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Good cell culture practices & *in vitro* toxicology

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ABSTRACT

Good Cell Culture Practices (GCCP) is of high relevance to *in vitro* toxicology. The European Society of Toxicology *In Vitro* (ESTIV), the Center for Alternatives for Animal Testing (CAAT) and the *In Vitro* Toxicology Industrial Platform (IVTIP) joined forces to address by means of an ESTIV 2016 pre-congress session the different aspects and applications of GCCP. The covered aspects comprised the current status of the OECD guidance document on Good *In Vitro* Method Practices, the importance of quality assurance for new technological advances in *in vitro* toxicology including stem cells, and the optimized implementation of Good Manufacturing Practices and Good Laboratory Practices for regulatory testing purposes. General discussions raised the duality related to the difficulties in implementing GCCP in an academic innovative research framework on one hand, and on the other hand, the need for such GCCP principles in order to ensure reproducibility and robustness of *in vitro* test methods for toxicity testing. Indeed, if good cell culture principles are critical to take into consideration for all uses of *in vitro* test methods for toxicity testing, the level of application of such principles may depend on the stage of development of the test method as well as on the applications of the test methods, *i.e.*, academic innovative research *vs.* regulatory standardized test method.

1. Introduction

Good Cell Culture Practices (GCCP) can have different levels of application, and yet are critical when making use of *in vitro* test methods for toxicity testing. On the 17th October 2016, the European Society of Toxicology *In Vitro* (ESTIV), the Center for Alternatives for Animal Testing (CAAT) and the *In Vitro* Toxicology Industrial Platform (IVTIP) joined forces to address by means of an ESTIV 2016 pre-congress session, the different aspects of GCCP.

At an international level, the OECD is developing a ***Guidance Document on Good In Vitro Method Practices***. This guidance document aims to ensure that the extensive process from *in vitro* method development to *in vitro* method implementation for regulatory use is as efficient and effective as possible taking into account the current scientific, technical and quality good practices. It targets all key players involved in that process and covers test method development, standardization, harmonization and international acceptance.

From a scientific point of view, a number of ***technological advances*** took place with the turn of the century including the use of more complex cell systems (stem-cells, co-cultures, scaffolds, extracellular matrices, 3D cultures), their combination in microphysiological systems; and the use of mechanistic biomarkers and assessment of cell responses by *e.g.* high-content methods. Progress in quality assurance, reporting on cell cultures as well as the validation of cellular test systems are pre-requisites for meaningful and reliable results in safety sciences, ultimately supporting risk assessment and product development decisions. Beside the technical development of new organotypic cultures (human-on-a-chip) and stem cell-derived models, there is a need for both conceptual steering and quality assurance of current practices. The concept of mechanistic validation is a possible way forward to quality-assure new cell-based tests.

In particular ***stem cells***, in contrast to many of the transformed and tumour-derived cell lines often used for *in vitro* toxicity testing, are genetically more stable and proficient in major cellular pathways necessary for accurate health hazard assessment. Furthermore, stem cells can be differentiated into a wide variety of cell types to study

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tissue-specific toxicity. The ToxTracker assay is an example of a novel mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways upon exposure to unknown compounds. An extensive validation of ToxTracker strictly following the GCCP guidelines and standardized protocols, confirmed the advantages of stem cells for *in vitro* carcinogenic hazard identification by unveiling activation of specific cellular signalling pathways upon exposure and delivering insight into the underlying mechanism of toxicity. The implementation of stem cells in combination with standardized cell culture protocols can significantly reduce misidentification of toxic properties of chemicals and improve *in vitro* human health hazard identification.

Finally, ***Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP)*** are in part or in all applicable to the conduct of *in vitro* studies intended for regulatory use. However, while the original principles were developed for chemical entities (GMP) and for *in vivo* non-clinical studies (GLP), they are often not fully deployable to *in vitro* toxicology studies. An optimized implementation of the (GxP) systems for regulatory purposes is needed to allow a dynamic and flexible quality system that reaches regulatory compliance while turning constrains into advantages and benefits whenever possible.

The main aim of the present manuscript is not to provide 'clear cut guidelines', but to report the current status of application of the GCCP principles in the different aspects above mentioned and as presented during the ESTIV-CAAT-IVTIP pre-congress workshop. A summary of the general discussions triggered with the attendees of the pre-congress session and the main conclusions achieved on the different possible applications of Good Cell Culture Practices are also discussed here.

