Japan, we should confirm the trends in practicing the 3Rs in Europe and the U.S. We must also evaluate our current situation objectively. Japanese strategy will be developed in this field once an understanding of the information from other regions is achieved.

In this paper, I present information on the 3Rs of animal experiments by describing EU trends, trends in the U.S., and cooperation of international cooperation agencies in this area. After that, I report on international efforts to present alternatives to animal experiments and international organizations for the replacement.

The FP7 Project AXLR8 – Accelerating the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally coordinated research and technology development

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The aim of the EU FP7 coordination support project AXLR8 (= accelerate) is to lay the groundwork for a transition in toxicology toward a more “pathway based” in vitro and computational approach, through enhanced networking and collaboration among scientists, regulators at European and international levels. The new concept entitled “Toxicology in the 21st Century”, which was proposed by the US Academy of Science in 2007, calls for a transition in toxicology toward a more mechanistic, cell- and computer-based approach applying human cells and tissues. To support European scientists in getting actively engaged in this research effort AXLR8 is assisting the DG RTD of the European Commission in coordinating the FP6 and FP7 projects of the Alternatives Testing Strategies activity.

The overarching goals of the AXLR8 project can be subdivided into the following specific objectives:

- 1) organise a series of annual workshops to map research progress, gaps and needs in the FP6/FP7 programme on alternative testing strategies;
- 2) provide a range of tools and opportunities for enhanced interdisciplinary and international communication, coordination and collaboration in order to maximise the impact of available resources;
- 3) work to streamline regulatory acceptance procedures to provide for the uptake of validated new methods, including a smooth transition to 21st century systems and
- 4) produce annual progress reports on the state of the science, including recommendations on priority research and funding targets to ensure a prominent role for European science in this rapidly developing research area.

The following conclusions and recommendations were provided at the first AXLR8 workshop in 2010:

1. Substantial progress has been made in Europe on the development of alternative test methods and integrated testing strategies. Examples include the EU-funded projects Sens-it-iv and ReProTect, which will deliver non-animal tests for the assessment of skin sensitisation and reproductive toxicity.
2. Opportunities exist for exciting cross-fertilisation and creation of synergies among EU research teams and international initiatives such as “ToxCast” and “Tox21” in the USA.
3. A coordinated, long-term strategy towards a common goal is urgently needed. From there a practical roadmap can be developed that integrates new and existing European and global research programmes and defines knowledge gaps and future research needs and allow for a more structured framework for transatlantic/pacific coordination and collaboration.

Given the substantial and increasing global investment in research aimed at developing new safety assessment methods and implementing the “3Rs” in toxicology, there is a need for better international. In response to this demand, AXLR8 provides the tools for effective real-time dialogue, information exchange, problem solving, and international cooperation.

The AXLR8 Progress Report 2010 is available on the AXLR8 website

<http://axlr8.eu/axlr8-2010-progress-report.pdf>

Approach for OECD Test guideline of alternative methods developed by Japan

Hitoshi Sakaguchi (Kao Corporation)

In the safety evaluation, the data obtained by global standard test methods that are widely used in the world and have a lot of background data, are judged as most dependable. Therefore, it is important whether an alternative method is adopted as OECD Test Guideline.

Currently we are developing in vitro skin sensitization test and eye irritation test, and the final goal of these tests is OECD Test Guideline. In developing the above test methods, I could notice the important points for developing test method as OECD Test Guideline, so I would like to present.

There are mainly four stages by acceptance as OECD Test guideline. 1) Test development, 2) Inter-laboratory study (Ring Study), 3) Validation, 4) Peer-review. In the 1st stage, the role of the lead laboratory is very large, and the development of SOP, making of database, and definition of applicability domain are important items. To evaluate predictive capacity, a database is needed. The number of test chemical in the database is reported about 100 for in vitro skin sensitization test or eye irritation test. The rapid evaluation for these test chemicals leads to shortening at the in vitro test method development period and it is important.

