Theme IX – Global Cooperation, Regulatory Acceptance and Standardization

Coordinators
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Session IX-1: Activity updates from international scientific societies

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Session IX-1: Oral presentations

IX-1-022

Activities of JSAAE (Japanese Society for Alternatives to Animal Experiments)

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The Japanese Society for Alternatives to Animal Experiments (JSAAE, http://www.asas.or.jp/jsaae/e_index.html) is a scientific organization that undertakes research, development, education, and surveillance activities for promoting international acceptance of the Three Rs as guiding principles for the proper use of animals in scientific testing. The following issues are general service of JSAAE.

1) Annual meeting of the society
2) Extraordinary symposium and workshops
3) Publication
   – J. Alternatives to Animal Experiments (AATEX)
   – Newsletters (in Japanese)
   – Home page
4) Financial support to related research
5) Validation and evaluation of new alternatives
6) Collect relevant information
7) Communication with other countries
8) Others
   – Support of International meeting
   – Collaboration with the other scientific associations Tissue culture Assoc, Mutagenicity assoc., etc
   – Communication with animal protection group

Our society was founded in 1989. There are the currently members, 389 regular members, 18 student members, three honorary members, supporting members (2 Platinum, Gold 4, Silver 23) in 29 companies.

IX-1-148

American Association for Laboratory Animal Science: past, present and future

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The American Association for Laboratory Animal Science (AALAS, http://www.AALAS.org) has a long and progressive history in the evolution of the field of biomedical research (McPearson and Mattingly, 2000). From its beginnings in the mid-twentieth century when five veterinarians met in Chicago to the current conferences of 5000 attendees, the association has been pivotal in the advancement of responsible laboratory animal care and use, including the 3Rs, to benefit people and animals. This presentation will feature updates on traditional programs and services such as certification, e-learning, print resources, scientific information distribution, and electronic resources. In addition, attention will be paid to new products such as "Laboratory Animal Science Professional", a magazine devoted to providing a wide range of resources and knowledge to laboratory animal science professionals and the Grants for Laboratory Animal Science program which provides funding for research about animal husbandry and welfare. The association recently established a new membership category, Global Partners, for individual member associations based outside the United States which allows members of the international association to obtain AALAS programs and services at the AALAS member prices. The presentation will conclude with predictions about the future of laboratory animal science and how AALAS will play a role in that future.

Reference

McPhearson, C. (2000). 50 Years of Laboratory Animal Science, *AALAS*.

IX-1-493

North American society promotes advances in *in vitro* and *in silico* toxicology

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As efforts to replace and reduce the use of animals in regulatory testing mature, it has become clear that collaboration between diverse stakeholders is necessary to ensure successful progress. The American Society for Cellular and Computational Toxicology (ASCCT) has, since 2010, provided a place for individuals representing non-profit, method developer, contract research, regulator, and regulated industry viewpoints to collaborate in order to speed the development and use of *in vitro* and *in silico* alternatives to animal tests.

The ASCCT offers traditional and innovative ways for members and others to collaborate, including yearly in-person meetings, an online newsletter, and an e-mail discussion list. The ASCCT's bi-monthly webinar program provides members access to in-depth presentations by experts in their fields, covering relevant new *in vitro* or *in silico* tools, concepts, and policy efforts and simultaneously providing a venue for interested members to share their research with colleagues.

While focusing on North American events and activities, the ASCCT also endeavors to collaborate with international societies with similar missions. Finally, the ASCCT offers discounted memberships, travel awards, and free annual meeting registration to students in order to encourage the involvement of young scientists in its activities.

IX-1-536

Cuban Group and the Latin-Ibero-American Network for Alternatives: initiatives for accelerating the implementation of 3Rs in our continent

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Latin-America has been a region with a low development of 3Rs Alternatives so far. Just few countries like Brazil can show some relevant progress in this field. In the 80s, Cuba formed its 3Rs Group with important results in the implementation of *in vitro* methods for Toxicology and Pharmacology research. Later, the development of the local biotechnological industry has increased the interest in 3Rs alternatives, mainly in the field of the quality control of vaccines. In spite of these advances, further implementation of alternatives is possible just in the framework of international cooperation. For that, National Groups working on alternatives in Latin America were gathered for creating the Latin-Ibero-American Network for Alternatives. Thus, it will be possible to reinforce our strengths and to overcome our weaknesses by means of the experience exchange and the scientific collaboration, courses and trainings, participation in proficiency and interlaboratory validation studies and platform of projects fully focused on the development and implementation of Alternatives. The definition of statements, a working structure (including a Board) and an Action Plan will be the priority of this Presentation in order to give the first step toward a near future with more development and implementation of Alternatives in Latin-America.

IX-1-676

ESTIV's activities for the promotion of *in vitro* toxicology

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ESTIV is a leading European organization for *in vitro* toxicology that aims to strengthen networks and foster scientific exchange between organizations and professionals. It promotes the regular exchange of information by organizing the well-established ESTIV Congresses, which takes place every two years at a location in Europe. ESTIV also organizes dedicated yearly workshops, joint activities with affiliated societies and publishes regular newsletter and the ESTIV website (http://www.estiv.org). In particular, ESTIV strives to attract and integrate young scientists.

Existing for over 20 years, ESTIV cooperates closely with various European scientific organizations, governmental bodies and industrial platforms to facilitate communication and encourage research and application and use of 3R alternative methodologies. In addition, ESTIV organizes international education and training courses dedicated to *in vitro* toxicology and aims to encourage and extend interest in *in vitro* toxicology in outreaching countries outside of Europe. "Toxicology *in Vitro*" is the official journal of ESTIV.

IX-1-769

Activities of EUSAAT, the European Society for Alternatives to Animal Testing

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Upon initiative of local animal welfare activists, in 1991 the first European congress on Alternatives to Animal Experiments was held at the University of Linz (Austria), sponsored by Austrian, German, and Swiss government institutions, industry, and private trusts. To ensure continuity in organizing the annual Linz conferences, in 1993, the Middle European Society for Alternatives to Animal Experiments was founded. Soon colleagues from neighboring countries joined (e.g., Czech Republic, Italy, Netherlands, Slovakia, Sweden). Therefore, in 2006, English became the official congress language, and in 2009, the society's name was changed to EUSAAT, European Society for Alternatives to Animal Testing – *the European 3Rs Society* (http://www.eusaat.org). During the last 20 years, the main topics of the EUSAAT conferences included ethical and legal issues of animal experimentation, *in vitro* pharmacology and (eco)toxicology, molecular modeling and education. EUSAAT has always maintained close cooperation with the European Commission and its services. In 2014, EUSAAT was accepted to the ECVAM Stakeholder Forum ESTAF. EUSAAT members have served internationally as co-chairs of world congresses on alternatives: Both co-chairs of WC9, Dagmar Jirova and Horst Spielmann, are EUSAAT members. EUSAAT has further established official cooperation with 3Rs societies outside Europe, such as ASCCT (USA) and JSAAE (Japan).

IX-1-901

Alternatives to animal experiments in Korea: Past achievements and present status of the Korean Society for Alternatives to Animal Experiments (KSAAE)

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The Korean Society for Alternatives to Animal Experiments (KSAAE) initially began as an informal study group among the concerned members from academia, industries, and government. It became a formal organization in 2007 through the efforts of many members. In the same year, it held WC-6 Seoul Satellite Symposium in Korea. Since the establishment of the KoreanCenter for the Validation of Alternative Methods (KoCVAM) in 2009, collaboration between KSAAE and KoCVAM has made a big progress in advancing alternatives in Korea. The Cosmetic Consortium, launched in 2013, is developing new alternative test methods for cosmetics such as eye irritation assay, skin sensitization assay and photosensitization assay. KSAAE members participate in international validation studies, Validation Management Teams (VMTs), and peer review panels led by member countries of the International Cooperation of Alternative Test Methods (ICATM). Also KSAAE and JSAAE agreed to exchange information mutually. In addition to chemical testing, alternative efforts were made in the areas of biological research. For example, the Korea National Institute of Health (KNIH) started a project to develop a new animal testing method with *in vivo* imaging tools to trace pathogens during infection. This can reduce the number of animals for the experiment of infectious disease. KSAAE will be continuing its efforts to promote both human health and animal welfare in Korea.

Session IX-1: Poster presentations

IX-1-159

The International Society for In Vitro Methods (INVITROM)

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Animal models have greatly advanced our knowledge of biological systems in many areas. Besides the ethical considerations, it is nowadays also recognized that they have major scientific limitations. Thanks to the continuous improvement and refinement of cell and tissue culture methods, the availability of (human) stem cells and in combination with advanced molecular techniques and the power of *in silico* analysis of large data sets, a new area for biomedical and toxicological investigations, now based on *in vitro* technologies, has emerged.

INVITROM (*the International Society for In Vitro Methods*) was founded to promote and support this new field of research and to create awareness and acceptance by the regulatory bodies.

INVITROM acts as a platform for discussion, contacts and co-operation for the enhancement and dissemination of *in vitro* technologies by organising annual workshops and joint symposia with related societies and by building an extensive network of experts (e-mail, website, LinkedIn).

INVITROM members believe in the strength of *in vitro* and *in silico* methodology in biomedical research and actively contribute to the improvement of these methods as new tools for expanding our knowledge in biomedicine.

Please visit the website to join and find information on INVITROM: http://www.invitrom.org

IX-1-198

'GUTS in the LAB' – Award for courageous scientists doing excellent 3R research

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European Directive 2010/63/EU as being implemented into national legislation of European member states, aims for more transparency in laboratory animal research and for the public to be better informed. Its ultimate objective is to fully replace animal use in science, industry and education through the promotion of 3R-alternative approaches.

In the Netherlands, since 2007, the Dutch Society for the Protection of Animals in collaboration with the Netherlands Knowledge Centre on Alternatives to Animal Use annually awards a prize to researchers who have shown remarkable courage in their lab, in applying the 3R's principle. Each year 4 or 5 scientists are nominated who tell about their research in a language that is comprehensible to laymen. The general public vote for their favourite candidate; the researcher with most votes is awarded the "Guts in the Lab"-award. The award is valued by both the scientific community and the public, as it bridges the gap between science and society. Transparent communication about the research is crucial, not only by the DSPA and NKCA, but also by and in close collaboration with the research institutes themselves. We hope our poster, describing the award, our strong collaboration and its past winners, will inspire many others.

IX-1-514

Science and awareness: a journey inside the 3Rs. Didactic exhibition project

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The exhibition project by IPAM, ECOPA affiliated, is intended as a journey to get to know the scientific value of the 3Rs, aimed at outlining the theoretical and practical evolution of research methods based on the principles of Reduction, Refinement and Replacement in various fields of biomedical research. It came into being from the idea of tracing a journey through time to show the gradual replacement of animals in testing, going on to predict a future in which highly advanced science will no longer have any reason to use them.

