Information for course users: E-learning module EU-60: "Developing Reliable and Relevant *In Vitro* Methods and Approaches for Scientific Purposes and Regulatory Use"

Welcome to this instruction guide for users. This document aims to provide information on the content of this eModule, and give some recommendations to better comprehend the module content and use it efficiently.

This instruction guide is sub-divided as follows:

1) Module content

    Target audience

    Prior knowledge required and expected level after module completion

2) How to use this eModule – some recommendations

    Where can I find reference material or additional materials in the eModule?

    What are the sources identified in part 2 and how should they be used?

    What may I expect from the additional materials?

    Can I divide the module in several parts?

    Should I prepare or study before this eModule?

3) In-depth explanation lesson by lesson

1) Module content

This module provides guidance to *in vitro* test method developers and others interested in improving the speed and efficiency with which new methods or approaches are developed, tested, optimised and approved by regulatory bodies. A self-assessment survey is available at the beginning of the module for users to rate their understanding of the contents covered by the module. Furthermore, a knowledge assessment based on case study exercises is provided at the end of the module for users to test the knowledge gained on the contents of the module. The module consists of 4 parts (Figure 1): 1) Context and needs for reliable and relevant *in vitro* methods, 2) Method development and implementation based on Good In Vitro Method Practices (GIVIMP), 3) Demonstrating the scientific validity of a new method or approach and 4) knowledge assessment.

To optimise learning, we would highly recommend dividing the module into several sessions. Parts should be followed in the order indicated above. You may divide the module into three or more sessions (e.g., session 1: part 1; session 2: part 2; session 3: parts 3 and 4). If you are new to the field of *in vitro* method development, we would advise you to further divide your time to complete part 2 into several additional sessions in alignment with the topic groupings presented in the module (e.g. first session for part 2: introduction and section 2.1, Purpose of GIVIMP; second session for part 2: 2.2, Test System Considerations; etc.). This division of the course content will provide enough time to look into references and further reading materials.

Target audience

The target audience consists of *in vitro* method developers, industry toxicologists, regulators, university students, biology teachers, established scientists new to the *in vitro* field, and providers of training on new approach methodologies or more generally anyone interested in learning more about the development and regulatory acceptance of non-animal testing methods.

Prior knowledge required and expected level after module completion

No specific previous knowledge is required. However, a basic understanding of cell culture techniques and general familiarity with established *in vitro* methods would be helpful.

After completion, you will understand the context in which new *in vitro* methods are developed; drivers for creation of new methods; recommendations to follow during development of novel *in vitro* methods that will help to improve the quality and robustness of your method; suggested elements for inclusion in reporting on your new method; and understand the process of method validation and approval by regulatory bodies.

		Learning Objectives
Introduction	Lessons 1-2	• Introduction to the course • Review your understanding
PART 1: Reliable and Relevant *In Vitro* Methods	Lessons 3-7	• Describe the various drivers for developing reliable and relevant non-animal methods or approaches • Identify the European Union (EU) directives, regulations and guidance documents that require the use of alternatives to animal testing in different sectors • Describe examples of existing non-animal methods or approaches that can replace, reduce, or complement the use of animals • Describe the different steps, from test method development, optimization, validation and peer review to regulatory implementatic
PART 2: Method Development and Implementation Based on Good *In Vitro* Method Practices (GIVIMP)	Lessons 8-11	• Summarize the aims of GIVIMP • Describe how and where GIVIMP can be used • Define the roles and responsibilities of the various actors in the *in vitro* method lifecycle
	Lesson 12-14	• Explain the importance of cell and tissue sourcing, authentication and documentation • Consider quarantine procedures, contaminant screening and safe handling practices for test systems
	Lesson 15-16	• Determine the appropriate reference/control items for a method • Define the appropriate acceptance criteria for method performance and results
	Lesson 17-18	• Define how to prepare test items • Explain the effects of solubility, stability, and interference of the test item with media components or test system • Define the applicability, limitations, and uncertainties of a method • Identify critical elements that may have an impact on the method
	Lesson 19-20	• Determine the proper level of detail in a standard operating procedure for an in vitro method • Apply the concept of quality risk assessment to *in vitro* method use
	Lesson 21-24	• Determine the level of calibration, testing or validation needed for the apparatus and equipment used to perform the method • Recommend experimental design and logistical considerations for in-house validation • Evaluate the intra-laboratory reproducibility and preliminary performance of a method, such as linearity, accuracy and precision, sensitivity and specificity • Compare options for training activities and identify those that are critical for the quality of the *in vitro* method • Decide when a method requires optimization or is ready for transfer and further validation
PART 3: Demonstrating the Scientific Validity of a New Method or Approach	Lesson 25-29	• Understand what is meant by a scientifically valid test method or approach • Determine the information required to demonstrate the reliability and relevance of a test method or approach • Understand the validation principles and study designs used to assess the reliability and relevance of a test method • Understand the OECD concepts of mutual acceptance of data (MAD), test guidelines, guidance documents, the OECD principles of Fair, Reasonable and Non-Discriminatory Intellectual Property Rights (IP FRAND principles)
PART 4: Knowledge Assessment	Lesson 30	• Knowledge assessment
GLOSSARY	Lesson 31	• Glossary

