# EUROoCS RAB (IN)FREQUENTLY ASKED QUESTIONS

This document compiles questions that are frequently asked or should be considered by Organ-on-chip developers regarding the use of this technology for regulatory purposes (according to the EUROoCs Regulatory Advisory Board [RAB]).

The different colour (shades) refer to the various categories of questions and distinct regulatory domains.

In the answers one can find references to documents (REF XXX_YY) that are found in the complementary list of resources.

| | |
|---|---|
| **1. What are the recommendations for cell quality assessment?** | Check the OECD (Organisation for Economic Cooperation and Development) guidance GIVIMP (Good In Vitro Method Practices) (Ref OECD_03, chapter 5) and the GCCP (Good Cell Culture Practice) document (Ref R_11). |
| **2. If I want to combine my OoC models with computational models, where can I find relevant regulatory information?** | Check the OECD GD (Guidance Document) on the Characterisation, Validation and Reporting of Physiologically Based Kinetic (PBK) models for Regulatory Purposes - No. 331 (Ref OECD_05). |
| **3. If I want to submit a validated method, what should I report?** | Check the EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) Test method submission (Ref ECVAM_01). Complete information on most of the test methods EURL ECVAM has validated over the years are reported in TSAR, the Tracking System for Alternative methods towards Regulatory acceptance (Ref ECVAM_03). |
| **4. How can I know the relevant compounds to test in my system?** | Check the EURL ECVAM library of reference chemicals (Ref ECVAM_02) |
| **5. Which scientific challenges are still limiting a reduction in animal testing?** | The main challenge concerning the reduction of animal testing is to sufficiently advance many areas of toxicological and biomedical sciences to deliver complete and effective non-animal solutions that can detect all the possible adverse effects that a chemical could possibly cause to an organism.<br>An example: human reproduction involves multiple organs, tissues, and cells together with hormonal systems that act at the level of the entire human body. Chemicals that are potentially toxic to reproduction could therefore act in a plethora of ways through many different mechanisms.<br>This level of complexity - most likely - cannot be modelled by one in vitro test. It is much more likely that a bank of different tests and/or computational modelling approaches would be needed to get close to mimicking real life human reproduction. |

| | |
|---|---|
| **6. What scientific improvements are still needed to address the most complex cases where alternative tests are not (fully) established?** | We still lack basic scientific understanding of how exactly some chemicals lead to toxicological effects. One common challenge for assessing a chemical against complex systemic health effects is to simply predict where the chemical ends up in the body and for how long it stays there.<br><br>Moreover, chemicals are often metabolised in the body and the resulting metabolites can actual be the source of toxicity.<br><br>Understanding the fate of a chemical in the body, i.e., how it is absorbed, distributed, metabolised (transformed) and excreted, and its so-called toxicological "mode of action" is essential. The identification of key events in toxicity pathways would enable the targeted development of specialised test methods to produce the information needed to decide if a chemical would trigger an adverse outcome, and hopefully, the intensity of this effect in the organism. |
| **7. Is it true that alternative methods are not yet available for some adverse health effects?** | It is correct to say that alternative test methods are not yet sufficiently developed to fully replace some standard animal tests. These include tests for the following adverse health effects or so-called "toxicological endpoints":<br><br>• Repeated-dose toxicity: this relates to issues associated with long term repeated exposure to a chemical.<br>• Carcinogenicity: the ability of a chemical to cause cancer.<br>• Reproductive toxicity: this refers to a wide variety of adverse effects that may occur in distinct phases within the reproductive cycle because of exposure to one or more chemicals. This includes effects on fertility, sexual behaviour, and embryo implantation.<br>• Toxicokinetic: this is the examination of the absorption, distribution, metabolism, and excretion characteristics of toxic substances in humans and for that matter any other living organism.<br><br>However, there exist methods that can be used for screening to understand whether there is a concern for adverse effects also for these endpoints. |
| **8. How to start to use NAMs in chemical risk assessment?** | A useful resource to start understanding chemical risk assessment would be the red book for risk assessment (Ref R_22). Scientific publications more specific on non- |

| | |
|---|---|
| | animal methods and OoC devices are also available (Ref R_20 and R_21). |
| **9. Can I use the same models for chemical risk-assessment and screening?** | No. The purpose of the model should be clearly defined *a priori*. For a simple screening purpose (qualitative information) simpler methods like 2D cell cultures are suitable, while OoC are more promising for risk assessment (quantitative information). |
| **10. Can I use the same OoC models for drug development and risk assessment?** | OoC models used for drug development purposes are usually perfectly tailored for specific (group of) drugs and for a (known) mode of action. To use the OoC for risk assessment, a more holistic understanding of the organ is required, since the pathways leading to toxicity can be multiple and regulators need a set of tools that allow to reproduce all possibilities. One interesting option is to link the OoC model to a specific effect in an AOP, thus making it clear in which part of the toxicological pathway the model can be used (see Ref OECD_09). OoC can be part of an IATA for a specific toxicological endpoint (see Ref OECD_08). Finally, OoC used for chemical risk assessment also need to account for the compound's ADME (absorption, distribution, metabolism, and excretion). |
| **11. The model I have developed investigates one or more of the mechanisms of action that leads to a certain adverse outcome. How can I bring this into a broader context?** | Have you thought of visiting the AOP-Wiki[1]? Have you thought of contributing to developing the Adverse Outcome Pathway (AOP) network? Some Key Events (KE)[2] assessed by your model could already be described in the Wiki and could benefit from the description of a novel method that can be used to detect or measure the biological state represented in the KE. AOPs are based on crowdsourcing from the scientific community!<br>Have you thought of developing a full AOP that causally link MIEs (Molecular Initiating Events) with AOs (Adverse Outcomes)? AOPs are intended to aggregate knowledge currently dispersed in journal articles, textbooks, reports, databases, and many other sources into a systematic and accessible format that facilitates use of that knowledge to support a broad diversity of applications and interests. The AOP concept can be applied as a framework to develop IATA.<br><br>More information on AOP can be found at OECD website[3] |
| **12. Do I need to have an AOP developed to support the mechanistic relevance of my method for chemical risk assessment?** | Although the AOP framework is a good tool to underpin the mechanistic relevance of the methods, it is not mandatory to use for Chemical regulatory applications. There is no need to develop an AOP, but some AOPs |