The exhibition consists of three sections – PAST, PRESENT and FUTURE. Each section has an introductory panel and panels dealing with specific topics. All the panels were produced by 25 experts in the various fields, who wrote the texts and provided illustrations.

A total of 30 panels have been prepared, dealing with subjects ranging from pharmacology to toxicology, legislation to didactics, animal housing to neuroscience and computer models to genomics.

The exhibition was designed and set up as an itinerant exhibition and will be on display in many universities and research institutes in Italy, starting off at the Rector's Office at La Sapienza University in Rome.

IX-1-718

COLAMA2012 – the first Latin American congress on alternatives

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The first Latin-American Congress on Alternatives to Animal Testing in Education, Research and Industry (COLAMA2012) was held in Niterói, Brazil as a joint initiative from the Brazilian National Network for Alternative Methods (RENAMA), the Postgraduate Program in Science and Biotechnology – Fluminense Federal University (PPBI/UFF) and the Brazilian Center for Validation of Alternative Methods (BraCVAM), supported by the National Institute for Quality Control in Health (INCQS/Fiocruz). COLAMA2012 aimed to create an opportunity in Latin America for gather researchers interested in 3Rs initiatives and, most of all, encouraging the development of new research groups on this field. The congress had 47 speakers and 226 attendees, in four different themes in alternatives related on humanities, 3Rs and training and education (in collaboration with InterNICHE and Latin American conference on Humane Education and Alternatives). Industry and academy were represented by more than a hundred works by participants from 9 Brazilian and 11 international companies, and institutes (from Cuba, Argentina, France, USA, Monaco, Germany, England, and others) and 26 institutes of education. One of the most striking results of COLAMA was the creation of the Brazilian Society for Alternative Methods. The second edition is programmed to be held at CUBA, 2015.

Session IX-2: Animal welfare implementation across the world

Co-chairs
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Troy Seidle, Humane Society International, Canada

Session IX-2: Oral presentations

IX-2-155

Is international harmonization of animal care and use standards progressing as hoped?

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Several international guidance documents have been published that suggest convergence of opinion as to appropriate program infrastructure, ethical oversight, as well as practices and procedures across country borders. For example, the OIE has published a chapter in its Terrestrial Animal Health Code that specifically addresses the care and use of research animals. One hundred and seventy-eight countries/territories are members of the OIE and thus have pledged to implement those standards. In partnership with ICLAS, CIOMS has updated its international guiding principles regarding the use of animals in research. In addition, numerous countries' regulatory frameworks and guidance documents urge implementation of the 3Rs. However, the rate of uptake of these standards at the institutional level is difficult to assess on a worldwide basis. AAALAC International is in the unique position of having an in-depth understanding of the status of animal care and use programs around the world. Thus, AAALAC has a bird's-eye view of the progress being made globally in harmonizing animal care and use standards. The status of this international harmonization based on this extensive experience will be described.

IX-2-488

What has changed for animal welfare in Europe?

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Animal welfare cannot be advanced by any single player. Real progress needs input and collaboration by all involved – be it animal users, animal welfare NGOs, scientists, and regulators at national, regional and international level as well as politicians.

Within the EU, the attitudes of different players in the policy scene in relation to animal welfare have shifted significantly since the late 1990s. From a typical "our side – their side of the meeting room" – approach with hardly any common ground to a collaborative productive partnership where all parties recognise the need to work together to make progress.

There are a number of contributors to this change in approach – some to do with a more general awareness of ethical issues, an increased understanding of animal needs and changes in approach to and purposes for which animals are used in the advancement of science; some linked to the economic situation and some driven by other policy changes.

By engaging actively all those parties with an interest in the use and care of animals used in scientific progress, much common ground has been found, enabling improvements in welfare without compromising scientific progress.

IX-2-728

CONCEA – Brazilian Council to Control Animal Experimentation

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Brazilian' Law regulates the use of animals in Brazil since 1,934 (Decree 24,645/1934 and Law 5,197/1967). However, the first Law aiming to regulate the use of animal for teaching and research activities was proclaimed in 1979 (Law 6.638/1979), although lacking enough details for implementation. In 1998, the Law 9,605 for environmental crimes was the first to consider crime animal experimentation when alternatives are available. The legislation was greatly improved with the Law 11,794/2008 (http://www.mct.gov.br/index.php/content/view/313144/Leis.html, known as Arouca's Law as tribute to Deputy Sergio Arouca, creator of the Law) and the Decree 6,899/2009 (http://www.mct.gov.br/index.php/content/view/313152/Decretos.html), creating the National Council on Animal Experimentation – CONCEA, establishes procedures for the scientific use of animals, organizes a "National System" of Animal Experimentation through a national Register of Institutions Conducting Animal Experimentation (CIUCA), determine the Concea competence to monitor and evaluate the introduction of alternative methods that replace the use of animals in teaching and research according to the 3R's principles. Representatives of four Ministries, Academic sector and two representatives from Animal Protection Societies constitute the council. During the last three years, directives (http://www.mct.gov.br/index.php/content/view/313178/Resolucoes_Normativas.html) concerning anesthesia, euthanasia, minimal infrastructure to produce, maintain, and use animals for teaching or research have been produced considering global best practices.

IX-2-760

A scientific approach to animal replacement policy

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The legal mandate to fully apply the 3Rs, including replacement, in biosciences research has existing in the European Union since the 1986 Animal Experiments Directive, and is becoming a common fea-

ture in new and revised animal welfare legislation worldwide. Yet a disconnect remains between this policy obligation and its implementation. Factors may include an over-emphasis on decision-making at the institutional level versus at the level of research funding bodies, and a failure to overcome a perceived schism between the objectives of advancing human health and avoiding animal use.

This presentation will explore the extension of the "adverse outcome pathway" (AOP) paradigm from toxicology into biosciences research as a science-based approach to the examination of human disease processes, from molecular to individual and population levels, linking environmental and genetic causes of disease via pathways at the cellular levels, through organ systems, to disease outcomes. The AOP paradigm would support a shift of focus away from animal models towards human-biology-based tools, such as patient-derived pluripotent stem cells, genome-wide association studies, computational systems biology, microfluidic chips, etc. Targeted research investment along these lines would, first and foremost, benefit human health sciences, while also addressing the replacement policy mandate in a meaningful way.

IX-2-846

InterNICHE activity in CIS countries and Iran: review and reflections

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InterNICHE has been active in countries of the Commonwealth of Independent States (CIS) since 1995 and in Iran since 2010. InterNICHE National Contacts and Partner organisations have worked with teachers, students, campaigners and others to introduce alternatives and replace harmful animal use. This presentation will describe the strategies employed to facilitate this curricular change within medical, veterinary medical and biological science education and training. These include organisation of outreach tours, seminars, training and exhibitions, establishment of Alternatives Loan Systems, translation and distribution of alternatives, signing formal agreements with universities for replacement, and securing media coverage. Despite significant obstacles, achievements have included replacement of the annual use of over 60,000 animals, and widespread awareness of alternatives in some countries. The positive results reflect teachers' acceptance of the pedagogical, ethical and economic advantages of humane education and alternatives, and a growing understanding of the potential of technology to support the learning process. They also demonstrate the opportunities for collaborative action in countries with challenging socio-economic realities.

IX-2-888

The state of animal welfare implementation in the United States from the perspective of the US National Academy of Sciences

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In the United States, animal use in research is governed by two distinct but overlapping legal entities. Provisions to safeguard and advance animal welfare are present in both but philosophical and practical differences distinguish the two systems. As part of session 9.2, this talk will discuss the state of animal welfare implementation in the United States, efforts to improve it and provide some thoughts on the goal of obtaining global consistency and best practices.

Session IX-2: Poster presentations

IX-2-056

3Rs dissemination in Asia: zebrafish embryo toxicity test transferred in Sri Lanka's universities

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Laboratory animal testing are accountable for at least 80 millions laboratory animals used each year worldwide. The ban on animal testing for cosmetics in Europe created a momentum. Since the enforcement of the ban, other countries took similar initiatives. Even by acknowledging that a lot of work still needs to be done, it is worth being mentioned that know how and confidence in validated and regulatory accepted alternatives to animal testing models for the last thirty years is robust. One should consider that those 3Rs models are ripe to be spread across the EU borders.

Under this scope, this presentation will describe the transfer of the regulatory accepted Zebrafish embryo toxicity test at Sri Lanka's universities. Besides being regulatory relevant for 3Rs (OECD TG 236, ISO 15008), Zebrafish is a native species in Sri Lanka and a low cost toxicity model. During the talk, the audience will learn about the obstacles, the lessons learned and the required preparation to ensure success of the establishment of a Zebrafish unit facility in Sri Lanka at Uva Welassa University for toxicity testing with Zebrafish embryos. The take home message will be that similar initiative can be repeated elsewhere with any appropriate models.

IX-2-088

Animal welfare implementation in Switzerland: a successful stakeholder approach based on a joint Animal Welfare Charter

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Animal research is a highly emotive topic. Many people are critical of animal experiments but at the same time are demanding pharmaceutical products that meet the highest quality and safety standards. While it is common for pharmaceutical companies to have internal standards for animal research, no country has previously succeeded in aligning and extending them across a national industry. After identifying four key elements, (a) open and constructive stakeholder dialogue, (b) foster education and training, (c) promote all aspects of 3R and (d) audits and certification, the 10 articles of the Animal Welfare Charter were developed. The commitments of the charter are not only applied within the companies but are also valid for all external research and development partners – on a global level (i.e. in countries with legal requirements below the Swiss or European standard or with no animal welfare acts in place). Based on the charter, industry working groups were built and collaborations with different stakeholders such as animal rights organizations and the academia have been established. The annually published report provides an overview of company initiatives and achievements. It gives proof that the commitments are sustainable and lead to constant progress in favor of the animals.

IX-2-091

5 years InVitroJobs – internet platform, job board and working group network for animal-free research

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The bilingual portal InVitroJobs is a project of the German Federal Association of People for Animal Rights that has the goal of promoting modern research without animal use. The development of new suitable methods is becoming increasingly complicated and their understanding demands an increasing degree of scientific expertise. The platform InVitroJobs provides pertinent background reports on the path from research and development to acceptance and inclusion in test guidelines and want to illustrate how and why a particular method may not yet be adequate, what is lacking and what demands must be met to facilitate development and financial support.

Young researchers and interested parties can get a quick overview of working groups dealing with animal-free research, vacancies and thesis. We regularly publish news on research results obtained without animal testing. Under the heading "Working group – a portrait" we introduce scientific teams and companies, and discuss current developments in detail. In 2013, major topics were advantages and disadvantages of primary cultures, human-specific cell culture systems and cell lines. In the future, relevant potential developments in this area could help to avoid the killing of many animals for organs or tissue samples, particularly in pharmaceutical research and the development of vaccines.

