



Meeting Report

Women in Alternatives

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The online Summer Meeting “*Women in Alternatives*”, held on September 8, 2022, organized and chaired by Francesca Caloni, Università degli Studi di Milano, brought together scientists to exemplify and evaluate the involvement of women in the 3Rs and alternative methods, their approach, contribution, role, career, and opportunities in this specific area of science. **Francesca Caloni**, opened the meeting by starting from an historical picture illustrating pioneering female toxicologists (Stirling, 2006), coming to a current and modern perspective of female participation in alternative methods and the 3Rs, and providing food for thought and discussion.

Isabella De Angelis, in “*Alternative Reflections*,” presented some initiatives organized by the Italian Platform on Alternative Methods (IPAM), of which she is president, and by the Italian National Institute of Health (Istituto Superiore di Sanità, ISS). IPAM was established about twenty years ago with the aim to promote and inform on the application of the 3Rs principle in scientific research and to speed up regulatory acceptance of replacement methods, now often called new approach methodologies (NAMs). To achieve these goals, the collaboration between the different areas involved in the development and use of NAMs, i.e., academic research, industry, governmental institutions, and associations for animal protection, was considered an essential requirement. IPAM, in compliance with this vision, promotes research and information on animal experimentation in Italy, building synergies to accelerate the development and acceptance of NAMs in basic, applied, and regulatory research. ISS has a solid background in the application and dissemination of the 3Rs principle as well. Several activities were realized in the last years, both to establish a robust internal scientific network on the development/application of NAMs in basic research and to communicate with researchers, citizens, and students on the responsible use of animals in scientific experimentation as well as on the innovative application possibilities offered by NAMs.

In her presentation “*Having a successful career in toxicology: what people see and what it takes*”, **Helena Kandarova** underlined the important role of toxicologists principally involved in the discovery of new knowledge and new models answering questions concerning the toxicity of substances, mixtures or products and how they produce their detrimental effects. The career path of a toxicologist usually starts with bachelor’s and master’s studies in biology, chemistry, or pharmacy or related fields, and toxicology, as a subject of science and/or research, usually appears later, typically during PhD studies or as a work experience. Besides universities, there are many commercial, governmental, and nonprofit laboratories that provide interesting

and challenging themes linked to toxicology that PhD students may explore. Building a career in toxicology as a post-doc opens various possibilities for early-career scientists to explore positions in industry, research institutes, and regulatory fields. Some may choose to combine the path with teaching, education, and dissemination. Regardless of the environment, interdisciplinarity of knowledge and networking abilities seem to be prerequisites for a successful career path as a toxicologist. The presentation summarized examples of how challenges and crossroads in the career path can be used for personal development and a successful switch between academia and industry.

Lena Smirnova presented “*Addressing autism with new approach methodologies*”. The strong increase of autism in recent years cannot be explained solely by genetics but rather by gene-environment (GxE) interactions. A combination of three technologies (induced pluripotent stem cells, CRISPR-Cas9 gene editing, and advanced 3D cultures) allows to produce an *in vitro* 3D brain model to address GxE *in vitro*. Mutation in the *CHD8* gene is one of the well-known genetic risk factors for autism. A heterozygous *CHD8*^{+/-} knockout in an iPSC cell line was generated using CRISPR-Cas9. Brain organoids were generated from *CHD8*^{+/-} iPSC and control iPSC from the same donor (*CHD8*^{+/+}) and were exposed to chlorpyrifos (CPF) and its active metabolite chlorpyrifos oxon (CPO) for 24 h. *CHD8*^{+/-} brain organoids were more sensitive to the CPF and CPF oxon as measured by a viability assay. Exposure to CPF and CPO reduced the level of *CHD8* protein in both control and mutant organoids, suggesting that the downstream target of mutation and exposure can be the same. Exposure led to reduced neurite outgrowth in both cell lines, which was rescued by tocopherol, while the ratio between excitatory and inhibitory neurotransmitters (glutamate/GABA) was significantly higher in mutant organoids versus control and was not changed by the exposure. They identified synergistic effects of mutation and exposure on the level of neurotransmitters and metabolites associated with autism spectrum disorders (ASD), thus validating their model against human data. Taken together, the data provides a proof-of-principle of how GxE can be addressed *in vitro* and for mechanistic validation.