Figure 1: Learning objectives per parts and lessons.

2) <u>How to use this eModule? – Some recommendations</u>

Q&A: how to use the eModule

Where can I find reference material or additional material in the eModule?

All references and further readings are provided at the end of each lesson. They are comprised of scientific articles, sections of books, websites, and videos. Clicking on any link will open a new window, from which you can download or visualise the additional material.

To note: the collection of references and further reading was created during 2020-2021 and was checked in May 2021.

What are the sources identified in part 2 and how should they be used?

Since part 2 provides training on the OECD GIVIMP document, the chapter and subpart of that document where the material presented in the lesson comes from has been identified in the "Source" section at the bottom of each lesson. It may be helpful to read those sections of the GIVIMP document for a more thorough understanding of the content presented within the lesson.

What may I expect from the additional materials?

The references and further readings at the end of each lesson are resources that provide additional details and more information on the topics discussed within the lesson. The lesson can be reviewed and completed without reading the additional materials, but they do enrich the content of the lesson. It is not necessary to review the references and further reading to complete the assessment in part 4.

Can I divide the module in several parts?

Yes, you can. The course has been divided into 4 parts. Each part can be done independently. However, some lessons within part 2 belong together and should be done in the order in which they appear, to sustain coherence. If you would like to divide the module into several sessions or focus only on some parts/lessons, we would advise that Parts 1 and 3 could be used together. Part 2 could be used as stand-alone material. To successfully complete the knowledge assessment in part 4, all previous lessons must be completed.

Should I prepare or study before this eModule?

The module will guide you step by step and no prior studying should be required. A comprehensive glossary for the module is provided after part 4 and is linked to at the bottom of each lesson. It may be helpful to reference this glossary and any sources of content identified within the module as you complete this section. It is not required that you do this ahead of completing the course.

Some of the questions and interactive activities within part 2, included to check your understanding of each lesson, rely on a basic understanding of cell culture techniques. If you do not have a background in cell culture techniques, it may be helpful to review the materials linked within lesson 13 provided by SaferWorldbyDesign prior to completion of the next several lessons.

(https://saferworldbydesign.com/saferliver/heparg/tutorials-and-support/protocols/)

3) In-depth explanation lesson by lesson

Lesson 1: Introduction to the course, background, and learning objectives

Lesson 2: Review your understanding

-A small survey to rate students' first knowledge and understanding of the topic addressed in this course

PART 1: Context and Needs for Reliable and Relevant *In Vitro* Methods

Lesson 3: An introduction to part 1

-Learning objectives for part 1

Lesson 4: Drivers for developing reliable and relevant non-animal methods or approaches

-Explanation of the different types of drivers of NAM development, i.e. scientific, regulatory, economic, and ethical

Lesson 5: Legislation that calls for the use of alternatives to animal testing

-Discussion of the EU Directive 2010/63/EU

-Examples of other EU legislation and guidance on the use of alternatives for different sectors (e.g. cosmetics, chemicals, mixtures, biocides, medicinal products)

