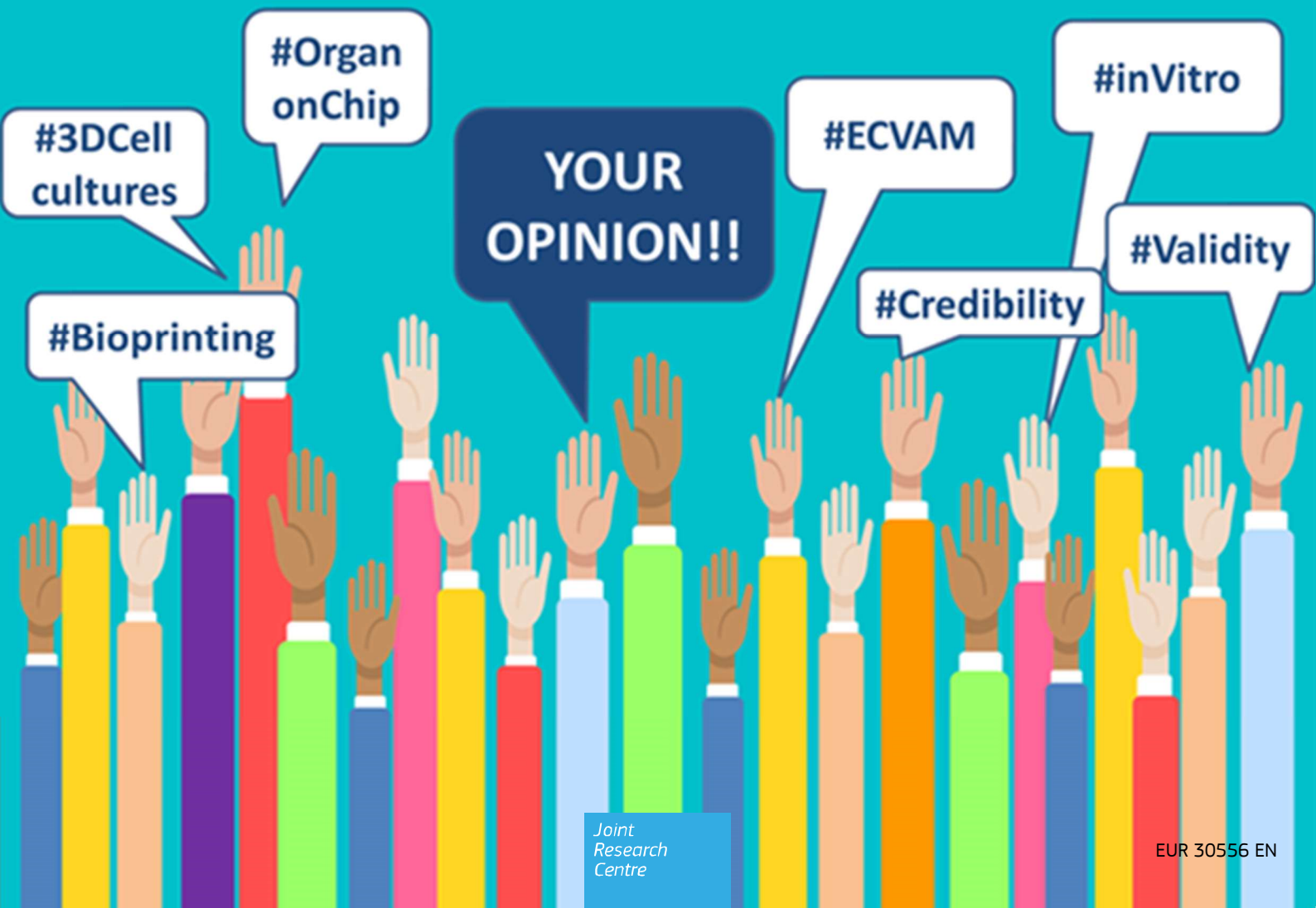
European Commission

Establishing the scientific validity of complex *in vitro* models

Results of a EURL ECVAM survey

2021



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Contents

Abstract

Complex *in vitro* models (CIVMs) - such as 3D cell cultures and spheroids, microphysiological systems including organ-on-chip devices, bioreactor cultures or bioprinted tissues – aim to represent higher-level anatomical and physiological aspects of human biology in experimental studies. The underpinning technologies are developing fast and the models and methods being created constitute promising approaches in several different scientific areas with many applications in research and regulatory testing. However, the successful implementation and acceptance of CIVMs is likely to require their proper characterisation, validation or qualification to demonstrate to end-users that they are fit for a particular purpose or context of use. To explore this, the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) of the European Commission's Joint Research Centre (JRC) conducted a survey to investigate stakeholder opinions and perceived needs. The outcome of the survey showed that i) there is high interest in establishing some kind of assessment approaches for CIVMs; ii) assessment approaches (even if conducted differently) should be adequate not only for regulatory use-contexts, but also address applications in research; and iii) CIVMs are still under (technological and biological) development and are thus not yet mature or standardised enough to enable a consensus on how their assessment should be conducted.

INTRODUCTION

1 Introduction

Animal studies have been the dominant means to increase scientific knowledge on human biology and disease. However in recent years, evidence has emerged that animal models may be a poor predictor of the human situation, particularly in the areas of safety pharmacology and toxicology. Already in 2004, the US Food and Drug Administration (FDA) stated in their innovation report [1] that traditional animal models are often deficient for the discovery of new scientific knowledge, the identification of promising therapies, or for reliable safety profiling of new drug candidates [1]. Since then, more and more work has shown the limitations of animals in mimicking several human biological processes such as inflammation [2], immune response [3], stroke [4], Alzheimer disease [5], cancer [6], ageing [7], diabetes [8] and cardiovascular diseases [9], among others.

For decades, typical 2D monolayers of immortalised cells have been the mainstay of *in vitro* methods. However, the simplicity and cancer-like phenotypes of these cell lines that allow an easy manipulation are also the reasons why they fail to recapitulate many aspects of normal human biology. Several shortcomings of the 2D cultures can be overcome when using more complex *in vitro* models (CIVMs) such as 3D cultures or organ-on-chip that better mimic the organ/tissue organization [10].

