Meeting Report

Application of Computational Methods in Replacement – an IPAM Webinar

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The IPAM webinar 2020 on the "Application of computational methods in Replacement" was organized by the Italian Platform on Alternative Methods (IPAM[1]) in November 2020 with the aim to inform on computational methods as replacement tools within the 3Rs (reduction, refinement and replacement) principle. On behalf of the IPAM board, the webinar was chaired by **Isabella De Angelis** and **Stefano Lorenzetti**, from the Istituto Superiore di Sanità (ISS, Rome, Italy), IPAM president and secretary, respectively. The webinar was targeted mainly at young scientists (De Angelis et al., 2019). Both ways to use computational methods as new approach methodologies (NAMs) and the management, organization and exchange of toxicological information and data were discussed. More than 60 participants from universities, research centers, and industry joined the webinar.

The webinar was opened by **Alessandro Giuliani** (ISS, Rome, Italy) with an introductory lecture on *Computational Methods in Toxicology*. Computational methods are used in all biological fields. Terms like "artificial intelligence" or "data mining" often convey the false impression of a sort of automatic solution to the management of the increasing mass of (largely unstructured and difficult to harmonize) toxicological results, ending in a definitive verdict on the toxicological risk of chemical agents. Moreover, often the work of "computational scientists" is perceived as separate from biological research and aimed at facilitating the emergence of a "latent truth" already present in the raw data. This is far from the reality of scientific practice, which works by "subtraction" (distilling the relevant regularities out of a sea of largely irrelevant details) and not by "addition" to an ever-increasing corpus of data. The analysis of successes and failures of quantitative approaches in pharmacological and toxicological research by demonstrating the decoupling between method sophistication and prediction power as well as the avoidance of common problems like overfitting and lack of well-defined boundary conditions is instrumental to a sensible approach to computational models. Overall, we must look for relevance, not only predictive power, and this requires integration between computational and biological knowledge.

Orazio Nicolotti, from the University of Bari, Italy, gave an introductory lecture on *Quantitative Structure-Activity Relationship (QSAR)*. QSAR practitioners seek causative correlations explaining the variance of a given endpoint based on the variance of properly selected, meaningful chemical descriptors (Nicolotti and Carotti, 2006). In this respect, QSAR models are employed to predict biological, toxicological and physico-chemical properties of new compounds based on existing experimental data. Two milestones in QSAR are the works of Hammet and Hansch, the former correlating the electronic properties of organic acids with their reactivity, the latter reporting the importance of lipophilicity for bioactivity. Even when using sophisticated approaches and overwhelming numbers of descriptors, the quality of data is pivotal to obtain successful QSAR, thus avoiding the "garbage in - garbage out" pitfall. In this respect, the need to derive trustable predictions has promoted new strategies to derive a model's applicability domain, which represents the interpolative space within which reliable predictions can be made. The entry into force of REACH, the European regulation on chemical substances (EC 1907/2006), has paved a new regulatory road to QSAR, furtherly supported by the OECD principles (Nicolotti et al., 2014). Accordingly, the paradigm "no data, no model" has been replaced by the motto "no data, no market". For toxicological purposes, the ultimate goal is that of minimizing false negatives, i.e., harmful chemicals predicted as harmless (Gissi et al., 2014). QSAR can promote innovation (Alberga et al., 2019, 2020), save time, reduce costs, limit animal use, make rational prioritizations for experiments, and indicate approaches to safer chemicals.

Pietro Cozzini, from the University of Parma, Italy, gave a lecture about molecular docking applications entitled *Big Data, Screening, Docking/Scoring et similia*. Molecular modelling can serve to reduce *in vitro* and *in vivo* testing, in particular when we manage a huge amount of data as for food contact materials (FCMs), substances occurring naturally or that are intentionally or unintentionally added to food (Cavaliere and Cozzini, 2018). Structural databases are essential for computational studies on biomolecular association, but they are not sufficient. Data can be defined from different points of view – identification, structural, legislative, toxicology – and Big Data technology is needed. In order to predict the possible endocrine disrupting activity of many FCMs, the following procedures have been applied: i) screening – filtering a reduced set from a huge number of molecules, ii) docking – prediction of the

