US Strategic Roadmap: New Approaches to Validation

- **OECD Activities to Increase the Utility and Uptake of AOPs in Regulatory Contexts Across Countries**

  Presenter: Mr. Bob Diderich, Organisation for Economic Co-operation and Development

  Regulatory authorities want to increase the efficiency and number of chemical safety assessment and reduce the need for additional animal testing. Integrated approaches to testing and assessment (IATA) are innovative approaches for combing chemical safety data to reduce or eliminate the need for animal testing. Adverse Outcome Pathways (AOPs) have emerged as evidence integration tools to organise diverse data and to better understand links between biological events leading to toxic effects, beginning with mechanistic chemical-target interactions and leading to an apical response. However, as of yet, there have been relatively few examples of AOPs used in regulatory contexts. Several ongoing projects in the OECD's chemical safety programme aim to apply AOPs frameworks to regulatory scenarios to: (i) identify chemicals that can trigger an AOP in order to building quantitative structure-activity relationships (QSARs) and group chemicals into categories for read-across, (ii) identify key events to be measured in new Test Guidelines, (iii) include non-guideline information in hazard assessments, and (iv) develop IATAs. This presentation will focus on examples of current OECD activities that use AOPs to inform regulatory decisions. In order to build QSARs, databases on chemicals that can trigger a molecular initiating event or are associated with key events included in AOPs are compiled. These databases, when added to the OECD QSAR Toolbox, can be used to design profilers for data-poor chemicals with similar structures. Users can group chemicals that trigger adverse effects through the same AOPs and with the totality of data available on key events for the group, increase the robustness of read-across predictions. There are a variety of current activities using AOPs to develop new test methods or methods used in combination to predict complex endpoints. For example, development of a battery of in vitro test methods for predicting developmental neurotoxicity includes several molecular initiating events and key events included in a network of AOPs. Despite gaps in the complete biological understanding of the toxicity pathway, activities to characterise chemicals that can disrupt retinoid pathway signalling are making use of mechanistic data to guide the development of new OECD Test Guidelines. Skin sensitisation is an example of a toxic effect with a complete AOP and a variety of guideline methods to test several key events in the AOP. IATAs can be built around this AOP and elements of the IATA can be fixed to develop Defined Approaches, using the results of in vitro test methods and QSARs in combination, to predict the in vivo response. It is expected that the Defined Approaches from skin sensitisation can be covered by the OECD agreement on Mutual Acceptance of Data. Work is underway to develop IATA for non-genotoxic carcinogens around AOPs. In this case, the AOP construct is used to anchor existing assays or those under development to Key Events involved in tumour formation. Together, assays will be used to construct an IATA for non-genotoxic carcinogenicity. In parallel with these activities, the OECD coordinates an

IATA case studies programme, where countries exchange experiences on the practical application of IATA for regulatory decision making. In many of the case studies, AOPs are used to support the scientific robustness of the IATA. Now beyond the proof-of-concept phase, the OECD's AOP development programme is evolving to better serve the regulatory needs of countries and support on-going and planned OECD projects for the development of IATAs and Test Guidelines.

- **Antibodies and Non-antibody Affinity Reagents Generated Using Animal-free Technologies, For Use in Research and Diagnostics**

  Presenter: Dr. João Barroso, European Union Reference Laboratory for Alternatives to Animal Testing

  Affinity reagents are binding molecules that have a high specificity for their unique target (antigen). They are crucial for research, diagnostics, therapeutic and regulatory applications. Based on their recognition properties and binding specificity, protein-based antibodies are currently still the most important tools for the specific detection of proteins or other molecules. However, with developments in protein and genetic engineering, new alternative binders are being introduced, such as aptamers, affimers, DARPINs, etc. These alternative binders can be based on peptides, proteins, ribonucleic acids, or single-stranded DNA. Although these new molecules are already being applied in diagnostics, antibodies are still the molecules of choice for many applications. In many cases, antibody production is still relying on animal-based methods. More recently developed animal-free technologies involve the use of large collections of recombinant forms of miniaturised antibodies such as single-chain variable fragment (scFv) and fragment antigen binding (Fab), or antibody analogues. These approaches require the ability to present large binder libraries on the surface of various available display systems (e.g., phage display), which permit the selection of peptides or proteins with high affinity and specificity for virtually any target. In line with the legal requirements of EU Directive on the protection of animals used for scientific purposes (2010/63/EU), animals should not be used in procedures, where a non-animal alternative exists, which provide the same or higher level of information as obtained from animal procedures. In April 2018, EURL ECVAM mandated its advisory board (ESAC) to review the available evidence and deliver an opinion on the scientific validity of antibodies and non-antibody affinity reagents produced using animal-free technologies for use in research, regulatory testing and diagnostics. Taking into consideration the available evidence, the ESAC has drafted an opinion on the suitability of existing animal-free technologies to produce affinity reagents with equal or better quality (purity, activity, specificity, affinity, stability) than that offered by antibodies produced using the conventional animal-based methods. In addition, ESAC commented on the scientific benefits of using animal-free affinity reagents and assess whether there are any production and/or application scenarios for which these are not fit-for-purpose and animal-derived antibodies are still indispensable. This talk provides an overview of the report findings, which are anticipated to be published later this year.