IX-2-145

Use of animals in military medical training by NATO nations

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Among the member nations of the North Atlantic Treaty Organization (NATO), a variety of training methodologies – including high-fidelity patient simulators, moulage scenarios, task trainers, didactics, and live animal laboratories– are used to prepare military personnel to treat injured civilians and soldiers. For ethical, educational, practical, legal, and economic reasons, the propriety of animal use for this purpose has come into question.

Our survey of NATO nations shows that 23 of 28 NATO nations do not use any animals for military medical training. The United Kingdom, United States, Norway, Denmark, and Canada continue to use thousands of pigs, goats, and sheep each year for emergency medical training drills in which live animals are stabbed, shot, burned, and otherwise harmed.

These exercises persist despite the various nonanimal training methods available and these countries' regulations requiring the use of alternatives to animals when they exist. Indeed, the few nations still using animals have acknowledged through NATO that the practice has come under "significant scrutiny" and may need to be "completely eliminated."

This presentation discusses the survey results, attendant scientific and legal issues, and recent developments in efforts to curb animal use in Europe and North America.

IX-2-328

A new guideline about euthanasia to laboratory animals in Brazil

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A new guideline about euthanasia applied to laboratory animals has been created in Brazil. Some methods which have been used up to last year are not permitted anymore. The focus of this work is to introduce the methods approved nowadays in Brazil in order to induce the euthanasia in rodents, amphibians, lagomorphs, birds, pigs and fish. In order to proceed these studies, we were based on the new Brazilian law to describe the methods approved to induce euthanasia in laboratory animals. For all animals previously mentioned the chemical methods with barbiturates or anesthetics are approved. Physical methods are approved only with restriction and only when we cannot use any chemical method. Carbon dioxide (CO_2) can only be used for rodents and birds when a chemical method cannot be used. Carbon monoxide (CO) and ether are prohibited. These results demonstrate that Brazil has changed the law on euthanasia and recital based on the ethical principles of the 3RS. The methods that are approved and recommended are chemists and the use of physical methods are used only with restrictions and some as CO and ether are forbidden.

IX-2-500

Legislation and implementation of alternative methods and outcomes

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The well-known 3R (reduce, replace, refine) concept was laid down by Bill Russel and Rex Burch in 1959. Now, on the next century we have reached to the stage we possess not only the ideas and soft law derived from guidances but international hard law with the examples of EU Regulation (REACH), EU Directive 2010/63/EU and European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of Council of Europe. Among the significant sanctions the mandatory stipulation to reduce, enhance and promote the alternative methods to reduce animal tests is assured. Maximising access to public legal information for all participants is part of the harmonisation and deserves common heritage of humanity.

In this paper the implementation of hard and soft law on 3 R in different aspects will be studied with the attention to regional pharmacopoeias and other relevant international institutions. Substantial case studies will be presented. In conclusion the support to intensive functional network instead of fragmented protectionist policy is expressed.

Acknowledgements: European Social Fund´s Doctoral Studies and Internationalisation Programme DoRa.

IX-2-604

The transposition of directive 2010/63/EU on the protection of animals used for scientific purposes – a comparison between France, Germany, the United Kingdom and Austria

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A comparison is drawn of the recent transposition of directive 2010/63/EU on the protection of animals used for scientific purposes, in the three major animal testing EU member states France, United Kingdom and Germany, as well as Austria, the author's country of residence. First, a few flaws in the directive's wording itself are pointed out, concerning for example the prospective evaluation of procedures, including the classification of severities. Subsequently, the different styles and emphases of the four transpositions are lined out, illustrated through the contrasting of a few particularly interesting articles of the directive with the wording of the corresponding national legal texts. Thus, the respective modalities for project evaluation, retrospective assessment, national animal protection committees as well as the differing penalties for non-compliance are described. Most notably, the UK has, for numerous rules laid out by the directive, not taken the opportunity to grant exceptions for scientific reasons, certain procedures or certain establishments. Austria has, but not in all allowed cases; whereas Germany and France have widely – emphasising clearly the facilities for research. France, for some articles, has not even transposed the directive correctly. Resuming, some possible consequences of the different wordings in transposition are lined out.

IX-2-790

3R work structured according to Lean Sigma results in increased refinement and reduction in toxicity testing in the pharmaceutical industry

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The aim of the project was to study effects of structured work with continuous improvements on the principles of the 3Rs in a large toxicology research unit. Would structured work according to Lean Sigma improve 3R results or would it hamper creativity? A specialized Refinement Team was set up in 2008, and a cross-departmental 3R Team was set up in 2009. Both teams worked with continuous improvements according to Lean philosophy, based on engaging all levels of staff. Implemented 3R ideas were mapped 2006-2011 and compared according to number of implemented ideas as well as cross-departmental collaborations for each R. The total number of implemented 3R ideas was 55 on Refinement and 39 on Reduction. The number of implemented Refinement ideas per year increased by almost five-fold after implementation of the Refinement team. Increases in cross-departmental projects were seen after implementation of the 3R Team; from 10% to 42% for refinement and 28% to 67% for reduction. The present study shows that Lean Sigma and continuous improvement can increase creativity and create a structured process of implementing 3Rs into toxicological research, as measured by increased number of implemented 3R ideas and increased levels of collaborations.

IX-2-806

Alternatives in South Africa: initiatives in education, research and testing

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South Africa's first series of workshops and seminars on alternatives was organised in 2012 following collaboration between InterNICHE and the NSPCA. Two successful workshops with international speakers were held in Pretoria. The first introduced replacement alternatives in education and training, and a multimedia exhibition provided hands-on experience. Alternative laparoscopic surgery training was demonstrated using a perfused ethically sourced animal cadaver. The second introduced the 3Rs in research and testing, with a focus on replacement. Legislation, ethics committees, the use of sentient animals in fundamental research, information retrieval, funding of R&D and validation of alternatives were addressed. Delegates from all relevant fields were present at the workshops, which were followed by seminars in universities across the country. The NSPCA/InterNICHE Alternatives Loan System, a South African library of learning tools, was also established. The events were significant for introducing the concept of alternatives in the country, and contributing to NSPCA guidance work within schools, universities and animal ethics com-

mittees. Alternatives in research and testing are increasingly being considered. Replacement of dissections and animal experiments with alternatives is ongoing, and most universities have now replaced the majority of the 2000 animals that each would use annually for comparative anatomy classes.

IX-2-821

India takes a giant step forward to protect dogs from testing, with more emerging evidence that dogs add no additional value in toxicity testing

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With repeated scientific evidence emerging that dogs provide no additional value in toxicity testing, there have been series of international discussions and publications on the pertinent question "Should dogs be put through the pain of experimentation?" (Hasiwa et al., 2011; Zurlo et al., 201; Turner, 2011) Ethically, there has been a societal demand for decades, calling for a ban on the testing on dogs on the premise that dogs are companion animals, deeply sensitive and intuitive. An analysis of dog toxicity studies showed that in 92% of the studies, safety data added no more relevant information to that provided by the rat, and the other 8% did not result in the withdrawal of drugs from development, indicating that dog studies are not required for the prediction of safe doses for humans (Broadhead, 1999). Recently, a robust analysis of the value of dogs for predicting drug toxicity, based on more than 2300 drugs, showed that a negative toxicity result in dogs added no evidential weight to the probability that a drug may not be toxic in humans (Bailey et al., 2013). In light of the above, India has taken a bold step, with the CPCSEA requesting the Drug Controller General of India to look closely at the use of alternatives to dogs in testing.

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Session IX-3: Activity updates from international validation centres

Co-chairs

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Session IX-3: Oral presentations

IX-3-017

JaCVAM update

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The Japanese Center for the Validation of Alternative Methods (JaCVAM, http://jacvam.jp/) was established in 2005 to promote the use of alternatives to animal testing in regulatory studies, thereby replacing, reducing, or refining the use of animals, according to the Three Rs principles. JaCVAM assesses the utility, limitations and suitability for use in regulatory studies, of test methods needed to determine the safety of chemicals and other materials. JaCVAM also organises and performs validation studies of new test methods, when necessary. In addition, JaCVAM co-operates and collaborates with similar organisations in related fields, both in Japan and internationally, which also enables JaCVAM to provide input during the establishment of guidelines for new alternative experimental methods. These activities help facilitate application and approval processes for the manufacture and sale of pharmaceuticals, chemicals, pesticides, and other products, as well as for revisions to standards for cosmetic products. In this manner, JaCVAM plays a leadership role in the introduction of new alternative experimental methods for regulatory acceptance in Japan.

IX-3-293

EURL ECVAM strategy to avoid and reduce animal use in acute systemic toxicity

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Assessing chemicals for acute systemic toxicity represents a standard information requirement within several pieces of EU chemicals legislation. Classification and labelling is the main purpose of conducting the test. Currently, only *in vivo* tests are accepted by regulatory bodies and cytotoxicity assays are recognised simply as additional tests that can be used for estimating the initial doses for tests *in vivo*. The development of mechanistically-based alternative methods and strategies in this area is still hampered by the limited understanding of the key toxicity pathways in humans. The EURL ECVAM strategy is based on the state-of-the art in the area, including recent and ongoing efforts. Available data indicates that the 3T3/NRU cytotoxicity assay can be used to support the identification of non-classified substances, although results should always be used in combination with other information sources to build confidence in the decision not to classify a substance for acute oral toxicity. EURL ECVAM is therefore focus-

ing its in-house activities on the better use of *in vitro* and *in silico* methods, and on exploring the usefulness of existing data from other systemic toxicity studies. EURL ECVAM will also continue to support activities aimed at the refinement of relevant *in vivo* studies.

IX-3-388

EURL ECVAM's approach to the global acceptance of alternative methods

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With the adoption of EU Directive 2010/63/EU on the protection of animals used for scientific purposes, ECVAM became the European Union Reference Laboratory on Alternatives to Animal Testing (EU-RL ECVAM). Its key responsibilities are to coordinate and promote the development and use of alternatives; coordinate the validation of alternative approaches at EU level; act as a focal point for the exchange of information on the development of alternative approaches; set up, maintain and manage public databases and information systems on alternative approaches and; promote dialogue between legislators, regulators, and all relevant stakeholders in view of the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.

This presentation will describe how EURL ECVAM responded to these key provisions by streamlining its validation workflow, ranging from test submission assessment and prioritization, over validation and peer review to publication of EURL ECVAM recommendations and leading projects on alternative test methods at OECD level. It will explain how regulators, stakeholders, international partners and test method users are involved in the process in a structured and systematic way. The ultimate aim is to facilitate and speed up the European and International regulatory acceptance, global recognition and use of standardised test methods and approaches.