There is in fact a growing interest in CIVMs. This is not only demonstrated by the increasing number of CIVMs, but also by: a) their capacity of representation of human physiology [11]; b) the continuous growing number of publications related to such models (annual increase of 4x from 2014 to 2018); c) the rising interest of biomedical researchers in CIVMs even in the absence of specific expertise [12]; d) the amount of institutions in the EU (e.g. H2020, ORCHID, CRACKIT[1], IMI) and USA (e.g. NCATS, FDA, CFSAN) investing in such technologies [13]; and f) several Pharma companies that already use and support CIVMs (some examples are GSK, Astrazeneca, Pfizer, J&J, Merck, Roche and Takeda Pharmaceutical) [14].

The technological aspect of these systems had a quicker initial development when compared to the biological counterpart. Nevertheless, in the last years a gradual improvement and attention to replicate human biological mechanistic effects is observed. These improvements have put these models in the spot light of many scientific communities. However, the wider use of CIVMs requires that trust is build, not only from the developers, but also from the end-users and evaluators points of view. As for any other model, this is achieved by a proper characterisation of the model and proving its relevance and reliability. Such an assessment is standard for methods used in the regulatory arena, but this is not always the case in research where a "reproducibility crisis" has been reported in a Nature survey in 2016 [15].

In regulatory toxicology, a way to increase trust in a method is through validation. The several rounds of performances challenging the method reproducibility and relevance towards validation gives the regulator (or anyone willing to take on the method) a higher level of confidence. Usually a test method is validated for a specific purpose. Would a similar approach be possible for a model that is used for different applications, and to address different scientific questions? These and other questions regarding the scientific credibility of these models have been raised in different fora with no harmonized agreement among the experts.

These challenges prompted EURL ECVAM to develop a **survey on CIVMs**. The **purpose** of the survey was to consult a broad community of stakeholders to get a better understanding of the level of uptake and satisfaction regarding such models, and the current thinking around how best to establish their validity for use in research and testing, with a view to building end-user confidence. The results of this survey are intended to be disseminated and shared with all the CIVMs community and the scientific community at large (including developers, potential users and assessors) starting from the respondents that showed interest of being further contacted.

[1] https://nc3rs.org.uk/crackit/crack-it-challenges

BUILDING THE SURVEY

2 Building the survey

The survey questions (Annex I) and its structure were built taking into account several publications addressing the theme of CIVMs and their implementation [11][22][17][23][24]. There are general questions regarding validation, qualification or assessment of models as well as on how to establish their relevance for particular contexts of use. The survey constitutes 14 multiple-choice questions divided into three main sections: I. respondent profile; II. respondent experience (general and with CVIMs) and III. opinion on the credibility and validity of CIVMs. The majority of the questions allowed the respondent to select more than one option.

In the context of the survey, CIVM was not explicitly defined but presented as referring to 3D cultures, spheroids, organoids, bioreactor cultures, 3D bioprinting, organ/body-on-chip, microphysiological systems, or similar. The only term for which a definition was provided was "validation", which according to OECD Guidance Document No. 34 [25], is , the process by which the reliability (i.e. reproducibility, repeatability) and relevance of a method is established for a defined purpose.

Questions were uploaded in the European Commission survey platform[2] and the invitation to complete the survey was disseminated among different EURL ECVAM networks and a broad community of stakeholders with potential interest in such models, independently from their experience. The survey was conducted over a period of 6 weeks (23rd April to 31st May 2018).

[2] https://ec.europa.eu/eusurvey/home/welcome

MAIN FINDINGS

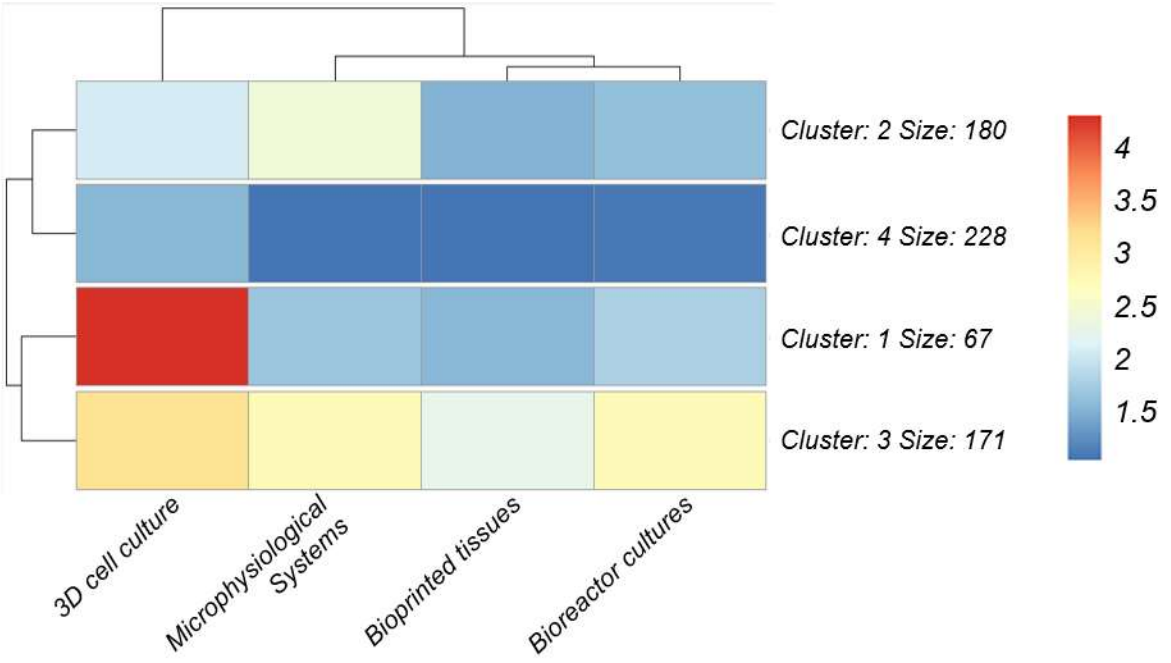
3 Main findings

3.1 Profile and experience of the respondents

A total of 646 replies were received from 36 countries and are freely available[3]. Of these, 54% of the respondents are from Academia, 23% from industry, 17% from governmental/public service while the remaining 6% are from non-governmental or other sectors. The majority of the respondents are from Europe[4] (89%). They are also quite experienced as 51% had more than 10 years' experience *in vitro* and 48% have more than 10 years of experience *in vivo* (these two groups were overlapping). From the listed areas of expertise, the majority of the respondents have experience in toxicology, followed by disease modelling, pharmacology and oncology/cancer.