Maria Pilar Vinardell described her career in toxicology, presenting “*3Rs activities at the Universitat de Barcelona from in vivo to in vitro*”. From 1996, after her attendance at the Second World Congress on Alternative Methods, she learned a lot about the 3Rs, and especially replacement. She involved her colleagues in 3Rs research and replaced the traditional practical classes with animals with classes without animals. She taught such courses at her own university and other academic institutions, especially in



different countries in Latin America. In all courses, the majority of students were women. Her research activity is focused on the development of alternative methods in toxicology and especially on skin sensitization. The main difficulties experienced in this kind of research have been the reluctance of many researchers to use methodologies without animals and rejections of project applications that did not involve animal experiments. Recently, after many years of requesting financial support for alternative methods, the Spanish Ministry of Sciences and Innovation has established the development of alternative methods to reduce animal experimentation in its strategic priorities for research projects in 2022. This prioritization will provide opportunities for researchers interested in changing the idea that animal experimentation is the gold standard.

Fenna Sillé described “*A path forward: Current and future perspectives of alternatives to developmental immunotoxicity testing*”. The development of non-animal new approach methodologies (NAMs) has been widely acknowledged as a critical need for toxicity testing. Although critical windows of developmental immunotoxicity (DIT) have been defined by the field, DIT testing currently is intimately linked to the use of whole animal studies with their inherent limitations in translatability to humans. Sensitive *in vitro* assays are hampered by the complex nature of the effects and the partially missing information on interrelationships in the human system, especially during the developmental period. Clinical information on the effects of drugs and other exposures on the developing immune system *in utero* are scarcely available. The status of *in vitro* and other alternative assays available for DIT screening is unclear as few alternative approaches have been developed and adapted by the greater toxicology community. Through expert workshops, the “*International Working Group on Alternatives to Developmental Immunotoxicity Testing*” is identifying and addressing critical knowledge gaps in the field of alternative DIT approaches. First, the working group has highlighted the need for alternative DIT testing strategies from applied and regulatory end-user perspectives. Second, the working group has brought experts across the field of developmental immunology and toxicology together for an updated comprehensive overview of the current status and advances in developmental immunology with relevance to DIT. The working group started a continuing project of mapping an updated and refined network of key molecular and biological events in developmental immunology that will be important to assess during DIT testing. Current efforts translate scientific advances into adverse outcome pathways (AOPs) that can inform regulatory hazard or risk assessment. The working group is currently evaluating the feasibility and test readiness status of existing alternative models and strategies across fields (for example CD34⁺ cell-based immune cell development assays) that may be useful and appropriate for DIT testing. In addition, areas in need of development of alternative DIT models and tests will be highlighted through the introduction of a novel framework to encourage the refinement and development of new alternative test methods suitable for screening of (large numbers of) DIT compounds. With that, the need for the identification of reference compounds and data

sets for DIT testing purposes has been established. Finally, the working group provides an outlook on how ground-breaking innovations and state-of-the-art technologies, such as SuPERR-seq (Surface Protein Expression, mRNA- and Repertoire-seq) and artificial intelligence can benefit the switch to alternatives for DIT. The ultimate goal is to develop a strategy for alternative DIT screening and test guidelines that can be incorporated into OECD guidance documents.