- Information on the broader international acceptance of scientifically valid test methods

Lesson 6: Examples of alternative approaches to animal testing

-Discussion on Adverse Outcome Pathways (AOP)s, Integrated Approaches to Testing and Assessment (IATA), and Defined Approaches to Testing and Assessment (DA)s

-Video on the use of new methods in risk assessment

Lesson 7: The process from developing a method to its regulatory implementation

-Describes the process for gaining regulatory acceptance of a new method

-Explains why scientific validation of test methods and approaches is useful

-Provides the minimum elements required to start a validation study

PART 2: Method Development and Implementation Based on Good In Vitro Method Practices (GIVIMP)

Lesson 8: An Introduction to part 2

-Background on this part of the module and learning objectives for part 2

Lesson 9: Aims of GIVIMP

-Discusses why GIVIMP was created

-Video introduction to GIVIMP

-Purpose of GIVIMP

Lesson 10: How and where to use GIVIMP

-Recommendations for how to use the GIVIMP document

Lesson 11: GIVIMP users

-Outline the groups that can benefit from using GIVIMP specific to method development

-Video testimonials of users on the GIVIMP document

Lesson 12: Test system characterisation and documentation

-Discusses documentation needed concerning the test system used

-Provides guidance on considerations for test systems modified in a proprietary way

Lesson 13: Quarantine and contaminant screening the test systems

-Shares how to handle newly acquired test systems

-Video showing cell culture techniques in a laboratory

-Protections against contamination for laboratory staff and the laboratory environment

Lesson 14: Considerations for handling test systems

-Handling techniques for preserving test system quality and integrity

-Suggestions for preservation for future use

Lesson 15: Selection and use of reference/control items

-How to determine the appropriate reference and control items for your method

Lesson 16: Define appropriate acceptance criteria

-How to determine if a method performs consistently

Lesson 17: Test item preparation and concentration range

-Ways to handle materials to be tested in the method

-Information on potential interferences with test items

Lesson 18: Assessment of method limitations, risks and uncertainties

-Information on elements in test method design that could have unintentional effects on the data

-Determining limitations of the method

Lesson 19: Preparation of standard operating procedures

-How to provide others with instructions for performing the method

-How to ensure the method is of high quality

-When to start documenting the steps for performing the method

Lesson 20: Determine the proper level of detail in a standard operating procedure

-How to know if an SOP is detailed enough

Lesson 21: Maintaining and calibrating equipment

-Controls to put in place for equipment used in a method

Lesson 22: Staff training and development

-How to promote correct performance of the method

Lesson 23: Assessing method performance using in-house validation

-What to do after method optimization

-Explanation of in-house validation

-Suggestion of things to analyse in an in-house validation

-How to share the method with others

Lesson 24: Reporting

-Documenting method information for manuscript publication or validation assessment by regulatory authorities

PART 3: Demonstrating the Scientific Validity of a New Method or Approach

Lesson 25: An introduction to part 3

-Learning objectives for part 3

Lesson 26: What is a scientifically valid test method or approach

-Explanation of when a test method or approach is considered valid

-Description of the information needed to demonstrate the scientific validity of a test method

Lesson 27: Information requirements to demonstrate the reliability and relevance of a method

-Outline of information required to complete the seven modules of the modular approach to validation

Lesson 28: Principles and study designs to assess the scientific validity of a test method

-Conditions to take into account when planning a validation study

-How to plan a validation study

-Possible designs for a validation study

Lesson 29: Understanding the principles of OECD test guidelines and guidance documents

-Requirements for OECD Test Guideline Programme consideration for a test method or approach

-Explanation of the OECD mutual acceptance of data (MAD) and its applicability

PART 4: Knowledge Assessment

Lesson 30:

-Knowledge assessment based on case study exercises

Lesson 31: Glossary

-Glossary of terms used throughout the eModule

We hope that this document provided you with enough information to fully comprehend the content and scope of this eModule. We sincerely wish you best of luck in your studies and thank you for considering our eModule.