In terms of **general experience** with the different CIVMs (question 4) the respondents have most experience with the 3D cultures (also the model that was developed first), while they have the least experience with the Bioprinted tissues. The ranking is: 3D Cell cultures/Spheroids (91%) > Microphysiological Systems (68%) > Bioreactor cultures (54%) > Bioprinted tissues (45%). The combined experience in the different CIVMs is depicted in Figue 1. Only about 1% of the respondents have no experience with any of the listed types of models.

Figure 1. Experience of the respondents with the different Complex *in vitro* models (CIVMs), results to question 9 (How do you define your experience with complex *in vitro* models?).
A score (ranged from 0.5 to 4.5) was assigned to each respondent depending on their experience (including generally information, experience in research, developmental and regulatory decision making, combined experience) in the four fields (3 D cell culture and spheroids, Bioprinting tissues, Bioreactor cultures, Microphysiological Systems as Organ-on-chip). By applying hierarchical cluster analysis, it was possible to classify the respondents and divide them in four homogeneous groups. The most numerous group was "Cluster 4" including 228 people with modest experience in all the complex systems. A restricted group was expert in 3D cell cultures ("Cluster 1", 67 people), whereas more respondents are highly experienced in Microphysiological System ("Cluster 2", 180 people). All the other participating ("Cluster 3", 171 people) have a general competence in all the complex systems..



Altogether, the distribution of the respondents over the different profiles and experience groups based on the replies to questions on parts I and II of the survey (Annex I) is quite uneven. When designing the questionnaire, the idea was to analyse the replies more in depth and by groups, but when retrieving the replies it was realised

[3] https://data.jrc.ec.europa.eu/dataset/57058ab5-e037-42c3-b664-80d4ccd8dbf8

[4] Austria; Belgium; Croatia; Czech Republic; Denmark; Estonia; Finland; France; Germany; Greece; Ireland; Italy; Latvia; Luxembourg; Malta; Monaco ;Netherlands; Norway; Poland; Portugal; Romania; Slovenia; Spain; Sweden; Switzerland; United Kingdom.

that this would lead to inaccuracies, since the group sampling (for group comparison) is quantitatively different. It was then opted to analyse the general opinions, treating the respondents as a whole and take the main results. In the future, if relevant, a group-based analysis might be conducted giving different weights to the different groups.

To set the scene and understand the respondent's uptake of the information available on CIVMs, the first opinion questions were related to what is captured in the **peer-review publications**. The majority of the surveyed people believe that the CIVMs described in scientific papers are relevant for scientific/biological applications but not for regulatory decision making. Regarding the different CIVMs level of satisfaction with the respective publications, the ranking (from the highest satisfaction to the lowest) is: 3D Cell cultures/Spheroids > Bioreactor cultures > Microphysiological Systems > Bioprinted tissues.

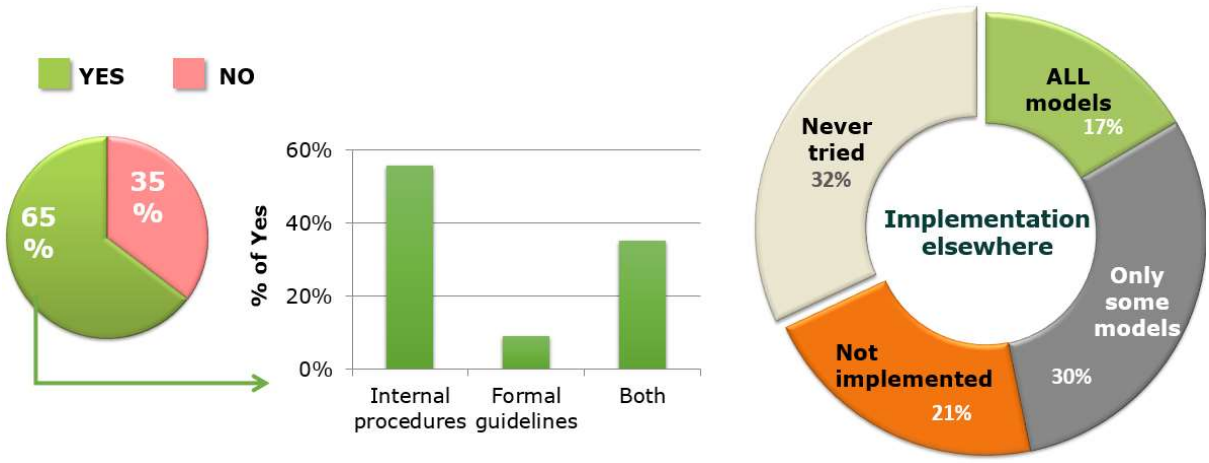
The main type of experience with CIVMs is in the research field (53%). Additionally, the physiological systems that the respondents have more experience with are liver/hepatobiliar, skin and nervous system (including brain). Since the development of the CIVMs, the biological (functional and morphological) reproduction *in vitro*, varies from organ to organ (some are more advanced than others). One should keep in mind that the experience of the respondents on a specific organ may influence their opinions towards the CIVMs in general.

The general satisfaction with CIVMs is high, but it should not be ignored that the sample being analysed might not be fully representative of the general scientific community, as the people more familiar with these models would feel more inclined to reply to the survey.

3.2 Current practices to assess CIVMs

The next question with pertinent results aimed at understanding if the respondents with experience in a certain type of CIVM would perform an **assessment to increase trust in their model**. When asked if they were already performing some kind of ***in house*, internal validation** of the models used, 65% of the respondents replied positively (Fig. 2a). Nevertheless, the majority of those used only internal procedures, without any type of formal/external acceptance/review (Fig. 2b). This could also be the reason why only one quarter of the respondents who tried to implement their models elsewhere were successful, while 51% managed to implement only some (30%) or none (21%) of the models (Fig. 2c).