[1] https://aopwiki.org/h

[2] A key event is a measurable change in a biological system

[3] https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

| | |
|---|---|
| | found in the AOPKB can be used to support method development. |
| **13. Is there a recommended standard template to describe a new OoC study protocol in e.g., the scientific literature?** | GD 211 (OECD_02): This guidance document lists elements considered relevant for providing a comprehensive description of an *in vitro* method to facilitate the interpretation of results and support scientifically defensible fit-for-purpose applications, to be suitable for their use in decision making processes by regulators or by the scientific community and end users in general. Tox-Temp can also be consulted (Ref R_23). |
| **14. I have developed a new test method or a technique that could fit in an existing OECD Test Guideline, who should I contact to discuss its inclusion in the OECD Test Guideline programme?** | You should contact your National Coordinator of the Test Guidelines Programme (list available at the OECD website[4]) to discuss the relevance of the test method to the TG (Test Guidelines) programme. New or updated Test Guidelines are continually developed to meet the regulatory needs of member countries, to reflect scientific progress in the area of hazard identification, to address animal welfare aspects, and to improve the cost-effectiveness of test methods. Proposals for the development of new or updated Test Guidelines can be submitted by the member countries, the international scientific community, industry, non-governmental organisations, all via a National Coordinator. |
| **15. The method I have developed includes elements protected by a patent. Is it possible to submit this method to the OECD for potential Test Guideline development?** | Yes. The IP (Intellectual Property) right owner should then follow the guiding principles developed by the OECD on good practices for the availability/distribution of protected elements in OECD test guidelines[5]. The OECD intellectual property rights policy will require participants wishing to have their protected elements included in the Test Guideline to provide an irrevocable commitment in writing to give access to all third parties and to license their protected elements on fair, reasonable and non-discriminatory terms ('F/RAND Commitment') for the use of the Test Guideline. |
| **16. What if the test method includes Confidential Business Information?** | Test developers are encouraged to use other protection means than confidentiality claim for protected elements in test methods candidate for Test Guideline development. The OECD will temporarily host information on confidential elements on a protected community webpage accessible to the Working Party of the National Coordinators of the Test Guideline Programme only, until the completion of the Test Guideline development. When a Test Guideline is adopted, this information becomes publicly available as supporting material to the Test Guideline. Test developers should seek other means of protecting their |

[4] https://www.oecd.org/chemicalsafety/testing/national-coordinators-test-guidelines-programme.htmh
[5] https://www.oecd.org/chemicalsafety/testing/intellectual-property-in-oecd-test-guidelines.htm

| | |
|---|---|
| | IP than confidentiality (e.g., patent, copyright, tradename, publication, etc.). |
| **17. I have heard of IATAs (Integrated Assessment and Testing Strategies), but what are they exactly? How would my model fit into an IATA? How could I receive guidance and feedback?** | Integrated Approaches to Testing and Assessment (IATA) are pragmatic, science-based approaches for chemical hazard characterisation that rely on an integrated analysis of existing information, coupled with the generation of additional information using testing strategies. IATAs can include a combination of methods and can be informed by integrating results from one or many methodological approaches [(Q)SAR, read-across, *in chemico, in vitro, ex vivo, in vivo*] or omics technologies (e.g., toxicogenomic). The OECD IATA case study project provides a forum for scientific exchange of approaches on how novel methods are applied to assess the hazard of chemicals and establish common and best practices for the use of these methods for assessing different types of chemicals. IATA case studies submitted by member countries are reviewed and discussed every year. Non-guideline/non-standard methods can be used within IATA but fall outside of the provisions of OECD Mutual Acceptance of Data[6]. |
| **18. Does the European Food Safety Authority (EFSA) have a qualification system for *in vitro*/non-animal models applicable to Organ-on-Chip (OoC)?** | Not yet, but it is under development. EFSA is following several EU research projects, and other methodological developments relevant to EFSA assessments may be proposed for case-by-case discussion. Proposals can be submitted to the Methodology and Scientific Support (MESE) Unit[7]. |
| **19. Does EFSA have a guidance for the use of data generated in *in vitro* non-validated methods?** | EFSA has developed guidance for the assessment of peer-reviewed open literature results (EFSA_02) it is not specific to OoC or NAMs (New Approach Methodologies), but the same principles apply. The guidance document (EFSA_01) also differentiates between "valid" and "validated" methods and provides recommendations for using results from not-validated methods. |

[6] The 1981 OECD Council Decision concerning the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals is built on the OECD Test Guidelines and Good Laboratory Practice Principles (GLP). The Council Act requires OECD governments to accept chemical test data developed for regulatory purposes in another country if these data were developed in accordance with the OECD Test Guidelines and GLP Principles. This means new data for notifications or registrations of a chemical only have to be developed once and are then used across OECD countries.

[7] MESE@efsa.europa.eu