In the Ring Study for evaluation of inter-laboratory reproducibility, the number of test chemicals and testing laboratories, and the selection of tested chemicals are important points. Especially it is needed that the test chemicals will be selected from the lists that were published from COLIPA or ECVAM as standard chemical for development of a new alternative method.

And, it is important to conduct by strategic approach in Validation and Peer-review. From now, the most of Validation and Peer-review will be organized under the ICATM framework as international work. Therefore, it is important that the validation and peer-review for a new alternative method developed in Japan will be done by collaboration with not only JaCVAM but also ECVAM and ICCVAM. The Japanese in vitro skin sensitization, h-CLAT are on going in Pre-validation by ECVAM, and the Japanese in vitro eye irritation test, STE test will be Peer-reviewing by ICCVAM in near future.

Finally our Japanese researchers need to be present and submit as manuscript timely for our data of new alternative methods. It is important to present at not only Japanese meeting but also EU/USA meeting actively, and to be published our data in an appropriate international journal by English.

Approach of pharmaceutical companies on 3Rs for the animal experiment

Fumio Sagami (Eisai Co., Ltd)

To study and confirm the efficacy and safety of pharmaceutical candidates, animal experiments are considered an essential process; however, this process is achieved at the sacrifice of precious lives. Internationally, the role and methods of non-clinical safety studies, which are essential before clinical trials in the pharmaceutical development, have been redefined by the ICH. Throughout the ICH processes, the necessity of each safety study was discussed and each experiment was standardized according to ICH safety guidelines based on science and the 3Rs. In the ICH, continuous efforts toward standardization are ongoing based on progress in science and technology. In Japan, the Japan Pharmaceutical Manufacturers Association (JPMA), a voluntary organization of research-based pharmaceutical manufacturers in Japan, established “the guidance for animal experiments” in January 2005, which can be used as a guide for member companies to establish a company standard for the management of animal experiments. Based on this guidance, each member formulated or revised their company regulations, and promoted appropriate self-management of animal experiments based on the 3Rs, addressing the reduction of animals use, development of alternative methods as well as the refinement of animal experiments such as modification of anesthesia. In June 2006, the revised “Law for the Humane Treatment and Management of Animals” came into effect and “Basic Policies for the Conduct of Animal Experimentation” was notified by the Japanese MHLW. Based on these law and policies, pharmaceutical companies have revised their own internal rules and self-management systems for animal experiments. Today, to increase the transparency of self-management systems, Japanese pharmaceutical companies have started to study external evaluation. In this workshop, I will introduce the activities of Japanese pharmaceutical companies, and introduce the current external evaluation system for the self-management of animal experiments.

JCIA's Activities on 3Rs

Shinji KOTACHI (Japan Chemical Industry Association ;JCIA)

The risk assessment and management of chemicals were addressed at UNCED in 1992. At WSSD (2002), countries adopted the goal that by 2020 chemicals are used and produced in ways that minimize significant adverse impacts on the environment and human health. Then, the strategic approach, SAICM, was adopted to meet the goal at ICCM-1 in 2006. To take examples from among many challenges, REACH was enforced in 2007 as the first legislation to force the industry to conduct risk assessment of commercially available all chemicals to achieve the goal. In Japan, revised Chemical Substances Control Law (CSCL), enforced in 2010, defined that the government assesses the risk of not only new chemicals but also existing ones in collaboration with industry. In addition, ICCA and JCIA launched a new voluntary program, GPS/JIPS, in 2010 to assess the risk of chemicals and publish the safety summaries. Consequently, it became a major issue for chemical industries to gather information, e.g. toxicity, of thousands of chemicals more efficiently.

On the other hand, regulatory authorities managing chemicals are seeking to develop 3Rs under the global trend of animal welfare. For example, REACH placed the vertebrate test as the last method to gather toxicity information. The revised CSCL's supplementary resolution of the House of Councilors also includes the promotion of 3Rs.