Figure 2. *In house* validation, a) and b) main results to the question 9.4 of the survey (Have you or your colleagues performed *in house* validation of any complex *in vitro* model?); c) main results to the question 9.5 of the survey (Have the models developed in your laboratory been successfully implemented and used in a different location/facility?).



3.3 Credibility and validity

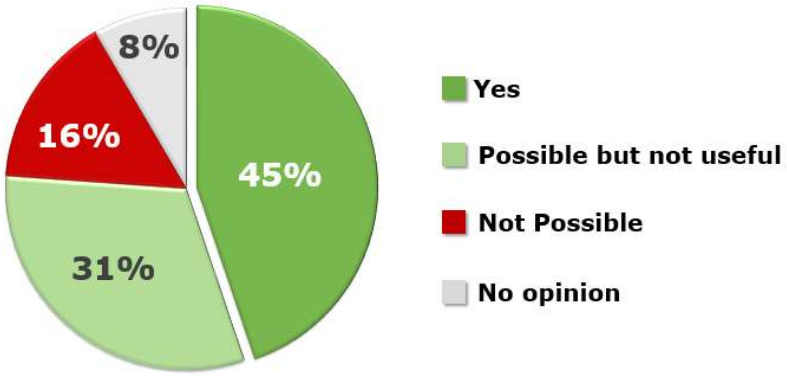
With question 10, people's opinion was assessed on whether **establishing the credibility or validity of a complex *in vitro* model outside a context of use would be possible and useful**. This would mean for instance that a liver organoid could be assessed independently from its use for hepatotoxicity testing or drug

efficacy and metabolism. This question was actually divided into two parts: first, to understand if the respondents find the assessment in such conditions possible and, in that case, if they find it useful to establish the validity of the CIVMs outside the context of use.

The majority of the respondents replied yes (45%) to both parts of the question, followed by the ones that believe that it is possible but not useful to increase its acceptance and use (31%) (Fig. 3).

Following the lead opinion of almost half of the survey experts, there should be a way to assess the model reliability that would then serve multiple purposes. Such activity would create a useful quality 'stamp' of a model that could feed both research and industry, making it more attractive. Still a bit more than one quarter of the respondents do not see any technical limitation for such untargeted assessment, however they believe that it is not relevant nor useful, unless the purpose is defined.

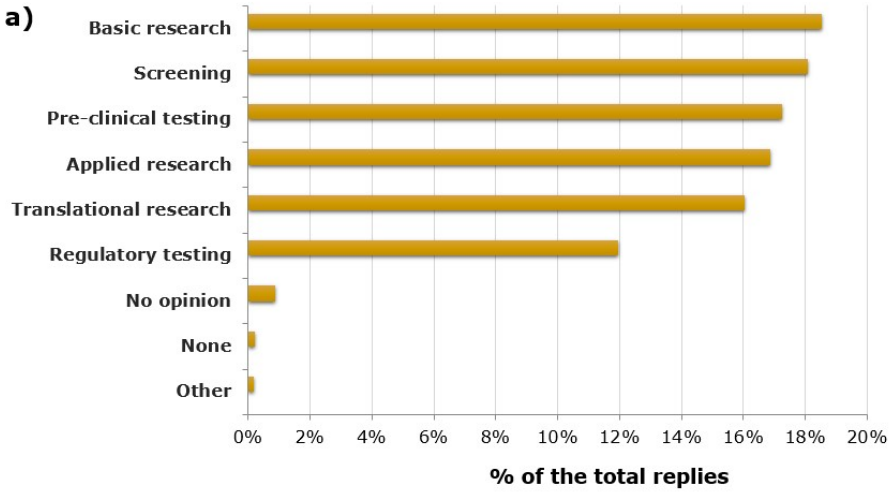
Figure 3. Possibility and usefulness of establishing the validity of a complex *in vitro* model outside a context of use to increase acceptance and use. Main results of question 10 of the survey (*In your opinion, establishing the credibility/validity of a complex in vitro model outside a context of use (i.e. without a clear definition of purpose) is*:)

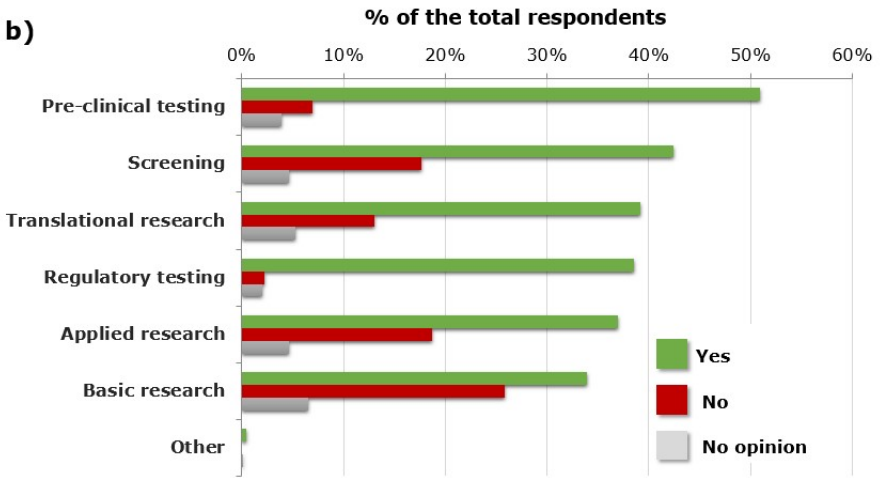


3.4 Factors that influence acceptance

It was subsequently asked for which of the listed scientific applications, would an **assessment of the CIVM lead to an increase of its acceptance.** All had similar levels of agreement except for regulatory testing which was lower (Fig. 4a). One explanation is that since this is the normal procedure in regulatory testing – the assessment of the methods – the level of acceptance could not further increase. Unfortunately, the data collected do not allow us to further interpret such distinction. In any case, there is a general trend that acceptance can be increased in all the other areas of application. Nevertheless, when the assessment is performed, there is almost a general consensus that it should be independently reviewed (Fig. 4b), especially in the case of regulatory testing (higher Yes/No ratio) and despite the fact that most of the respondents do it using *in house* procedures (Fig. 2b).