IX-3-560

A new vision and direction for ICCVAM and NICEATM

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The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is reorienting itself to better address advances in toxicological science while reflecting the needs and priorities of its partner regulatory agencies. ICCVAM includes representatives from 15 U.S. agencies that generate or use toxicological testing information. It promotes scientifically valid test methods that better protect human health and the environment while advancing the 3Rs. In 2013, ICCVAM initiated an effort to (1) explore new paradigms for validation and utilization of alternative toxicological methods, (2) identify areas of scientific focus, and (3) improve communications with the public. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which provides support for ICCVAM, is broadening its scope to support the National Toxicology Program and the Tox21 interagency consortium. Specific programmatic changes are described herein.

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IX-3-570

Alternative methods to animal experiments – ongoing activities in Germany

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Approximately a quarter of all animals used in the EU for scientific and regulatory purposes is used in Germany. This reflects Germany's traditionally active role in basic and applied life science research, and in the development, safety assessment and quality control of medical, industrial and agricultural products and devices. The German government has early recognized the need to support the development of alternative methods to animal experiments according to the 3R principles (replacement, reduction and refinement) by Russel and Burch (1959). Since the 1980s, research projects intending to develop alternative methods were funded with a total volume of about 150 million Euros. In 1989, the National Centre for the Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) was founded at the BfR to pursue the 3R goal by supporting the development and validation of alternative methods, advising expert panels, and providing a forum for information and education. In addition, the new legal tasks derived from the Directive 2010/63/EU have been assigned to the BfR. At the same time industry and NGOs have continuously driven forward the implementation of the 3Rs in animal experiments with scientific and regulatory purpose. An overview of ongoing activities in Germany will be given.

IX-3-619

Activities of Korean Center for the Validation of Alternative Methods (KoCVAM) to promote alternative test method in Korea

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The Korean Center for the Validation of Alternative Methods (KoCVAM) was established within the National Institute of Food and Drug Safety Evaluation (NIFDS), which is a part of the Ministry of Food and Drug Safety (MFDS), in November 2009 under the Laboratory Animal Act of 2008. The mission of KoCVAM is supporting policies on the development and approval of alternative methods, coordinating validation studies and peer reviews, and providing recommendations to regulatory authorities. Furthermore, KoCVAM promotes cooperation among domestic and international organizations, and provides information and education on alternative methods. In March 2011, KoCVAM joined the

International Cooperation on Alternative Test Methods (ICATM) and has, since then, been collaborating with ICATM partners. KoCVAM has recommended 9 alternative test guidelines on Cosmetics, including RHE test method, and MFDS has accepted all of them. From 2013, KoCVAM launched a Consortium of Alternative Methods for Safety Evaluation of Cosmetics which aims at developing and validating new alternative test methods for safety assessment of cosmetics in the areas of eye and oral mucosal irritation, skin sensitization and photosensitization. KoCVAM will continue working on alternative test methods in collaboration with different national and international organizations to improve public health and animal welfare.

IX-3-867

The Brazilian Centre for Validation of Alternative Methods (BraCVAM) and the establishment of the validation process in Brazil

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The need for creating BraCVAM arose in 2008 and, immediately, members of academia, industries and validation centers engaged this idea. In 2012, cooperation between Oswaldo Cruz Foundation (FIOCRUZ) and the Brazilian National Agency of Health Surveillance (ANVISA) started the establishment of BraCVAM, created in 2013. The Brazilian validation process will follow the OECD Guideline 34 where BraCVAM will identify methods for entering the validation process and/or receive requests from test submitters. BraCVAM will inform the Brazilian National Network on Alternative Methods (RENAMA) about promising assays, which will in turn prioritize and contribute to the validation study of the selected assays. The validation study will be supervised by a Validation Management Group, and the obtained results will be peer-reviewed by an ad-hoc Scientific Review Committee, organized under the auspices of BraCVAM. Based on the peer-review outcome, BraCVAM will prepare recommendations on the validated test method and send these final recommendations to the National Council for the Control of Animal Experimentation (CONCEA). CONCEA will in turn be in charge of the regulatory adoption of the validated test methods in Brazil following an open public consultation.

Session IX-3: Poster presentation

IX-3-870

The Brazilian Center for Validation of Alternative Methods (BraCVAM) and the National Network on Alternative Methods (RENAMA): the first years of activities

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Animals are still used in Brazil for many purposes but the Law 9,605/1998 and the Law 11,794/2008 state that when there is a validated alternative method, animals are not allowed to be used for the same subject. Although BraCVAM was proposed in 2008 (Presgrave, 2008) and its organization was subsequently described (Eskes et al., 2009; Rivera et al., 2010), its creation only occurred in 2013. In 2012, the National Network on Alternative Methods (RENAMA) was created. In the same year, the Ministry of Science, Technology and Innovation (MCTI) and the National Council on Research (CNPq) published the first specific calling for funding alternative studies. Ten laboratories received fund for introducing already validated assays and one laboratory was funded for developing a "Brazilian" reconstituted human skin model, since, due to the expiration period, it is very difficult for Brazilian labs to import this kit. The way of working is being designed so that RENAMA organize all the studies, BraCVAM recommends the approval of the study and the Council for Controlling Animal Experimentation (CONCEA) becomes the test officially accepted in Brazil.

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IX-3-895

In vitro laboratory GLP-certified in Brazil: a successful public-private partnership to achieve international harmonization

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The boost of the *in vitro* methods, to meet global ethical and regulatory requirements that head nonclinical safety and toxicology stud-

ies, has resulted in an increased need to use of accreditation programs in Good Laboratory Practice (GLP), which determinates the reliability and relevance in the assessment's safety for *in vitro* trials.

On 2014, the Laboratory of In Vitro Assays CB-BIOSINTESIS installed on IPEN-USP, in Brazil, received the Certificate of GLP Conformity, as the first GLP test facility in country with a compliance statement for "In Vitro Toxicity", "In Vitro Biocompatibility" and "Cell Viability".

This initiative are according with most advanced programs of OECD countries to reduce uncertainty of data, increase the normalization of standard operating procedures (SOPs), quality control systems, safety procedures, records and reporting applied for *in vitro* studies.

The aim of Brazil, and other emerging markets, to an *in vitro* GLP-compliant infrastructure within international requirements and higher standards is fundamental to establish international harmonization and guarantee confidence, accuracy and integrity of data for *in vitro* studies produced by laboratories at different countries.

Session IX-4: Novel approaches to validation

Co-chairs
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Session IX-4: Oral presentations

IX-4-634

Systematic reviews of test method performance: a case study using the Zebrafish Embryo Test for developmental toxicity

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Systematic reviews, with their emphasis on transparency, objectivity, and consistency, provide a framework that can be adapted to assessing the literature on the performance of alternative test methods or testing strategies, a process that can be loosely likened to retrospective validation. The Evidence-based Toxicology Collaboration (EBTC) (http://www.ebtox.com) is pioneering this application of systematic reviews, translating the Cochrane Collaboration's guidance for conducting systematic reviews of diagnostic test accuracy in medicine to the toxicological context (Cochrane Collaboration, 2014; Hartung, 2010; Stephens et al., 2013). Our case study assesses the performance of the Zebrafish Embryo Test (ZET) in predicting the results of prenatal developmental toxicity tests in rats and rabbits, as typified by the Organization for Economic Cooperation and Development's Test Guideline 414 (Selderslagh et al., 2012). We have written a protocol that describes the various steps to be taken in our systematic review, such as the literature search, eligibility determination, data extraction, and risk-of-bias assessment. An overview of our approach, the protocol, and its implementation via a pilot study will be provided. While our primary aim is to assess the ZET's performance vis-à-vis the established mammalian tests, we are also seeking to operationalize systematic review methods for the toxicology domain.

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IX-4-713

Evolving validation practice to meet the demands of predictive toxicology

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For validation to be a key enabling factor in the acceptance of alternative methods for regulatory use, the principles, purpose and process underlying a validation study need to be carefully considered to ensure that expectations of decision makers are properly met. The validity of a method or approach can be established in a variety of ways but ultimately the aim is to demonstrate the reliability and relevance of the data generated while describing the associated prediction uncertainty. Although reliability assessment is somewhat of a technical task, the relevance aspect has become increasingly challenging due to the fact that prediction of more complex toxicity endpoints relies on the optimal combination of multiple complementary methods. Moreover, with the emergence of mechanistic frameworks such as Adverse Outcome Pathways (AOP), the relevance of a method is often more related to its ability to capture one or more Key Events of an AOP rather than its ability to predict an apical *in vivo* effect. However, validation practice is evolving to meet the demands of predictive toxicology and solutions lie in the innovative use of the same tools and thinking behind the new paradigm for safety assessment.

IX-4-799

The role of European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) towards internationally accepted harmonised *in vitro* method standards

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In cooperation with EU Member States and in response to provisions of Directive 2010/63/EU on the protection of animals used for scientific purposes, EURL ECVAM has recently established the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL, http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eu-netval). Its mission is to provide support for EURL ECVAM validation studies that serve to assess the reliability and relevance of alternative methods that have a potential to replace, reduce, or refine the use of animals for scientific purposes. The network aims to increase Europe's validation capacity. The first pilot project of selected EU-NETVAL test facilities is the AR-CALUX validation study to support the development of an OECD performance-based test guideline and associated performance standards for Androgen Receptor Transactivation Assays (ARTA) for the detection of compounds with (anti)androgenic potential. Future tasks for EU-NETVAL will be the generation of high quality data based on currents best scientific practices for *in vitro* methods targeting EURL ECVAM's priority areas. To accelerate actively test submissions for these priority areas, EURL ECVAM identifies *in vitro* methods using test submission e-survey tools and subsequently defines the necessary efforts towards internationally accepted harmonised *in vitro* method standards.

IX-4-854

Regulatory acceptance of 3R testing approaches for medicinal products

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Regulatory testing of medicinal products is carried out to support first administration to humans or to target animal species; before carrying out clinical trials in larger populations; before marketing authorisation and to control quality during production.

In line with Directive 2010/63/EU, the 3Rs are embedded in the drafting process of regulatory guidance (European and (V)ICH). Regarding non-clinical testing for human medicinal products, new 3R methods have been accepted via multiple and flexible approaches, either as pivotal, supportive or exploratory mechanistic studies.

The recently approved ICH guidelines, ICH M3(R2) and ICH S2(R1) are good examples in this respect. Current efforts related to the revision of ICH S1 and ICH S5 illustrate ongoing work.

Although regulatory acceptance of 3Rs is possible, a formal process has been lacking and implementation of new test methods in routine regulatory testing has sometimes proven problematic. Therefore, the EMA JEG 3Rs drafted a Guideline on regulatory acceptance of 3R testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012) that clearly defines regulatory acceptance and provides guidance on the scientific and technical criteria for regulatory acceptance of 3R testing approaches, including a process for collection of real-life data. Pathways for regulatory acceptance and a new procedure for method submission and evaluation are described.

