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Meeting Report

Virtual Class on Alternative Methods: Ethics & Science

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The international Virtual Class on *Alternative Methods: Ethics & Science* (http://ames.lakecomoschool.org), aimed at young scientists, was focused on alternative methods and the 3Rs (Reduce, Refine, Replace animal use), from the ethical and scientific points of view. The purpose was to provide an overview, through an ethical, scientific, and philosophical approach, to illustrate the history, the application, and the future of alternative methods. The class was chaired by Francesca Caloni, Università degli Studi di Milano, Department of Environmental Science and Policy, and was attended by 27 participants from all over the world with backgrounds ranging from agronomy, biology, environmental science, veterinary medicine, to economy, and computer and social sciences.

Francesca Caloni started the class with a brief overview entitled "Alternative methods: Educational experience". The main aspects underlined during the presentation were related to the importance of an "inclusive" education on the 3Rs, not only focused on scientific aspects but promoting and developing a mental attitude through a multidisciplinary 3Rs educational approach, answering the increasingly tangible needs of a complex knowledge. An educational experience of the 3Rs, merging different disciplines through a well-structured pathway and a multistep approach, aimed at undergraduates to early career researchers would address the future requirements of a global society.

Marco Pedrazzi, Università degli Studi di Milano, Department of International, Legal, Historical and Political Studies, presented a lecture entitled "Ethics and science: Which role for research integrity?" Research integrity (RI) has become a central topic worldwide, particularly in Europe. The presentation defined RI and looked at some of the main themes of RI, such as the duties existing within a research group and towards external participants and society as a whole, plagiarism, and self-plagiarism.

Isabella De Angelis, Environment and Health (ISS), spoke on "Alternative methods move towards new approach methodologies: Reflections and perspectives". Since the early 2000's, an impressive acceleration in efforts to develop non-animal approaches for investigating hazardous properties of chemical substances and drugs has taken place. These efforts have gone hand in hand with the new, exciting possibilities offered by human biology-based innovative technologies and approaches. For this reason, researchers and legislators now prefer to address their attention to new approach methodologies (NAMs) instead of "sim-

ple" alternative methods. NAMs mainly include: i) *in vitro* and *in silico* approaches connected to modern technologies and "big data"; ii) human-relevant models; and iii) mechanism-based models. To date, the replacement of traditional toxicity testing with NAMs to determine human hazards and risks is constantly increasing, but it still needs to be consolidated. Recent evidence indicates that NAMs data are relevant for human risk evaluation of chemicals.

Gianfranco Mormino, Università degli Studi di Milano, Department of Philosophy, gave a lecture entitled "Instrumental and terminal values in experimentation". The distinction between "means" and "ends", crucial to every ethical argumentation, is usually invoked in order to legitimate actions that cause harm: the goodness of the goal is the justification for the infliction of an evil, insofar as it may be argued that the former exceeds the latter. The correctness of a comparison between good and evil, however, should depend on a precise evaluation of all other conditions: comparing the harm inflicted to A to the good received by B, e.g., is a fault in reasoning, as it is the comparison between a certain and present evil and an uncertain and future good. In considering the terminal values of experimentation, therefore, it would be misleading to apply the same measurements we use for instrumental values without taking into consideration the quite different implied perspectives of the subjects and the different epistemological status of the means and the ends.

"Animal-free and chemically-defined media for cell culture: An ethical and scientific duty" was presented by **Yula Sambuy**, Council for Agricultural Research and Economics (CREA). In the last fifty years, advances in cell culture techniques have allowed propagation and maintenance of eukaryotic cells from different tissues and organs in a highly differentiated state. Fetal bovine serum (FBS) has been, until recently, an important and often essential supplement in cell culture media, providing important factors for cell adhesion, growth and differentiation. However, its widespread use has increasingly been criticized for both ethical and scientific reasons. Strong ethical objections to its use arise from the collection procedures from bovine fetuses that do not rule out animal suffering. But there are also many scientific reasons that advise against using it. Among the disadvantages of FBS are the chemically undefined and variable composition of different commercial lots, the high costs, the risk of transmitting unknown pathogens, and the high protein concentration that interferes with purification procedures of recombinant cell culture products. The rapid increase in the production of biopharmaceutics (recombinant proteins, monoclonal antibodies, viral vaccines) from cultured mammalian cells and in the therapeutic use of stem cells has boosted the development of chemically-defined media without animal-derived products to reduce the risk of potential contamination from known and still unknown pathogens. Among the problems of developing a chemically-defined animal product-free medium is the need to adapt it to the specific needs of the different cell lines in the proliferative and differentiation stages. This is particularly relevant in the case of stem cells that need to maintain in culture both their proliferative capacity and their pluripotent ability to differentiate into distinct cytotypes. For *in vitro* toxicology, the use of chemically-defined cell culture media is particularly important to guarantee a high reproducibility in dose-response curves of toxic substances to allow more accurate prediction of *in vivo* toxicity. In addition, the presence of serum or other undefined proteins in the medium can interfere with the assays by binding and reducing the availability of the substance under investigation.

Paola Fossati, Università degli Studi di Milano (ESP), with a lecture entitled "Alternative methods in animal research: a look into the law", gave a complete overview of the legislative aspects related to 3Rs and alternative methods. The 3Rs principle, which is the basis for the ethical approach applied to the use of animals in research, is incorporated in systems that are referred to as new approach methods and has been expressed in the legislation pertaining to animal experimentation in Western societies.

A final interactive discussion with the participants on the trans-disciplinary vision of the 3Rs and alternative methods concluded the Virtual Class. A special thanks to the 27 active participants of the Virtual Class.

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