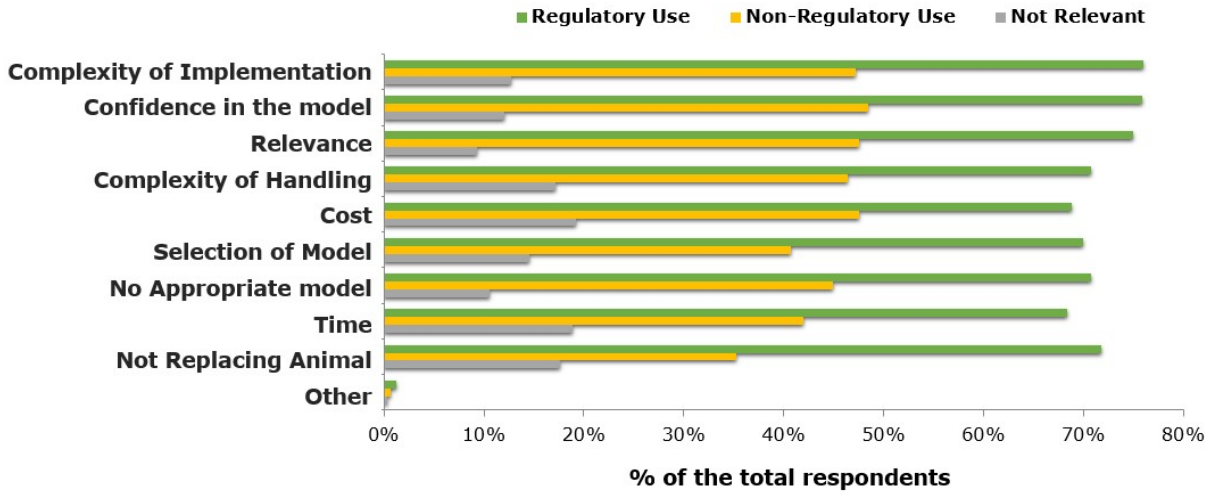
Figure 4. Applications where assessment: a) could increase acceptance and b) should be independently reviewed. Main results to the questions 11 and 11.1 of the survey (*For which of the following applications do you think an assessment of a complex in vitro model could potentially increase its acceptance and use?; Should the assessment of a complex in vitro model be independently reviewed (e.g. by an independent body) in order to guarantee a wider acceptance and use?*)





Assessment, through a formal validation or even a simpler approach is normally seen in science as a way to build trust in a system. Questions 11 and 11.1 (Fig. 4) intended to understand in which areas a simple model assessment would increase its use. Nevertheless, it was clear that this is not the only factor. Therefore, in question 12, a list of nine **possible factors that would also contribute to restricting a wide use of the CIVMs** was presented. All nine factors were considered equally limiting for the wide acceptance of the models (Fig. 5). The only difference observed was that a higher percentage of the respondents believe that such factors would affect more the incorporation of the CIVMs in regulatory use (70%), while a lower percentage believe that they would affect its acceptance in non-regulatory use (40%). In order to increase the acceptance of these models, efforts should be made at all the factors considered. Such efforts do not only include the further development of the model, but also communication endeavours. For instance, are the developers expecting that such models would replace animals? It is important to invest in the proper description, framework, simplification, training and transferability of CIVMs.

Figure 5. Factors that compromise acceptance. Main results to the question 12 of the survey (*Which factors/perceptions may compromise a wide acceptance and use of complex in vitro models?*)



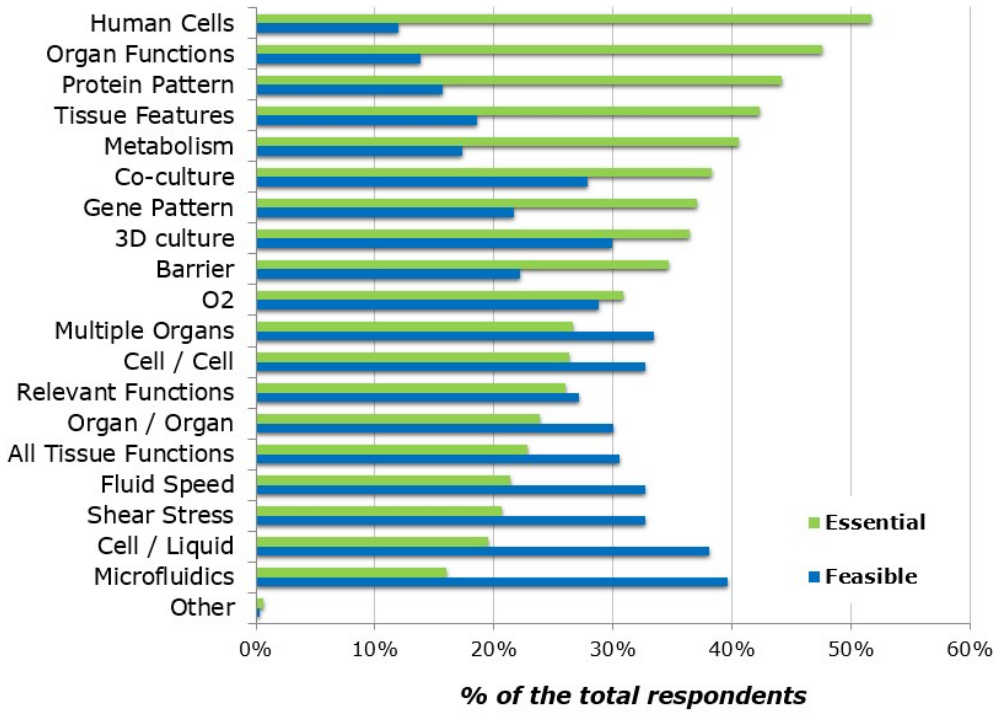
Additionally, different features were proposed whose **assessment could be prioritized in order to improve acceptance based on how essential they are considered**. The top ones were (a) (Human) Physiological and Biological relevance, (b) Reliability (reproducibility, repeatability), (c) Predictive Capacity and (d) Relevant Endpoints. Furthermore, the respondent needed to select what the purpose of such assessment was: regulatory, non-regulatory or both. It was interesting to note that the group that valued the most the assessment of

features for both fields was Industry – 51% on average – against 43% for Regulators and 35% for Academic Researchers.