IX-4-925

Validation and qualification of new *in vitro* technologies for drug development

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Over the past two years, the American Institute for Medical and Biological Engineering and the National Institutes of Health have held a series of workshops on Validation and Qualification of New *in vitro* Tools and Models for the Pre-clinical Drug Discovery Process. The overall goal of this series of workshops is to develop guidelines for investigators developing new technologies for the drug development process on how to validate these new technologies so that they become useful, meaningful tools. Specific emphasis has been on model systems, such as "organs on a chip", that may augment existing models, especially animal models, in the US Food and Drug Administration drug approval process. The workshops have mainly been focused on drug toxicity evaluations with new *in vitro* systems but have also addressed efficacy issues, not only for pre-clinical drug development, but also for use during clinical trials and potentially in lieu of clinical trials for diseases with small populations of patients. A summary of the outcomes of the workshops will be presented.

IX-4-939

Alternative toxicological methods for drug safety assessment in China

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Bringing together the recent progress in alternative toxicological methods in China, this presentation introduced the development and validation of replacement, reduction, and refinement alternatives (the 3Rs) to animal testing for drug safety assessment. Current, in China, zebrafish was a model under validation for early screen of reproductive/developmental toxicity and neurotoxicity testing. In carcinogenicity, Bhas 42 cell transformation assay was validated with intra-laboratory and within-laboratory and has being used for the early toxic test of Chinese traditional medicine (TCM). Also, embryonic stem cell test, micro mass test, and whole embryo culture were validated and recommended to be used for early test for developmental toxicity. A computational toxicological model is under developing by China FDA to predict TCM toxic, especially for injection products. Also, in this presentation it addressed what has been accomplished thus far in developing acceptable alternatives to traditional animal toxicological assessment and provide potentially new initiatives in China, including the use of stem cell. Finally, the abstract discussed regulatory acceptance of alternatives in China.

Session IX-5: Regulatory acceptance of alternatives

Co-chairs
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Derek Knight, ECHA, Finland

Session IX-5: Oral presentations

IX-5-107

Regulatory acceptance and use of the Extended One Generation Reproductive Toxicity Study within Europe

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Two-generation study (OECD TG 416) is the standard procedure within REACH to test reproductive toxicity effects of chemicals with production volumes >1000 tonnes and encounters ethical, economical and scientific objections. The Extended One Generation Reproductive Toxicity Study (EOGRTS) was incorporated in the OECD test guidelines in 2011 (OECD TG 443) and thereby became an internationally validated method. This protocol reduces animal use for reproductive testing by about 40% and is far more informative. However, its regulatory acceptance within Europe is a challenging process. This research describes the factors influencing the three stages of regulatory acceptance and use of the EOGRTS, using literature research and expert interviews. The stage of Formal Incorporation into the OECD was stimulated by retrospective analyses of the F2 value, strong advocates and the push of US and EU chemicals legislation. The Actual Regulatory Acceptance within REACH is withheld by several legal, economic and scientific factors. The Use by Industry lingers due to uncertainty about the regulatory acceptance, costs and manageability of the EOGRTS. The existing debate is fed by two opposing frames i.e. the frame of precautionary and the frame of innovation. The 4C's of commitment, communication, collaboration and coordination are vital to enhance the process.

IX-5-425

Promote the use and outspread of alternative methods in China through standardization and administration acceptance

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The development of 3R alternatives in China is full of conflicts and hope. The lack of national 3Rs regulations meets increasing conflicts with industries' needs. The slow popularization of 3Rs concepts and alternative technology in China makes it difficult to keep pace with the rapidly-developing modern bioscience. The absence of national level coordination mechanism also restricts the 3Rs development. Under this background, the major governmental department AQSIQ system, takes the leading position in promoting understanding and acceptance, effectively moves forward the alternative methods across the country. AQSIQ has accepted and implemented more than 20 alternative-testing standards. The National Standardization Administration also adopted some *in vitro* methods from OECD guidelines. The China National Accreditation Service for Conformity Assessment has accelerated the inter-laboratory transfer and laboratory network construction process of alternative methods. AQSIQ also released the validation guideline according to international standards and accomplished the first multi-center validation of the *in vitro* skin-irritation-test in China. Actively driven by AQSIQ, with the benefits from international communication and cooperation, through education and training, the China Food and Drug Administration panel also puts forward a five-year planning advice regarding alternative methods. China is now in a new stage of development towards 3Rs and alternative methods.

IX-5-652

Can the ADAPT principles help regulatory authorities implement the 3Rs?

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Within the regulatory sector there are a number of stages after validation that must be passed before an alternative method can be considered to have replaced a specific animal test. Failure by regulatory bodies to recognise and take responsibility for each stage has, in our opinion, been a major reason for some of the delay in the implementation of methods replacing acute toxicity, skin irritation, pyrogenicity and reproductive toxicity amongst others.

We have created the ADAPT checklist for regulatory bodies to ask themselves to ensure alternatives are not being unnecessarily delayed. Assessment -does the body have a proactive mandate to assess the suitability of new methods for their sector? Decision -who takes responsibility for deciding whether an alternative method is suitable? Acceptance -have all bureaucratic hurdles to acceptance such as the need to revise guidance and/or legislative text been identified? Policing -are there mechanisms in place to monitor the use of alternatives and will action be taken if animal tests are done unnecessarily? Transparency -does the authority inform all stakeholders of their actions at each stage?

In this presentation we provide examples of each ADAPT stage where alternatives have struggled and what action should be taken.

IX-5-671

Possible roles for non-standard methods in the REACH registration

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The various regulatory schemes for chemical and other specific products have their own data requirements: one illustration is the EU REACH Regulation for chemicals. The registrant can "adapt" the standard information requirements under REACH, and use other information instead: non-standard or non-GLP studies, *in vitro* studies, human epidemiology data, information from structurally-related substances (i.e. "read-across" and "chemical categories"), predictions from valid (Q)SARs and use of the weight of evidence (WoE) approach. Such non-standard information has to be equivalent to the standard studies, in that the key results from the standard method should be addressed and the result must be suitable for adequate risk assessment and classification. There is an R&D need to develop rational combined approaches for integration of tests/data/predictions into ITSs & IATAs & "test batteries" for use by industry and regulators in assessing the properties of substances. For example the JRC, with steering from ECHA, are working on a flexible "framework" Integrated Assessment Strategy for skin sensitisation to apply the OECD AOP by means of "assessment blocks" for the Molecular Initiating Event and the Key Events. Each "block" will be populated with a selection of assays and *in silico* assessment tools in a flexible manner.

IX-5-797

Regulatory acceptance of methods intended to become OECD Test Guidelines

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OECD countries have tasked the Environment, Health and Safety Program to develop harmonized methods for testing chemicals. The methods are intended to generate valid and high quality data to support regulations in member countries. The regulatory acceptance is an ultimate step leading to their full implementation. Other upstream factors come into play, namely a balance between different policy mechanisms that enable the development of the alternative methods. In Europe, the regulatory framework for cosmetic products aims at striking the right balance between different policy mechanisms that facilitate the regulatory acceptance of non-animal methods (e.g., joint public-private investments in research; changes in the regulation preventing use of animals setting time pressure to get valid test methods). The validation process has been applied to filter methods of sufficient relevance, reliability and predictivity. In recent examples of OECD Test Guidelines, the regulatory acceptance has only been possible when protection of human health was not jeopardized. For more complex endpoints, the task will no doubt be harder. More work is needed to understand toxicity pathways, build integrated approaches to testing and assessment, in supplement to rigorous validation, in order to provide the context under which alternatives to animal testing can be safely applied.

Session IX-5: Poster presentations

IX-5-478

Read across strategy for the assessment of dyes with the aid of a new QSAR system

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Dyes are commonly used in a variety of applications, including textile, wood, leather and paper coloring. In spite of the large diffusion of those substances, little is known about their toxicological profile in particular for the most complex endpoints such as reproductive toxicity, carcinogenicity and the fate in the environment. Beyond the social concern, a better definition of the risk posed by the use of dyes is now requested as mandatory by REACH. About fifty dyes have been already registered under REACH, but more than 500 will require a registration dossier by the 2018 deadline. Testing all of them is too demanding in terms of cost and animal lives. In order to set up an effective testing strategy, an advanced approach on grouping and read across will be applied, based on a new QSAR system. This is a unique project because no software is available that can process such complex molecules. The achievement is possible due to the availability of a very large number of data. In fact, the authors have received the permission to use all proprietary data that were acquired in the latest 30 years and own by the main manufacturing companies.

IX-5-530

SLiM: a smart way from innovations to humans

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Safety assessment of pharmaceuticals and chemicals requires significant numbers of animal experiments. Although methods that replace, reduce or refine animal experiments (3Rs) for the safety assessment became available over the past decades, the number of accepted and

fully implemented methods within industry and regulations is low. The slow implementation process delays the innovations in product development, which is undesirable for scientific, societal and economic reasons.

Within the SLiM project good practices were obtained for a smarter and faster development, acceptance and implementation of 3R methods by intensifying the collaboration between companies, research institutes and regulatory bodies at an early phase in the method development process.

Factors that drive or withhold (regulatory) acceptance and use of 3R methods were identified by two expert panels (pharmaceuticals and chemicals), consisted of three stakeholder groups: regulatory authorities and legislators, industry and academia. To accelerate the process of acceptance, developments at micro-, meso and macro levels need to be aligned and the drivers need to outweigh the barriers. The dominant factors that are perceived to influence the process at these different levels revealed largely similar in both sectors. The four Cs: Communication, Cooperation, Commitment and Coordination are considered of key importance to augment the process (Schiffelers et al., 2014).

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IX-5-691

Developing regulatory acceptable *in vitro* methods

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Change in the safety assessment paradigm has resulted in the introduction and, increasingly, regulatory acceptance of alternative *in vitro* methods as replacement for, or supplementary to, existing *in vivo* tests. Change was often driven by scientific or animal ethical considerations resulting in regulatory legislation for chemicals (REACH), consumer products (7th Amendment to the Cosmetics Directive) and biological therapies (FDA, ICH).

A challenge in developing alternative methods is the need to validate against known human endpoints. Frequently, there is only animal data to validate against and this can result in a false positive or negative being attributed to the human *in vitro* model which, in reality was due to the weakness in the animal *in vivo* model (e.g., skin sensitisation models against LLNA).

Assays for dermal absorption, skin and eye irritation, phototoxicity, genotoxicity, drug transporter, hepatic metabolism, functional immunoassays (cytokine release, ADCC and NAb) are now used and accepted as standard tests. Screening *in vitro* models are generating improved drug candidates for selection into preclinical testing. These, and other tests, continue to help achieve the goals of the 3Rs. The creation and validation of future methods may now be more affected by human ethical considerations.