[1] http://www.ipamitalia.org

best-fitting small molecule within a receptor cavity, iii) molecular dynamics – study of the time evolution of a protein or protein-ligand complex, and iv) consensus scoring techniques – obtaining agreement from different prediction methods on the same results. Some case studies have illustrated ligand-nuclear receptor (NR) interactions and their importance for multiple important diseases or toxicological purposes. For instance: i) bisphenols, molecules used to produce plastics against estrogen and androgen receptors, of interest for cancer (Cavaliere et al., 2020), ii) *Daphnia magna* and RXR for water environment control, iii) *in vivo* test reduction for multiple sclerosis (Cavaliere et al., 2017).

Olga Tcheremenskaia (ISS, Rome, Italy) gave a lecture on *Adverse Outcome Pathways (AOPs)*. AOPs are novel mechanistic-based tools in toxicology, which provide a clear representation of critical toxicological effects over different layers of biological organization. An AOP describes a sequence of events, starting from an initial interaction of a stressor with a biomolecule within the organism causing a biological perturbation (molecular initiating event, MIE). MIE can progress through a dependent series of intermediate key events (KEs) and culminate in an adverse outcome (AO) considered relevant to risk assessment or regulatory decision-making (OECD, 2016). To maximize the utility of AOPs, their aggregation and standardized organization is essential. The OECD has developed a series of tools, collectively known as the AOP Knowledge Base (AOP-KB), to provide a standardized, systematic structure for AOP development and dissemination. The AOP-Wiki[2] facilitates collaborative AOP development by collecting and linking expert-curated AOP information through a controlled vocabulary (Ives et al., 2017; Wittwehr et al., 2017). An AOP allows for the mapping, organization and integration of various types of information such as *in silico*, *in chemico*, *in vitro* and *in vivo* data, which is essential to support integrated approaches to testing and assessment (IATA) (Sakuratani et al., 2018). The AOP on skin sensitization has been recently implemented in a defined approach (DA) and will be published soon in an OECD guideline. This is an excellent example of a scientifically valid and sustainable application of AOPs in regulatory toxicology (Kolle et al., 2020).

Cecilia Bossa and **Chiara L. Battistelli** (ISS, Rome, Italy) presented on the *FAIR (Findable, Accessible, Interoperable, and Reusable) approach to nanomaterials databases*. Availability of experimental data on physico-chemical and (eco)-toxicological properties of nanomaterials is essential to enable their risk assessment, including the possibility to apply *in silico* methodologies, like QSAR, grouping and read-across. Although a large amount of nanosafety data has recently been produced in international collaborative initiatives, their reuse is hampered by several obstacles, e.g., poorly described (meta) data, non-standard terminology, lack of harmonized reporting formats and criteria (Jeliazkova et al., 2021). To guide the scientific community in good data management and stewardship, the FAIR principles have been established (Wilkinson et al., 2016). However, being a relatively young research area, their implementation in nanoscience is particularly challenging. The definition of the methods, protocols and parameters that guide data generation is in fact evolving together with knowledge on the determining factors of the physico-chemical and toxic effects. The eNanoMapper database and related tools are ongoing efforts to improve the FAIRness of data infrastructures (Kochev et al., 2020). One of the strengths of this FAIR-compliant data repository is the creation of a communication channel between laboratory experts and data modelling experts to maximize the understanding, exchange, availability and, ultimately, the reuse of data.

The webinar was concluded with the presentation of the winner of the IPAM 2020 award, assigned to a scientific master degree or PhD thesis in which the relevance for the development or improvement of alternative methods is clearly highlighted. The aim of the award is to encourage and disseminate the application of the 3R principle in the field of university and post-graduate training. IPAM's board awarded the prize to Daniela Ricci for her master thesis in medical biotechnology on *Study of the immune response stimulated by the vaccine against tick encephalitis virus (TBEV): possible applications for the evaluation of vaccine immunogenicity*. The study characterizes the innate and adaptive immune response induced by the vaccine Encepur® directed against TBEV, identifying possible predictive biomarkers of vaccine immunogenicity through the use of human primary culture-based assays as an alternative to tests performed on animals (Etna et al., 2020).

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