When selecting Human Physiological and Biological relevance, in the previous question, a sub-question appeared, to understand **which model features would be essential to ensure such relevance**. Not surprisingly, human cells were at the top together with organ functions and protein pattern (Fig. 6, green bars). In contrast, fluidics and other features characteristic of Microphysiological Systems appear at the bottom.

However, when trying to understand how feasible such features would be to assess, there is an inversed trend pattern (Fig. 6, blue bars), making prioritization difficult.

Figure 6. Features essential for assessing human physiological and biological relevance together with feasibility to assess. Main results to question 13.1 of the survey (*Which of the following features do you think are essential to have in a human physiologically and biologically relevant complex in vitro model, and/or are feasible to assess in view of establishing its human relevance?*)



3.5 Additional general findings from the results

It is not surprising that a survey with 14 main questions with several sub-questions offering multiple choice answers, with 646 respondents, has collected a huge amount of data, making it challenging to report them in a simple manner. This report, tries to recapitulate the main and more relevant results. When the survey took place, the major message was actually that this was **considered a hot topic and of interest to many scientists, but there was still no consensus in the interested community on how the assessment of CIVMs should be performed**. The heterogeneity of respondents has most probably led to the non-uniform replies, but it is believed that this constitutes at the same time part of the richness of the results, and that the multidisciplinarity of the interested community should be embraced. Nevertheless, it might help to understand the tendencies in the different communities, even if a preliminary analysis did not show any clear conclusions. Below are some additional interesting facts that appeared in the results:

- Toxicologists tend to have a more regulatory way of thinking with respect to the respondents that do not have experience in toxicology.
- Among the respondents with high *in vitro* experience (10+ years), the ones that also had significant *in vivo* experience (10+ years) tended to be less satisfied with the CIVMs.
- Respondents with experience in the development and research related to CIVMs perform the same level of *in house* validation as the other profiles, but they use mostly internal procedures (developed *in house*) and not formal guidelines.

The great majority of the respondents perform *in house* validation. It seems to be more common in the USA than elsewhere. Still, despite showing less effort in implementing the models in different laboratories, USA success rates are higher.

CONCLUSIONS

4 Conclusions

The EURL ECVAM survey on CIVMs aimed to investigate stakeholder opinions and perceived needs on the most appropriate approach to reach their scientific credibility.

Based on the answers received from the nearly 650-respondents to the survey, it was not evident to find a consensus on which approach should be followed. Such uncertainty might reflect the early stage of development of the models or even the mixed profiles of the respondents.

Nevertheless, some conclusions can be drawn:

- Although it is well recognized that CIVMs demonstrate increased physiological relevance, these models are still not widely accepted and used.
- There is general support for some type of assessment of CIVMs.
- Such assessment and standardisation of assessment are considered relevant, not only for regulatory purposes, but also in research (although at different degrees).
- Although the way to conduct such assessment is still not clear, there was general support for the need of independent reviews of the CIVMs assessments, especially for regulatory purposes.

At the OECD level there are two highly relevant guidance documents for *in vitro* method development and use: the Guidance Document on Good *In vitro* Method Practices (GIVIMP) [26] and the Guidance Document for Describing Non-Guideline *In vitro* Test Methods (GD211) [27]. These documents have been developed by groups of experts from relevant fields (including researchers and regulators) for the purpose of regulatory acceptance of methods. The combination of these two documents can improve the performance and credibility of *in vitro* methods and models. While the GIVIMP may help in improving performance of the models and methods, GD211 supports their thorough description and characterisation, thus enhancing the credibility. Despite these existing guidelines regarding *in vitro* methods, the survey suggests that there is a general feeling within the CIVMs community that more guidance tailored to the specific characteristics of these models is still needed. Towards this end, there are several initiatives ongoing within different dedicated working groups [17] [28] [29], especially in the **organ-on-chip** community [5,6,7] [20] [18] [19].

CIVMs are proving to be an exceptional life science tool to add to the existing research test systems and continues to be attractive for the research community. CIVMs provide exciting prospects for future use in regulatory decision-making, diagnosis and treatment planning but their standardisation and validation need to be addressed in an appropriate manner.

[5] https://www.iqmps.org/
[6] https://www.h2020-orchid.eu/
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List of abbreviations and definitions

2D	Two-dimensional
3D	Three-dimensional
CFSAN	Center for Food Safety and Applied Nutrition (at FDA)
CIVMs	Complex *in vitro* Models
EMA	European Medicines Agency
EURL ECVAM	European Reference Laboratory European Commission Validation Alternative Methods
FDA	Food and Drug Administration
GIVIMP	Guidance Document on Good *In vitro* Method Practices
IMI	Innovative Medicines Initiative
IQ	Innovation and Quality in Pharmaceutical Development (Consortium)
JRC	Joint Research Centre
NCATS	National Center for Advancing Translational Sciences
OECD	Organization for Economic Co-operation and Development
ORCHID	Organ-on-Chip in Development (H2020 consortium)
USA	United States of America

Annex 1. Survey run by ECVAM

Credibility/validity of complex *in vitro* models:

A survey on the scientific and technical issues that influence end-user confidence and uptake of complex in vitro models

BACKGROUND

Complex *in vitro* models aim to represent higher-level anatomical and physiological aspects of human biology in practical experimental setups and are often referred to as 3D cell cultures, spheroids, bioprinted tissues, bioreactor cultures or microphysiological systems (e.g. tissue-/organ-/human-on-chip). The range of potential applications is considerable and thus new experimental methods based on complex *in vitro* models have the potential to not only provide an attractive alternative to animal models, but also offer the promise of advancing scientific methodologies underpinning research, development and testing. As this new generation of models and methods matures therefore, it is important to facilitate their translation from research and development into useful application by considering the scientific and technical issues that influence end-user confidence and uptake.

Objective of the survey

The purpose of this survey, conducted by the Joint Research Centre's European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM), is to consult a broad community of stakeholders to get a better understanding of the current level of uptake and satisfaction regarding complex *in vitro* models, and the current thinking around how best to establish their validity for use in research and testing with a view to building end-user confidence. The survey seeks the opinion of all those interested in complex *in vitro* models and their potential applications irrespective of how familiar one may be with the subject area. Therefore, please reply to the questions below even if you don't consider yourself an 'expert'!