IX-5-800

Good In Vitro Method Practice (GIVIMP): guidance on the implementation of *in vitro* methods within a GLP environment to support regulatory human safety assessment of chemicals

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In vitro methods, often based on the use of human cells and tissues, are submitted to international validation bodies (Rispin et al., 2004; Gupta et al., 2005; Coecke et al., 2005). Well-designed, robust, reliable *in vitro* methods that can run in a GLP environment for generating data sets are becoming more and more instrumental for supporting regulatory decisions. Good In Vitro Method Practice (GIVIMP) is a proposal from EURL ECVAM to issue an international guidance on the implementation of *in vitro* methods within a GLP environment to support regulatory human safety assessment of chemicals. GIVIMP will contribute to increased standardisation and harmonisation in the generation of *in vitro* information on test item safety. The Guidance will further facilitate the application of the OECD Mutual Acceptance of Data agreement for data generated by *in vitro* methods and as such contribute to avoidance of unnecessary additional testing. GIVIMP will take into account the requirements of the existing OECD guidelines and advisory documents to ensure that the guidance is complementary and 100% in line with these issued documents (OECD, 2004, 2005). In conclusion, GIVIMP will contribute to the use of *in vitro* method data to support regulatory human safety assessment of chemicals by striving that such data are being generated in compliance with GLP and based on current good scientific practices.

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IX-5-880

Considerations for alternatives to non-human primates in preclinical safety assessments

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Recent, we reviewed all the toxicity studies in National center for safety evaluation of drugs (NCSED) in the last 10 years. Total 43 non-human primates (NHP) repeated dosing studies have been performed. Most of test articles were biopharmaceuticals and about 1600 NHPs were used. However, only chimeric anti-EGFR Mab and humanized anti-EGFR Mab caused obviously toxicity. We compared the data between NHPs and rodents in all studies. No more valuable information was got from these NHPs. It suggested that we may need to consider to decrease animal number or use alternative way.

By using PBMCs obtained from monkey and human, we investigated cytokines secretion, proliferation of lymphocytes, and gene expression after antibody, phytohemagglutinin, lipopolysaccharides, and Fluzone vaccination stimulating. We found that there were significant difference in function of T cell proliferation and cytokine secretion between human and cynomolgus. The gene expression profiles data confirmed that the differentially expressed genes involved in immune regulation and response in human are more complex and sensitive than that in monkey. It suggested that we should be caution when predict T cells related toxicity got from animals to human, and *in vitro* tests used human peripheral blood may be a useful method to evaluate immunotoxicity.

Session IX-6: Breaking down barriers and promoting international cooperation on 3Rs

Co-chairs
Rodger Curren, IIVS, USA
Nick Jukes, InterNICHE, UK

Session IX-6: Oral presentations

IX-6-006

EPAA – a key player in shaping the future of 3Rs

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Since its creation in 2005, the vision of the European Partnership for Alternative Approaches to Animal Testing (EPAA) has been to promote the 3Rs approaches in regulation through better and more predictive science. Our mission is to promote the development, validation and implementation of alternative approaches, but also enhance the acceptance, harmonization and mutual recognition of tests by regulators at national, European and international levels.

EPAA brings together 37 companies from 7 industry sectors and 5 DGs of the European Commission: this unique knowledge-sharing platform launches working groups or studies to define research gaps in the development of 3Rs as well as to improve their implementation in safety regulation.

In 2013 for instance there were 11 ongoing projects related to Science and Regulation, and 6 scientific workshops were organised on topics as varied as stem cell research, skin sensitisation or vaccines consistency. In 2013, EPAA also organised events with external partners such as the European Parliament and SEURAT-1, an illustration of the EPAA's wide network of contacts.

The unique nature of EPAA, its broad membership, its proven results and the width of its mission make it an effective cross-sector platform to further support the development and acceptance of alternatives.

IX-6-039

The International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) in 3Rs Leadership Group: the pharmaceutical industry's promotion of alternatives

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In January 2012, the IQ 3Rs Leadership Group (LG) of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) was established. The IQ 3Rs LG is made up of senior veterinarians, biomedical scientists and 3Rs specialists from IQ member pharmaceutical/biotechnology companies. The mission of the 3Rs LG is to promote sharing and integration of high quality scientific practices to advance the Replacement, Reduction, and Refinement of animals used in the discovery and development of new medicines, vaccines, medical devices and health care products for humans and animals. Our first two years were dedicated to creating a variety of Working Groups (WGs) to promote 3Rs in a range of areas and ini-

tiating several 3Rs projects. One of the main goals of the IQ 3Rs LG is to gather benchmarking information about alternatives from across the industry to highlight strengths and identify gaps and to promote scientific research to further 3Rs innovation. A third goal is to facilitate communication and education about 3Rs advances in a more systematic manner across the biomedical research community via WebEx conferences, journal articles and seminars at international scientific meetings. Another important goal is to develop industry consensus on scientific positions related to alternatives to advance the science, animal welfare, and innovation on 3Rs issues with external stakeholders (legislators, regulators, NGOs, CROs, academia). This presentation will provide a more detailed overview of the IQ 3Rs LG and its various WGs and current 3Rs initiatives to further our collaborative reach and progress our goal of communicating 3Rs advances globally. We will also present our different collaborative efforts in the advancement of the 3R's globally. Members of the IQ 3Rs LG are serving in various functions, e.g., theme coordinators, co-chairs and presenters to support the success of this World Congress.

IX-6-496

A seat at the table: advocating for replacement at the OECD

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The current multi-national and multi-sector scope of many companies requires a coordinated approach to toxicity testing and assessment guidance. The International Council on Animal Protection in OECD programmes (ICAPO) is uniquely placed to provide a united voice for the replacement of animals in toxicity testing and chemical hazard assessment guidelines and programmes. ICAPO comprises groups from Asia, Europe, and North America, and provides consensus policy and scientific interventions to expert meetings, test guidelines, guidance documents, and work plan proposals.

Since 2003 ICAPO has worked to reduce and refine existing and new *in vivo* test guidelines, though most of its efforts aim to increase the portfolio of *in vitro* and *in silico* methods and tools available to companies and regulatory agencies. For example, ICAPO helped to draft guidance on strategically reducing fish testing and recommendations for furthering *in vitro* thyroid disruption test methods. Nine out of the last 11 test guidelines published by OECD are *in vitro* guidelines. Today OECD leads the international coordination of adverse outcome pathway development and AOP-informed testing strategies, and ICAPO is committed to making financial and scientific contributions to this process to ensure the ultimate adoption of a non-animal predictive toxicology paradigm.

IX-6-503

Lack of infrastructure can be a barrier to the acceptance of 3Rs methods; education and training can provide the solution

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Policy changes, especially those addressing regulatory requirements for the safety of new products, can be impeded for several reasons. In some cases decision makers in national regulatory bodies are unaware of the science supporting proposed new methodologies and are hesitant to adopt them solely on the insistence of other countries. This is not entirely unexpected since such decision makers may be more frequently exposed to political concerns rather than the applicable scientific ones. Another barrier exists when the technical infrastructure to properly conduct new *in vitro* methods has yet to be established in a country's domestic laboratories. Both barriers exist in the area of non-animal methods for toxicity testing where significant international differences in acceptance exist. Europe and the US, for example, are quickly moving towards using human-derived cells and tissues – rather than animal based models – to assess many toxicological endpoints, while other countries may be reluctant to make a change because their scientists have not had sufficient time to develop sound data bases of information. We have found that providing specific hands-on training and education on standard methods directly to regulators and scientists in these countries has significantly improved the recognition and acceptance of new 3Rs approaches.

IX-6-585

A process for regulatory science development and application

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The Critical Path Initiative: From Stagnation to Innovation provided a strategy for strengthening the biomedical enterprise. This document outlined mechanisms by which FDA could work with the regulated industries and other stakeholders to further regulatory science. Specifically, this process was developed for genetic and genomic information to be reviewed under the Voluntary Genomics Data Submission process (VGDS). This process was extended to include other types of data including proteomics, metabonomics, and imaging. Submission of these data types to the Agency permitted the development of internal expertise, tools, and processes for the analysis and exploitation of new data types in the regulatory review process. In addition to the regulated industry, consortia such as ILSI/HESI that are umbrella consortia have contributed to developing the regulatory review process by forming tripartite relationships and public-private partnerships to enable data sharing, collimation, and use by the Agency and its stakeholders. Qualification of biomarkers through the VXDS process has led to a focused method for context dependent biomarker and regulatory tool qualification through the activity of the Biomarker Qualifi-

cation Review Team. In this manner, FDA and its collaborators and stakeholders can work together to harness biomedical innovation in the quest to improve public health.

IX-6-608

Implementing the 3R methods and hurdles for their application – a perspective from the chemical industry

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The toxicological potential of chemicals must be determined by law and often requires animal studies. We are commitment to animal protection and apply 3R principles whenever possible. Here, we report on some hurdles that BASF SE has encountered in the recent years.

Hurdles for reduction of animal numbers used for regulatory purposes exist in several forms

1) timeliness for the acceptance (e.g., validation/acceptance for sensitization may come too late)
2) changes in the original designs to reduce animal testing in the extended one-generation reproductive toxicity study may result in the performance of an extended 2-generation study
3) Different international standards (e.g., eye irritation *in vitro* works for GHS classification, but not for the Brazilian system), in this context, there is a need for harmonization of regulations all over the world to use the same study types and avoid unneeded animal studies that can be address already with alternatives.
4) Technical issues, most *in vitro* methods are water based systems, this can potentially limit the testing and identification of the hazard potential of certain test substance classes.

Consequently we observe that there still is room for improvement for effective use of 3R studies for regulatory purposes.

IX-6-883

International collaboration: case study of InterNICHE in India

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InterNICHE is a volunteer-based organisation and network with National Contacts and Partners in over 40 countries. The opportunities and obstacles of working across national and cultural boundaries, and of negotiating for replacement across differences of opinion, have provided experience that feeds into all InterNICHE activity. With successful outreach performed, projects developed, and resources provided, this presentation will explore the nature of successful international collaboration for progressing replacement. The case study of InterNICHE work in India will be used to illustrate its potential.

Session IX-6: Poster presentations

IX-6-075

Science communication in alternatives to animal testing: raising awareness beyond the community

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This presentation intends to illustrate with concrete examples how to spread the word as well as raise and maintain awareness beyond our 3Rs community. In the first part of the talk, the author will discuss multiple social media tools available (e.g., Twitter), participative platforms (e.g., Wikipedia) or websites, which ensure dissemination of the knowledge. It will be the opportunity to discuss who are the main actors on the web and where they are located. In the second part of the talk, the author will focus more on legislative proposals linked with societal demands (e.g., European citizenship initiative). This will also be the opportunity to map and present ongoing worldwide initiatives e.g., North & South America and Australia. In the last part of the talk, the author will provide an overview on the progress on alternatives to animal testing worldwide as well as the details the functioning of the 3Rs at the policy level.