Silvia Letasiova in her presentation “*Can work in a private company change life?*” provided an overview on how work at a private company, i.e., MatTek, changed her life when she took over the position of managing director of MatTek In Vitro Life Science Laboratories, Slovakia, in January 2019. MatTek Life Science, a BICO Company, is a biotech company that is at the forefront of tissue engineering technology, and its lab-grown human tissues of the skin, eye, respiratory, digestive, and other systems are used worldwide as ethical and human-relevant alternatives to animal testing. In 2009, MatTek Life Science, USA, established its only facility in Bratislava, Slovakia, where four types of reconstructed human tissue models (EpiDerm, EpiOcular, and EpiIntestinal (partial and full thickness)) are produced, focusing on the development, optimization and validation of new methods and providing training for *in vitro* methods. Over the years, they have been able to grow in different areas and extend the mission of their company, to implement the production of new tissue models, and hopefully, soon they will be able to officially announce the production of the MelanoDerm tissue model in their facility. They have increased not only the number of employees but also the number of different laboratory devices, and moreover, they have expanded their R&D laboratories, so they are able to perform internal research, find new applications areas for the tissue models, and collaborate on research projects with partners. These areas consist of, for example, combining EpiDerm phototoxicity testing of different compounds with their ability to form ROS, sub-categorization of ocular irritants using EpiOcular tissue models, development of physiologically relevant tests using the EpiAirway tissue model to predict acute respiratory toxicity of mists and volatile liquids, studies of EpiDermFT for wound healing effects of different products, studies of oral toxicity using the EpiOral tissue model, and many more. They actively participate in workshops, hands-on training, conferences, and meetings, where they present methods and models that can be used in the reduction and replacement of animal experiments in regulatory as well as in non-regulatory toxicology. The work at a private company has given Dr Letasiova great opportunities to personally grow, but as Steve Jobs said “*Great things in business are never done by one person. They’re done by a team of people.*” Everything that they have reached so far is the success of the whole MatTek team, who are deeply dedicated to their work.

Thomas Hartung related on “*Animal tests are from Mars, new approaches come from Venus*”. This analysis of the field of alternative methods used John Gray’s 1992 book “*Men are from Mars, Women are from Venus*”, which sold 15 million copies and, according to a CNN report, was the “*highest ranked work of*



non-fiction” of the 1990s, to describe the field of new approach methods. Gray describes males as “*Martians value power, competency, efficiency, and achievement. They are always doing things to prove themselves and develop their power and skills. Their sense of self is defined through their ability to achieve results. They experience fulfillment primarily through success and accomplishment.*” This reminds of animal tests and their “Protect at all costs!” approach. In contrast, Gray describes “*Venusians have different values. They value love, communication, beauty, and relationships. They spend a lot of time supporting, helping, and nurturing one another. Their sense of self is defined through their feelings and the quality of their relationships. They experience fulfillment through sharing and relating.*” This reminds much more of alternative methods, e.g., mirroring the world through mechanistic approaches, a community collaborating toward change, a willingness to share (data and methods). Gray notes that males do not like to change: “*If something is working, their motto is don’t change it.*” In contrast, “*Venusians firmly believe that when something is working it can always work better. Their nature is to want to improve things.*” Again, similar things could be said about the world of animal-based regulation versus the alternatives sector. This corresponds with the author’s personal experience in the field of alternatives, where his groups at the University of Konstanz, ECVAM, and CAAT always attracted more women than men, at roughly 70%. In closing, Gray was cited with “*With new insight you have the added wisdom and power to change your approach rather than seeking to change your partner*” and the author hoping for a life on Venus with flourishing new approach methods.

Tuula Heinonen presented her experience on “*How to get NAMs high priority on the state’s agenda.*” To raise the priority of NAMs on the state’s agenda, there must be a clear and broad need for new methodologies, a doable and concrete action plan that is in line with the needs of stakeholders and national legal requirements, such as set by Directive 2010/63/EU, and a team that is shown to possess sufficient knowledge, experience, and motivation. The prerequisites of success include a good cooperation with key stakeholders and broad dissemination of the concept. The Finnish Centre for Alternative Methods (FICAM), which served as the solid basis for additional national tasks in Finland, was established in 2008. Very importantly, many parliament members in Finland found promotion of non-animal methods and approaches from science, animal welfare, regulatory, legal, and financial perspectives valuable. Significant support was also provided by Finland’s Members of Parliament (MPs) and by EURL-ECVAM. Because MPs have many and often pressing duties, they were approached in several ways to keep FICAM’s issues high on the parliamentary agenda: having personal meetings to update on legal requirements and scientific developments