EURL ECVAM's intention is to make the findings of the survey publicly available once the responses have been analysed. It is hoped that the results of the survey can be combined with information gathered from other sources to eventually inform validation strategies and technology-transfer initiatives to expedite the uptake and use of complex *in vitro* models and methods in a variety of sectors.

Complex *in vitro* model

The term "complex *in vitro* models" is used in the context of this survey to describe 3D cell cultures, spheroids, bioprinted tissues, bioreactor cultures, microphysiological systems (e.g., tissue-/organ-/human-on-chip), etc. intended to represent complex aspects of human biology in practical experimental setups. It includes both the biological/cellular system and its physical environment (usually referred to as test system in a regulatory context).

Questionnaire

The survey consists on multiple choice questions where some offer the possibility to leave additional written comments. Some questions are conditional to previous answers and may therefore not be visible to all respondents. It should take about 15-20 min to respond to this survey. If the system times out while you are replying, your previous answers should be automatically saved and accessible by logging back in.

It is also possible to save completed answers as draft and return to the survey at a later stage to finalise and submit your answers. All the data will be treated anonymously. Fields marked with * are mandatory.

Thank you!

Annex 1. Survey run by ECVAM

PROFILE

* 1. In which country do you work?

2. From the lists below, how would you define your professional profile?

*A. Type of employer

[Please select all that apply]

- Academia
- Industry
- Governmental/Public Service
- Non-Governmental Organization
- Other
- Other *Please specify

*B. Career level

- Head of Institution/Top Management
- Senior Officer/Middle Management/Principal Investigator/Associate Professor
- Scientific Officer/Technical Officer/Professional/Post-doctoral Fellow
- Student/Trainee
- Other - *Please specify

*C. Field

[Please select all that apply]

- Academic research
- Cosmetics industry
- Chemical industry
- Food industry
- Clinical/Medical devices
- Pharmaceutical industry
- Regulatory affairs
- Therapy/Regenerative medicine
- Other *Please specify

*D. Area(s) of expertise

[Please select all that apply]

- Pharmacology
- Toxicology
- Disease modelling
- (Pharmaco/Toxico) Kinetics and/or Dynamics
- Bioengineering, Biophysics and Biomaterials
- Regenerative medicine/Tissue engineering
- Oncology/Cancer
- Biopharmaceutics
- Advanced drug delivery systems
- Advanced drug delivery systems
- Nanomedicine
- Stem cell applications
- Drug discovery
- Medical devices
- Other - *Please specify

*3. For how many years have you worked in the area of *in vitro* methods?

- 0
- 0-3

- 3-5
- 5-10
- >10

*4. For how many years have you worked in the area of *in vivo* methods?

- 0
- 0-3
- 3-5
- 5-10
- >10

EXPERIENCE

5. How do you evaluate the amount of available information on complex *in vitro* models?

	Good	Fair	Poor	No opinion
*3D Cell cultures/Spheroids				
*Bioprinted tissues				
*Bioreactor cultures				
*Microphysiological Systems (e.g. tissue-/organ-/human-on-chip)				
Other				

*Please specify the other type of complex *in vitro* model(s) considered.

6. How do you evaluate the quality of the available information on complex *in vitro* models?

	Good	Fair	Poor	No opinion
*3D Cell cultures/Spheroids				
*Bioprinted tissues				
*Bioreactor cultures				
*Microphysiological Systems (e.g. tissue-/organ-/human-on-chip)				
Other				

*Please specify the other type of complex *in vitro* model(s) considered:
- Comment (optional)

*7. How many manuscripts on complex *in vitro* models have you (co-)authored?

- 0
- 1-3
- 4-10
- >10

*8. Which of the following tissues/organs/systems have you worked with/studied (regardless of context)?

[Please select all that apply]

- Adipose tissue
- Blood/Vascular system
- Bone/Cartilage tissues
- Brain/Nervous system
- Eye
- Gastrointestinal tract system
- Heart
- Immune system
- Kidney

- Kidney
- Liver/Hepatobiliar system
- Lung/Respiratory system
- Muscle
- Pancreas
- Peripheral nervous system
- Reproductive system (female)
- Reproductive system (male)
- Skin
- Thyroid/Endocrine system
- Other - *Please specify
- None

– Comment (optional)

9. How do you define your experience with complex *in vitro* models?

[Please select at least one option per mandatory row]

	No experience	Only generally informed (e.g. through publications, conferences, etc.)	Develop	Use in research (basic, applied and/or translational)	Use in regulatory decision making	Evaluate
*3D Cell cultures/Spheroids						
*Bioprinted tissues						
*Bioreactor cultures						
*Microphysiological Systems (e.g. tissue-/organ-/human-on-chip)						
Other						

*Please specify the other type of complex *in vitro* model(s) considered:

- Comment (optional)

9.1. In your opinion, approximately what proportion of publications on complex *in vitro* models:

*a) Are relevant from a scientific/biological perspective

[Please give your best estimate]

- 0-25%
- 25-50%
- 50-75%
- 75-100%
- No opinion

- Comment (optional)

*b) Describe useful models for application in regulatory decision making?

[Please give your best estimate]

- 0-25%
- 25-50%
- 50-75%
- 75-100%
- No opinion

- Comment (optional)

*c) Describe useful models for application in basic/applied/translational research?

[Please give your best estimate]

- 0-25%

- 25-50%
- 50-75%
- 75-100%
- No opinion

- Comment (optional)

*9.2. Which of the following tissues/organs/systems were represented in complex *in vitro* models that you developed, used and/or evaluated?