IX-6-109

Regulatory acceptance and use of 3R models for pharmaceuticals and chemicals: Expert opinions on the state of affairs and the way forward

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Pharmaceuticals and chemicals are subjected to regulatory safety testing accounting for approximately 25% of laboratory animal use in Europe. This testing meets various objections and has led to the development of a range of 3R models to Replace, Reduce or Refine the animal models. However, these models must overcome many barriers before being accepted for regulatory risk management purposes. This paper describes the barriers and drivers and options to optimize this acceptance process as identified by two expert panels, one on pharmaceuticals and one on chemicals (Schiffelers et al., 2014). To untangle the complex acceptance process, the multilevel perspective on technology transitions is applied. This perspective defines influences at the micro-, meso- and macro level which need alignment to induce regulatory acceptance of a 3R model. This paper displays that there are many similar mechanisms within both sectors that prevent 3R models from becoming accepted for regulatory risk assessment and management. Shared barriers include the uncertainty about the

value of the new 3R models (micro level), the lack of harmonization of regulatory requirements and acceptance criteria (meso level) and the high levels of risk aversion (macro level). In optimizing the process commitment, communication, cooperation and coordination are identified as critical drivers.

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IX-6-195

A decision tree to facilitate the replacement of laboratory animals in Brazil

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We aimed to develop a decision tree to facilitate validated alternative methods (VAM) implementation. First, does a VAM exist? If yes (Y), is the laboratory director (LD) motivated to change? If Y, is/are there: Branch 1 (B1) Knowledge about VAM costs?; (B2) Qualified human resources?; (B3) Resistance to change by the staff?; and (B4) Incompatibilities between specific norms and Brazilian Animal Protection Law (1)?. For B1, if Y and VAM costs less than animal use, go to B2; if no (N), costs should be studied. For B2, if Y, go to B3; if N, training should be sought. For B3, if Y, staff should be educated about law, ethics and the 3Rs (2); if N go, to B4. For B4, if Y, norms should be denounced to appropriate instances; if N, implement VAM. If LD is unmotivated, does he/she know Brazilian Animal Protection Law (http://www.planalto.gov.br/ccivil_03/Leis/L9605.htm)? If Y, law enforcement is required; if N, LD should be educated about law, ethics and the 3Rs (Russel and Burch, 1959). If LD becomes motivated, he/she is ready to move to B1. If a VAM is not available, it should be developed. This decision tree provides guidance to address the main obstacles for laboratory animal replacement (Bones et al., in press).

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IX-6-199

Ban safety tests on animals

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Every day we surround ourselves by products and substances that have been tested on animals to establish their safety. And every day, new products and substances enter the market. Many of the legally required safety tests have existed for decades and new ones are being drafted. A very important institute in safety testing is the OECD. Many safety tests rely heavily on laboratory animal use. Yet, do animal tests reliably show that substances and products are safe? Are these tests predictive for humans? More and more studies are being published that cast doubt on the value of animal tests. They plead to change the protocols and for a paradigm shift, but this takes (too much) time.

The Dutch animal protection organisation, Dierenbescherming, believes that change can be accelerated by a ban on safety testing, say as of 2025, in comparison (not equal) to the cosmetic campaign. A petition was started to ask the support of the public, to make them aware of the problem and to involve them in the discussion on this subject. Safety is regarded as self-evident, but not at the cost of animals. Ideas of how to accomplish this, we want to present at the World Congress.

IX-6-214

Animal use and cost estimates for the proposed US policy on cosmetics. Comparison with EU and other World States

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The proposed US Safe Cosmetics and Personal Care Products Act (H.R.1385) asks for highly demanding *in vivo* safety evaluations of all cosmetic ingredients that are marketed in the US, including both existing and new chemicals. A detailed analysis (Knight and Rovida, 2014) demonstrated that approval of that bill would cause animal use in a ten year period to increase up to more than 11 million animals with a cost over 9 billion US dollars. It also demonstrated the impossibility that the evaluation process could keep pace with the large and ever-growing number of cosmetics ingredients.

In contrast to that proposal, a new US Bill, the Humane Cosmetics Act, was recently presented to phase out cosmetic animal testing and the sale of cosmetics tested on animals, harmonized with the EU provisions (EC 1223/2009).

The situation worldwide is now confusing as other countries, for example, Japan, are requesting animal tests to authorize the use of cosmetics ingredients.

Details of the US proposal in comparison with the provisions of other countries will be presented.

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IX-6-255 *

Cost comparison between the mouse inoculation test (MIT) and the virus isolation in cell culture (VICC) for rabies diagnosis

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Because the decision for using laboratory animals is frequently based on cost aspects (Bones et al., in press), our objective was to compare the costs to perform the Mouse Inoculation Test (MIT) and the Virus Isolation in Cell Culture (VICC) for rabies diagnosis in Brazil. Based on the observation of laboratory routine at Pasteur Institute,

São Paulo, we listed fixed and variable cost items (Bertó and Beulke, 2005) necessary to perform both tests. We calculated the average total cost per sample and the costs of 1) implementation, and 2) routine use of both diagnostic tests. Considering that 200 MIT tests are equivalent to 350 VICC tests in terms of facilities and staff hours needed per month, one sample analyzed by MIT costs around 193% more than by VICC. MIT is also 67% and 406% more expensive than VICC considering implementation and variable costs for routine use per month, respectively. Such variations are mainly due to the higher cost of MIT variable items, as the animals themselves (76% of variable cost). Our results contribute to the resolution of cost obstacles that hinder the replacement of laboratory animals for rabies diagnosis in Brazil. The presented methodology may be useful for other situations of animal use when validated alternatives exist.

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IX-6-444

Combining 3R strengths in the international arena: together we are strong!

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Advancing the application of 3R-methods requires international cooperation. Almost every step from development to regulatory acceptance and implementation has an international dimension. Organisations, companies and social organisations that promote and advance 3R application can strengthen their impact by combining their knowledge and skills in this area. However, in the light of diversity of promising innovations and professional networks that can play a role, it is essential to create focus.

In a workshop organised by NKCA and RIVM, commissioned by the Dutch Ministry of Economic Affairs, five top priorities were identified, in which national cooperation can contribute to advancing 3R development and application in the international arena. The workshop built on the study programme, Alternatives to Animal Experiments, which identified a number of promising research areas.

A plan of approach was made to maximise the international impact of 3R activities in the Netherlands. An inventory was made of organisations that must be involved in preparing these plans. An organisation must be identified for each priority to take the lead in strengthening national cooperation. The National Committee can take a leading role bringing together the relevant parties. Social organisations are part of the international network and can add strength to the message.

IX-6-697

Working to reduce the use of animals in scientific research – a national approach

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EU Directive 2010/63 places the 3Rs at the core of a Europe-wide programme to protect animals used in science. The UK recognised successful implementation of the Directive would require a collaborative effort across UK government as well as the scientific community and non-governmental organisations (NGOs). New initiatives were needed to promote the development and widespread adoption of 3Rs advances.

The UK Coalition Programme for Government (http://bit.ly/Ublpr6) committed to a Delivery Plan (http://bit.ly/1eNCLBh) to work to reduce the use of animals in research. The Plan has three strategic priorities: putting the 3Rs at the heart of a science-led programme; influencing their adoption internationally; and promoting understanding about the use of animals where no alternatives exist.

The Plan uses the UKs expertise in science and innovation to support its delivery and has been well received by scientists, government officials and welfare communities, many of whom are involved in the delivery of the plan. Measures of success and key milestones have been identified and will be reviewed annually, starting in 2015.

Through this unique and coordinated national approach we are demonstrating how, whilst rigorously promoting and implementing the 3Rs, we are able to continue to deliver scientific benefits for people, animals and the environment.

IX-6-795

Cooperative industry activities to support international advancement of non-animal testing methods

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Although many cosmetic, personal care, consumer product and raw material supplier companies have been working for decades to eliminate animal testing, in some countries regulatory authorities still require animals for product safety testing. While there are differing hurdles to acceptance of non-animal methods around the world, a common difficulty is lack of technical training. IIVS, a non-profit and world leader in the validation, training and application of non-animal test methods, has organized a group of companies to form the Industry Council for the Advancement of Regulatory Acceptance of Alternatives (ICARAA). ICARAA is a working group which provides counsel and financial support of IIVS' mission to increase the use and adoption of *in vitro* methods internationally. Led by IIVS, ICARAA activities focus on educational programs that include lectures, hands-on training and data interpretation. Many of ICARAA's activities are currently in

China where there is keen interest on the part of the regulatory authorities to understand how non-animal approaches can be used to substantiate safety. This collaboration between regulatory agencies, industry and a technical institute serves as a model example of how to promote the practical acceptance of non-animal techniques and facilitate the movement away from animal testing for regulatory purposes.

Session IX-7: Harmonising ways to capture pathway-knowledge in toxicology

Co-chairs
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Session IX-7: Oral presentations

IX-7-286

Adverse Outcome Knowledge Base (AOP-KB)

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An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between the two anchor points – the Molecular Initiating Event (MIE), and an Adverse Outcome (AO), connected by a causal chain of Key Events (KE).

To give the scientific community the possibility to enter, share and discuss their AOP related knowledge at one central point of information, the OECD has launched a project to develop the "Adverse Outcome Pathway Knowledge Base" (AOP-KB – http://aopkb.org/aopwiki/index.php/Main_Page), where AOP developers can create an AOP wiki page and then build an AOP by linking related information about MIEs, KEs, AOs and Chemical Initiators. Controlled-vocabulary drop-down lists from which to select Methods, Actions, Biological Objects, Life stages, Species, etc. related to the AOP simplify the entry of ontology-based information. Information regarding KEs shared among multiple AOPs is stored on a single page to eliminate redundant entries and make the collective knowledge about those entities available in all AOPs containing them.

The presentation will give an overview of the ICT architecture of AOP-KB, its main user interface elements, the AOPs currently contained within the KB and ways to interact with the system as data provider or data user.

IX-7-426

Organizing the adverse outcome pathways knowledge – the Effectopedia way

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Adverse Outcome Pathways (AOPs) describe the causal linkages in a chemical induced cascade of biological responses, across different levels of biological organisation, leading to an adverse outcome over time. The knowledge needed for AOP development is distributed across disciplines that do not normally collaborate. Evolving a common language and understanding requires interactions of experts within a clearly defined context. Effectopedia (http://www.effectopedia.org) – an online collaborative platform – defines this context as multidimensional organizational space, which can visualize AOPs against chosen pair of dimensions (e.g., time to effect vs. level of biological organization). The pathway space helps scientists with different backgrounds determine where their knowledge belongs, and also aids them in identifying both the larger scope of their research and the individual experts who might be actively interested in it. New contributions are immediately distributed to interested parties, keeping all information current, documented and open for discussion, whilst giving credit to original authors and reviewers. Effectopedia space also helps biological responses (effects) to be defined just once and shared across pathways that include them. Shared effects become common nodes in the network of connected pathways which can be utilized for vulnerability analysis, hazard assessment or identifying the most used/needed/resource consuming test assays.