2. Development of an OECD guidance document on good *in vitro* method practices

Safety testing of chemicals is required under several directives of the European Union (EU) and international regulatory environments. Each of these legislative mandates requires submission of sound scientific data to assess the potential chemical hazards and risks for humans and the environment. Data obtained mainly by using *in vivo* toxicity testing methods are submitted to regulatory authorities for safety assessment. To use toxicity data in a regulatory context, an important requirement is that they are of high scientific relevance and quality, reproducible and internationally accepted.

A guidance document (GD) on Good *In vitro* Method Practices (GIVIMP) for the development and implementation of *in vitro* methods for regulatory use in human safety assessment was identified as a high priority requirement. Such a GD aims at reducing the uncertainties in cell and tissue-based *in vitro* method derived predictions by applying all necessary good scientific, technical and quality practices from *in vitro* method development to *in vitro* method implementation for regulatory use. The draft GIVIMP guidance is coordinated by EURL ECVAM and was accepted on the work plan of the OECD test guideline programme in April 2015 as a joint activity between the Working Group on Good Laboratory Practice and the Working Group of the National Coordinators of the Test Guideline Programme (WNT). During the first drafting stage, expert input was received from European regulatory agencies [*i.e.* the European Food Safety Authority (EFSA), the European Medicine Agency (EMA), the European Chemicals Agency (ECHA)], the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), the EU and OECD Working Groups on GLP, Three Rs Centres, a regulatory agency (RIVM), from scientists of large industries and SMEs and from international scientists with expertise in stem cells, cell biology, GLP and *in vitro* methods. The draft document was sent for review to the members of the EU-NETVAL (37 laboratories across Europe), to the experts of the Working Group on Good Laboratory Practice and the WNT.

The Guidance aims to 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. It describes the areas related to *in vitro* method development, standardisation, harmonisation and international acceptance that would benefit from more detailed scientific, technical and quality guidance. This guidance is not intended to duplicate or replace existing OECD Guidance Documents but rather to complement them by addressing specific gaps and collecting available references and information on current best scientific, technical and quality practices in one document.

The GIVIMP GD is divided into 10 sections covering:

1 **Roles and responsibilities**: Targets all players involved in the process *e.g. in vitro* method developers, test system (cells, tissues) providers, validation bodies, producers of equipment, materials and reagents, *in vitro* method end-users (*e.g.* EU-NETVAL, testing laboratories, large industries and small to medium enterprises), receiving authorities and GLP monitoring authorities.
2 **Quality considerations**: Discusses quality assurance *versus* quality control, quality risk assessment and details quality control requirements for development and implementation of *in vitro* methods, the types of documentation needed and quality considerations regarding the integrity of the data.
3 **Facilities**: Elaborates safety, risk assessment and management, on proper facility design to ensure integrity of the cell and tissue cultures used, the *in vitro* methods themselves and the resulting data. The document describes guidance on level of separation to avoid cross-contamination, air handling, water supply, environmental control, heating and cooling, and quarantine measures for new test systems.
4 **Apparatus, material and reagents**: Equipment requirements and quality requirements for material and reagents (*e.g.* use of serum, alternatives to the use of animal sourced serum, antibiotics, special media, certificate of analysis, stability and traceability) are detailed.
5 **Test systems**: Elaborates on Good Cell Culture Practice (Coecke et al. 2005; Pamies et al. 2017), logistics, cryostorage, handling, identification, containment, authentication and characterisation of the test system (*e.g.* cell lines, stem cells, primary cells, engineered tissues, *etc.*) already at the development stage.
6 **Test and reference/control items**: Test item characterisation, solubility and handling, test system and test item interaction, biokinetics, method design considerations during development to ensure test item compatibility and correct and reliable exposure are described.
7 **Standard operating procedures (SOPs)**: The section elaborates on the correct documentation of *in vitro* methods for routine testing including requirements for clear and concise SOPs.
8 **Performance of the method**: Elements of *in vitro* method design in the developmental stage are detailed including aspects of the statistical methods used, plate layout examples, data analysis, examples of data-intensive *in vitro* methods, acceptance criteria, signal intensity, signal variability and plate uniformity assessment, reliability of endpoint calculations, accuracy, reliability and uncertainty.
9 **Reporting of results**: Guidance is given on publishing and reporting of *in vitro* method studies and on data reporting for regulatory purposes.
10 **Storage and retention of records and materials**: The section gives insight on how to archive and retain key records and materials including their retrieval, back-up and restoration. It also details adequate document and record management of processes and the traceability of origin of materials and key decisions.

When developing and implementing *in vitro* methods intended for regulatory purposes, good practices, *e.g.* good scientific practices and good quality practices, are a critical prerequisite (Rispin et al. 2004; Gupta et al. 2005; Coecke et al. 2014; Coecke et al. 2016). Due to