Under such circumstances, the international organization OECD has partly changed the structure of Task Forces (TFs) to promote the voluntary chemical evaluation program in 2009. One of the established TF focuses on developing new techniques, such as Adverse Outcome Pathway (AOP), and refining structure-activity relationships to estimate the hazard of a large number of chemicals efficiently. These techniques are highly expected to replace the existing toxicological studies using mammalian animals. JCIA, having been focused on such activities and attended the TF as the representative of industry as long as regulatory authorities, established a new working group to promote our activities last year. We will further strengthen the cooperation with governmental regulatory authorities and agencies to promote the development of alternative test methods and disseminate them among the industry.

JCIA has also been promoting Long Range Research Initiative (LRI) program supported by member companies over 10 years. This program has developed test methods for chemical management and the outcomes, such as *in vitro* screening methods, have been highly praised. Developing alternative test methods is one of the priority issues for JCIA among ICCA, and we will continue supporting the program to promote 3Rs.

Contribution to 3Rs by Japan Cosmetic Industry Association

Hiroshi Itagaki, Ph.D

Alternatives for Animal Testing Task Force, Technical Committee,
Japan Cosmetic Industry Association

3Rs' activities by Japan Cosmetic Industry Association (referred as JCIA hereto) is featured mainly by the development of alternative testing (for the Replacement of animal testing), as well as other activities such as collection of information related with Alternative Testing. These are tasks of Alternatives for Animal Testing Task Force as well as Safety Subcommittee which is the superior organization of the task force. JCIA has been contributed to the development of alternative methods by participating to the validation of *In vitro* eye irritation test, drafting the guidelines incorporating this validation results, submitting comments to draft OECD safety guidelines, and also participating to the Scientific Research Group of the Ministry of Health, Labour and Welfare. JCIA takes an active role from the beginning as one of special supporting members in the Japanese Society for Alternatives to Animal Experiments (JSAAE) providing updated information on alternative methods and international regulatory progress related with animal testing. JCIA also published a document titled "The present status of alternatives to animal experiments" in JCIA Technical Bulletin (No.91) in 1990, followed by "Guidance for Cosmetic Safety Evaluation 2001", incorporating alternative testing methods. This Guidance has been revised in 2008.

JCIA member companies have actively engaged in development of alternative tests as seen with various publications in the Journal of JSAAE. They are playing important roles in various committees organized in JSAAE, not only scientific contribution to researches, but also to the management of this society. There are 2 types in the development of new alternative methods by JCIA member companies: inter-laboratories research and individual in-house research. As inter-laboratories research, there are the validation of a skin irritation alternative method using 3D human skin model (commercialized kit), 3T3 NRU PT assay, and *In vitro* sensitization tests using expression patterns of CD86/CD54 in cultured cells as indices. As JCIA member companies' individual in-house research, various methods substituting Acute toxicity, Eye irritation test, Skin irritation test, Phototoxicity test, Sensitization test, Skin absorption tests, etc have been studied since years, as ways to assess the safety of cosmetic products.

At present, there are not many alternative methods in OECD guidelines applicable to cosmetics and quasi-drugs. JCIA has established Task Force groups organized by experts of each testing method, and participate to JaCVAM Peer Review Panels and Regulatory Acceptance Board for the contribution to the adoption of OECD guidelines. However, further step is needed in the application of OECD guidelines in the safety assessment of cosmetics and quasi-drugs because these guidelines have been developed to identify the hazard with new chemicals. To solve these issues, JCIA is engaged with positive contribution to a committee organized by Ministry of Health, Labour and Welfare, titled "Committee for examination how to compile safety reports for the application of the marketing approval for quasi-drugs (referred "Committee" herein after").

As stated so far, JCIA and JCIA member companies have been made devoted contribution to substitute animal tests, by participating validation process as well as drafting of OECD guidelines, and also by having an important part in the Committee. However, it is extremely difficult to abolish all animal tests with scientific level we have today. To promote further the development and use of alternative methods, the support from various social sectors which enable further studies of basic sciences, and also in-depth discussion how to utilize the developed methods are now awaited. I believe this meeting will become the first milestone for this discussion.