in the field, with supporting letters from other groups and organizations acting in the 3Rs field, being heard in parliamentary committees, and inviting MPs to visit FICAM. Most important was that the ministry responsible for implementing Directive 2010/63/EU, the Ministry of Agriculture and Forestry, found FICAM to be a valid tool to promote the 3Rs and included FICAM in their annual budget for approval by the Parliament. The strong support from Tampere city and its regional area and from Tampere university provided credibility and plausibility. The international cooperation of FICAM was highly acknowledged. Significant financial support for FICAM, which focused on developing and disseminating NAMs, providing education and training, and acting as a GLP-validation laboratory (NETVAL) for EURL-ECVAM, was included in the state’s budget. Due to a merger with another unit at Tampere University after Tuula Heinonen’s resignation from FICAM, the name was later changed to FHAIVE¹. The sustainable public utility role of FICAM in the promotion of NAMs would not have been possible without the state’s specific financial support, which gave it significant visibility and plausibility. Later, another initiative proposed by Tuula Heinonen, which was promoted by the Ministry of Agriculture and Forestry and the Council on the Protection of Animals Used for Scientific or Educational Purposes to further promote NAMs and the 3Rs in general, was to establish a 3Rs center in Finland (FIN3R², founded 2022), which was also financed by the state. Finland has been and is active in the promotion of the 3Rs and of NAMs. The most important drivers were the many dedicated people and a long-term financial support and acknowledgement on state level.

Vivian Kral presented the activities performed at the Freie Universität Berlin in “*Experiences in teaching alternatives to animal testing*”. At the institute of pharmacy, teaching of alternatives to animal testing started in 2005. Within the invitrotrain³ project, national and international participants were provided with theoretical and practical training on selected 3Rs methods. In 2014, BB3R⁴, the research platform with the world’s first integrated 3Rs graduate program, was established. The aspiration is to provide all doctoral students within the program with fundamental knowledge on all the 3Rs, independent of their specific research topic. The 3Rs-related seminars and spring schools that are organized for the BB3R graduate students are also open to other interested researchers. Also, students in the life and natural sciences should have at least some basic knowledge of the 3Rs. Therefore, alternative methods are part of the curriculum for pharmacy students. As it is time-consuming to extract learning material that is appropriate with regard to content, knowledge level, and scope, the idea came up to create the 3Rs info hub⁵: the hub for learning material dealing with the 3Rs. Suitable media were selected and categorized (e.g., according to the

¹ <https://research.tuni.fi/fhaive/>

² <https://fin3r.fi/en>

³ <https://www.bcp.fu-berlin.de/pharmazie/faecher/pharmakologie/invitrotrain/index.html>

⁴ <https://www.bb3r.de/>

⁵ <https://www.3rsinfohub.de/>



R addressed, the required prior knowledge, etc.) and compiled on an easy-to-use website (for free, no registration needed). In addition, learning scenarios (3Rs courses) that include, e.g., video material on a specific organ system and a learning success control (3Rs quiz) have been developed and integrated into the hub. Another idea to bring students closer to the topic, was to set up a podcast series. The StudentCast on 3Rs⁶ has been recording interviews that students conduct with experts in the field of alternatives since 2021 and is available on various public podcast platforms.

In her presentation on “*Human health and animal welfare: the ONE wellbeing concept*”, **Laura Gribaldo** described how her work at the European Commission was inspired by two women, Jane Goodall and Dian Fossey, working with great apes, and by the founding mothers of the European Union. Dr Gribaldo spent most of her career at the European Union Reference Laboratory for Alternatives to Animal Testing (EURL), which was redefined under Directive 2010/63/EU on the protection of animals used for scientific purposes, subsuming the European Centre for the Validation of Alternative Methods (ECVAM). Among its responsibilities, she mentioned guiding research on alternative methods, coordinating validation within the EU, disseminating information on the 3Rs, facilitating stakeholder dialogue, and promoting international acceptance. In the last years, a new activity in biomedical research was started. Animal models are traditionally used in biomedical research to recapitulate human disease features and develop new drugs; however, animal models do not develop disease as it occurs in humans, and their use has not supported the development of drugs efficiently. Particularly when human tissues are used, non-animal models may generate faster, cheaper results, more reliably predictive for humans, whilst yielding greater insights into human biochemical processes. Therefore, the JRC’s EURL launched a series of studies to identify current and emerging non-animal models used in the biomedical realm. The final goal is to disseminate and improve knowledge-sharing on the potential and limitations of

human-based models at different levels: scientific communities, universities and secondary schools, national committees for animal welfare, and the public at large.

A final discussion with the participants concluded the meeting, opening new scenarios and opportunities toward a more inclusive vision in science.

Reference

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⁶ <https://www.3rsinfohub.de/podcast-media/index.html>