[Please select all that apply]

- Adipose tissue
- Blood/Vascular system
- Bone/Cartilage tissues
- Brain/Nervous system
- Eye
- Gastrointestinal tract system
- Heart
- Immune system
- Kidney
- Liver/Hepatobiliar system
- Lung/Respiratory system
- Muscle
- Pancreas
- Peripheral nervous system
- Reproductive system (female)
- Reproductive system (male)
- Skin
- Thyroid/Endocrine system
- Other *Please specify

9.3. What is your general level of satisfaction with complex *in vitro* model performance?

[Please select at least one option per mandatory row]

	Very satisfied	Satisfied	Slightly satisfied	Dissatisfied	Never used
*3D Cell cultures/Spheroids					
*Bioprinted tissues					
*Bioreactor cultures					
*Microphysiological Systems (e.g. tissue-/organ-/human-on-chip)					
Other					

*Please specify the other type of complex *in vitro* model(s) considered:

- Comment (optional)

*9.4. Have you or your colleagues performed *in house* validation of any complex *in vitro* model?

- NO
- YES, using internal procedures
- YES, referring to formal guidelines
- YES, both using internal procedures and referring to formal guidelines

*9.5. Have the models developed in your laboratory been successfully implemented and used in a different location/facility?

- YES, all models
- Only some models

- NO, they were not successfully implemented
- Not applicable

*Please specify which models have been transferred to another laboratory and, if any, explain the reasons why the others weren't transferred
- Comment (optional)

CREDIBILITY / VALIDITY

According to OECD Guidance Document No. 34, validation of a particular method or combination of methods for regulatory acceptance/use (e.g. as coordinated by EURL ECVAM) is defined as the process by which the reliability (i.e. reproducibility, repeatability) and relevance of the method(s) are established for a defined purpose. In this context, a method is defined as the combination of a test system (model), the endpoints measured, a protocol and a prediction model.
Validation can in fact be seen as a way to increase credibility and facilitate acceptance and use. This last section of the survey aims to explore if/how a process to establish the credibility of a complex *in vitro* model outside a specific context of use (i.e. only a test system), by means of an assessment of the model, could be useful to increase its consideration in a research and/or regulatory context.

*10. In your opinion, establishing the credibility/validity of a complex *in vitro* model outside a context of use (i.e. without a clear definition of purpose) is:

- Possible and useful to increase its acceptance and use
- Possible but not useful to increase its acceptance and use
- Not possible
- No opinion

Comment (optional)

*11. For which of the following applications do you think an assessment of a complex *in vitro* model could potentially increase its acceptance and use:

[Please select all that apply]

- Basic research
- Applied research
- Translational research
- Screening
- Pre-clinical testing
- Regulatory testing
- Other - *Please specify the other applications considered.
- None
- No opinion

– Comment (optional)

11.1. Should the assessment of a complex *in vitro* model be independently reviewed (e.g. by an independent body) in order to guarantee a wider acceptance and use?

[Please select one option per row]

	Yes	No	No Opinion
*Basic Research			
*Applied research			
*Translational research			
*Screening			
*Pre-clinical testing			
*Regulatory testing			
Other			

– Comment (optional)

12. Which factors/perceptions may compromise a wide acceptance and use of complex *in vitro* models?

[Please select at least one option per mandatory row]

	Yes	No	No Opinion
* Cost			
* Time constrains (e.g. it would take too long to implement)			
* Complexity of implementation			
* Complexity of handling			
* Does not replace the animal model (1-to-1 replacement)			
* Lack of availability of an appropriate model			
* Difficulty in selecting the most appropriate model			
* Questionable relevance			
* Lack of confidence/experience on the available models			
Other			

*Please specify the other factor(s) considered.
- Comment (optional)

13. Which of the following options do you think are essential to assess in a complex *in vitro* model to facilitate its consideration in a research and/or regulatory context?

[Please select at least one option per column]
at least 1 answered row(s)

	Regulatory use	Non-Regulatory use
(Human) physiological and biological relevance of the model		
Viability/Shelf-life of the model		
Functional stability		
(Relevance of) endpoints measured		
Reliability (reproducibility, repeatability)		
Availability of detailed protocols with control substances, acceptance criteria, prediction model/data interpretation procedure		
Blind testing of a number of reference compounds		
How well the *in vitro* data correlate with in vivo data (predictive capacity)		
Compatibility of the materials used in the model for *in vitro* testing applications (e.g. potential adsorption of test items to the materials used in the model)		
Other		
None		
No opinion		

*Please specify the other option(s) considered.
- Comment (optional)

13.1. Which of the following features do you think are essential to have in a human physiologically and biologically relevant complex *in vitro* model, and/or are feasible to assess in view of establishing its human relevance?

[Please select all that apply; it is possible to select both 'Essential' and 'Feasible'; leave blank when no opinion]

	Essential	Feasible
Human cells		
3D culture		
Multiple cell types in co-culture		
Physiologically matched cell/liquid ratios		
Physiologically matched cell/cell ratios (in case of co-culture)		
Physiological O2 concentration/pressure		
Microfluidics		

Perfusion with physiological fluid speed		
Perfusion with physiological shear stress		
Multiple organs/tissues connected in the same system		
Physiologically matched organ/organ ratios (in case of multi-organ)		
All of the biological functions of the tissue(s)/organ(s) being mimicked (enabling multiple applications)		
Solely the biological functions known to be relevant for its application		
Tissue/organ specific features (e.g. co-cultures, scaffolds, membranes, cell culture media)		
Having the gene expression pattern of the tissue(s)/organ(s) being mimicked		
Expression (at protein level) of a set of tissue/organ specific markers		
Tissue/organ specific function		
Presence of a tissue/organ specific physiological barrier		
Metabolic capacity		
Other		

*Please specify the other factor(s) considered.
- Comment (optional)

14. The output of an assessment of a complex *in vitro* model in view of establishing its credibility/validity should be:

[Please select at least one option per mandatory row]

	Regulatory use	Non-regulatory use	Not useful	No Opinion
* Statement/recommendation on the biological relevance of the model (tissue/organ specific)				
* Statement/recommendation on the usefulness, limitations and potential application(s) of the model				
* Standards to facilitate the assessment of other (similar) models				
Other				

*Please specify the other factor(s) considered.
- Comment (optional)

PERMISSION FOR FURTHER CONTACT & LAST COMMENTS /SUGGESTIONS

* I am interested in receiving information regarding the outcome of the survey. I give my consent to being contacted by the JRC / EURL ECVAM for this purpose using the contact details provided below.

- Yes
- No

Additional Comments/Suggestions

The survey is now complete. Thank you for your time

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