IX-7-699

Using the AOP knowledgebase to record an adverse outcome pathway for respiratory sensitisation

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The aim of the OECD Adverse Outcome Pathway (AOP) programme is to organize and harmonize available knowledge about toxicological pathways in order to facilitate the development and use of chemical grouping, QSARs, and *in vitro* methods.

There is considerable interest in developing test methods to identify chemicals that cause respiratory sensitisation, and especially methods that discriminate between respiratory and skin sensitisers. Using the already well-supported pathway for skin sensitisation as a guide, a group of experts surveyed the literature to identify evidence to confirm the AOP; this information was entered into the AOP Wiki, part of the AOP Knowledge Base (AOP-KB).

In order to avoid redundancy and divergent descriptions of the same key events (KE) and to encourage the inter-connectivity of different pathways, shared molecular initiating events and KEs should be shared between AOPs in the Wiki. For example, covalent protein binding is a shared MIE between skin and respiratory sensitisation; other KEs within the two pathways are similar. This leads to consideration of whether, and how, the two pathways should share MIE/KE descrip-

tions. AOP authors must consider not only how the AOP fits into the Wiki but how the AOP, once built, will be used.

IX-7-771

Using DRAGON to organize data and decisions for AOPs

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ICF International's DRAGON is a database platform designed to store qualitative and quantitative results from scientific literature to enable the data to be searched, analyzed, synthesized, and reported in support of systematic literature reviews for risk assessments. DRAGON consists of modules for literature categorization and evaluation as well as data extraction from animal toxicology studies, human epidemiology studies, and *in vitro* studies. EPA and NTP have provided funding and content support.

To facilitate development of Adverse Outcome Pathways, DRAGON users can import literature citations from multiple sources, use DRAGON-Screen to review titles and abstracts of scientific articles to identify literature relevant to a given AOP, tag literature to specific key events along an AOP, and, in cellDRAGON, extract data from the literature to serve as evidence supporting a key event. The DRAGON evaluation module provides a place to record decisions on the quality and applicability of studies and can be customized for each assessment. Common language and ontologies used in data extraction modules facilitate synthesis of evidence streams to connect, for example, mechanistic data indicating a molecular initiating event and apical adverse outcomes reported in an animal study. Data and decisions entered in DRAGON can be exported in various formats.

IX-7-829

Skin sensitization AOP proof of concept implementation in the OECD QSAR toolbox

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Adverse Outcome Pathway (AOP) concept provides a transparent mechanistic justification and weight-of-evidence approach to reduce uncertainty in the predictions for complex toxicological endpoints. As part of OECD QSAR Toolbox development a proof-of- concept AOP for skin sensitization was implemented. The skin sensitization AOP demonstrates the new functionalities using data rich chemicals, by connecting existing *in-silico* alerts, *in-chemico*, *in-vitro* and *in-vivo* test assays as nodes of a directed graph. When a chemical is subjected to the AOP first the in-silico alerts profilers are executed. If a chemical has the potential to cause skin sensitization according to protein binding profiles then it can "pass" to the next level of *in-chemico* tests. If experimental data is available for the chemical it can pass or not each test and move to next downstream nodes of the graph. Alternatively If measured data is not available a category of similar analogues can be used for read across and once again verify if the node is passed. This process continues until all nodes of the graph are marked. The Toolbox can also be used for simulating skin metabolism allowing to identify the outcomes for both the parent chemical and metabolites.

Session IX-7: Poster presentation

IX-7-860

Quality assurance of emerging technologies in toxicology

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Recent publications from the National Research Council, the EPA and the European legislations are among the drivers of the current landscape change in risk assessment and toxicity testing. At the center of this advance is the conviction that emerging technologies such as -omics-technologies, high throughput screening and computational toxicology could make toxicity testing more efficient in terms of time, cost and relevance to human exposures. This conceptual framework offers many opportunities; challenges, however, need to be addressed to ensure a sufficiently robust and informative outcome. The ongoing "Human Toxome" research project (Bouhifd et al., 2014; Hartung and McBride, 2011) is making use of transcriptomics and metabolomics technologies for Endocrine Disruption pathways of toxicity elucidation. The degree of maturity of these technologies and acceptance of the scientific and regulatory community is dissimilar. Although microarrays have been extensively used for more than a decade, debate is still ongoing about the reproducibility of experiments and the comparability of results at different sites and platforms. Moreover, consensus is still to be achieved concerning best practices in critical aspects including data generation, analysis and interpretation and pathway information generation (Leist et al., 2012). A major challenge is becoming obvious: How to make sure sound and relevant information (e.g., toxicity pathways) is derived from these new tools?

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Session IX-8: Towards harmonisation in the application of alternative approaches within chemical regulation and management

Co-chairs
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Session IX-8: Oral presentations

IX-8-633

Addressing residual uncertainties to enhance read-across: an Industry perspective

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Arguably the single largest challenge associated with the regulatory use of read-across is the perception of additional uncertainty arising from a hazard characterisation not based solely on substance specific toxicological data. Addressing such uncertainty holds the key to assure scientific confidence in the use of read-across without the need for new animal test information.

At the same time it is important to acknowledge the shift in focus in the field of toxicology towards the use of technologies such as high-throughput assays, high-content assays, and their associated predictive models all of which are anchored to adverse outcome pathways (AOPs). Read-across offers a convenient platform for implementing and evaluating these tools while benefitting from the potential reduction in uncertainty as a consequence of the additional information they bring. This presentation will therefore highlight cases where read-across has been used successfully and unsuccessfully to support regulatory submissions with an emphasis on what factors contributed to the ultimate acceptance/rejection of the approach. It will then consider future opportunities in terms of incorporating biological activity, high throughput/content assays, cheminformatics, AOPs and other emerging approaches to support read-across application, thus identifying and reducing uncertainty and therefore increasing the confidence in the predictions made.

IX-8-749

OECD revised guidance on grouping of chemicals

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The OECD is actively working on the development of tools and approaches to reduce or replace animal testing such as chemical categories and (Q)SAR models. The OECD would like expand the concept of grouping chemicals using different approaches of data gap filling as outlined in the revised OECD guidance for grouping of chemicals (OECD, 2014). New ways of grouping chemicals into toxicologically appropriate categories include for example grouping based on adverse outcome pathway (AOP), which is a framework for describing the events at the different levels of biological organization and other key dimensions and their causal relationship to the *in vivo* endpoint under consideration. In fact AOPs shift the emphasis from just intrinsic chemical activity to chemical activity plus the key events that occur across the different levels of biological organization. In this way, AOPs form a solid mechanistic reasoning to support the use of read-across and categories, thus reducing the need for toxicity testing of a substance. The presentation will outline the revised grouping guidance, including the role and application AOPs in forming chemical categories and read-across, and how the AOPs could be implemented into the OECD Toolbox.

Reference

OECD (2014). Guidance on Grouping of Chemicals, second edition, No. 194. Series on Testing and Assessment, ENV/JM/MONO(2014)4, OECD, Paris

IX-8-896

Assessment of read-across: an ECHA perspective

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Read-across data under certain conditions are accepted under REACH to address information requirements. The legal requirements are described in Annex XI (1.5) of the Regulation, specifically the results should be "adequate" for classification and/or risk assessment, have "adequate and reliable" coverage of the key parameters as in the standard test method, cover a comparable or longer exposure duration, and there must be "adequate and reliable" documentation. There is no explicit guidance to interpret what constitutes "adequate" and "reliable", how these relate to the "acceptance" of read-across or how to deal with any uncertainty that is introduced. In an effort to address such

questions, ECHA have developed a framework for the assessment of read-across cases called "The Read-Across Assessment Framework" or RAAF. This framework presents a structured tool for the assessment of read-across cases by ECHA evaluators though this will also be of use to Industry registrants.

At the same time there is growing interest in the development of Adverse Outcome Pathways (AOPs) and the role that *in vitro* data may play in substantiating read-across within chemical categories. This presentation will describe the RAAF and offer some perspectives on how in future read-across could be enhanced by exploiting the AOP framework.

IX-8-924

Application and uptake of alternative methods in China: where does read-across fit?

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The 3Rs principles started to become known in China from the 1990s onwards. Although the practical implementation of the 3Rs in China has progressed more slowly compared to other regions such as Europe, significant advances have been made in the last decade. The Alternative Animal Test Group of GDCIQ has established and is using more than 10 *in vitro* test batteries for the toxicological assessment of cosmetics. The major governmental department AQSIQ has accepted and implemented more than 20 alternative-testing standards. The use of non-testing approaches such as (Q)SARs, TTC and read-across have also gained traction by the different researchers that is helpful to promote the new chemical substance notification legislation process. China is now in a more open attitude to engage in the development and application of alternative approaches.

This presentation will provide an overview of how alternative approaches are being applied and accepted for regulatory purposes through scientific pathway within China with particular focus on non-testing approaches such as read-across. Perspectives on the opportunities, challenges and difficulties in the use of alternatives will be highlighted.

Session IX-9: Establishing criteria for an independent 3R-index: "Access to 3R's"

Moderator

Herman Koëter, Orange House Partnership, Belgium

Session IX-9: Oral presentation

IX-9-531

3Rs in Corporate Social Responsibility programs – possibilities for an independent 3R-Index

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Consumers, patients and animal welfare organisations ask for more openness and transparency from public and private organisations, which is in line with the new European Legislation (Directive 2010/63/EU). Smart companies and research organisations see the interesting possibilities of reduction, refinement and replacement of animal experiments as part of their Corporate Social Responsibility. A Dutch consortium has looked into the feasibility of setting up a 3R-Index as a benchmarking tool, in line with the "Access to Medicine Index" http://www.accesstomedicineindex.org. This 3R-Index will add insight into what is already possible in the field, identify gaps and new opportunities. Additionally it will identify frontrunners and recognizes the effort of industry and research organisations to improve the implementation of 3R methods. A brainstorm session with relevant stakeholders was initiated and themes and indicators for benchmarking are identified, such as 3R policy, stakeholder engagement, quality standards, capacity building and governance. It is clear that there is much that can be achieved with personal commitment and more openness of companies and research institutes. Not only for the animals, but also for science and innovations. The global 3R-Index could be a powerful incentive for industry and research institutes, as the Access to Medicine Index has already been demonstrated.