NC 3R^s

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Organ-on-a-chip Report and Recommendations

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NC3Rs organ-on-a-chip report and recommendations

Methodology

1. We have carried out a business intelligence analysis of the organ-on-a-chip (OoC) field to provide an evidence base to inform the NC3Rs future strategy and identify key areas with significant unmet need in the 3Rs. The analysis also provides a useful resource on the current landscape for technology developers, end-users, investors and regulators.

2. This report includes a literature review and the results of a survey from end-users that provides insight into how the technology is being used across sectors, the 3Rs impacts, and the barriers to and opportunities for adoption. There have been a number of surveys targeted to the OoC community in recent years (1,2,3,4). Our survey is unique in that it captures metrics on the current and future 3Rs impacts of the technology. The survey results can be found in Annex 1.

Introduction

3. OoC technologies, also known as microphysiological systems (MPS) or tissue chips, are microfluidic cell culture devices that closely recapitulate the structure, function and (patho)physiology of human tissues and organs *in vitro* (3). The technology provides a promising tool to reduce the reliance on animal models by providing faster, cheaper and more physiologically relevant human cell-based models for use in basic and applied research.

4. Applications of the technology include disease modelling, toxicology/safety, efficacy, ADME (absorption, distribution, metabolism and excretion) and personalised medicine, with end-users working across academia, industry and regulatory bodies. The most cited applications are in the pharmaceutical industry and it has been estimated that OoC technology could reduce R&D costs by 10 to 26%, mostly at the lead optimisation and preclinical stages (1,5,6,7). The technology has the potential to revolutionise drug discovery and development to improve and accelerate the delivery of safe and efficacious medicines to patients. Pharmaceutical companies such as AstraZeneca, Bayer and Roche are evaluating the technology for multiple applications, which is starting to be used for internal portfolio decision making (1,3). The technology is also being explored by other sectors (e.g. cosmetic and chemical industries) for toxicology hazard identification and risk assessment (1,8).

5. There has been considerable global growth in the field over the last ten years with initiatives in the USA and Europe providing funding and partnerships critical to supporting OoC development, commercialisation and adoption. These include the Tissue Chip for drug screening programme led by the National Centre for Advancing Translation Sciences (NCATS), part of the National Institute of Health (NIH), the Defense Advanced Research Projects Agency (DARPA) Microphysiological Systems programme, the Institute for human Organ and Disease Model Technologies (hDMT), the Netherlands Organ-on-Chip Initiative (NOCI) and the Organ-on-Chip in development (ORCHID) project (1,3,8,9,10,11,12,13,14,15). There has been a concomitant increase in the number of publications and patents, OoC companies and end-users

evaluating and, in some cases, adopting the technology for internal decision making (1,3,16). The global OoC market was valued at $29.6M in 2018, a four-fold increase from 2016 ($7.5M) and is predicted to increase at a compound annual growth rate of 28.6% (2018 to 2024) to reach $133M by 2024 (17,18).

6. OoC models have been developed for multiple organs and tissues covering major organ systems (6,8,16,19). These have been used to study both healthy organs and a number of diseases including cancer, neurodegenerative, cardiovascular and respiratory diseases and diabetes (1). OoC technology can replicate single organ systems or can be interconnected to form multi-organs-on-chips (two or more organs or tissues) or human/body-on-a-chip to study pharmacokinetics, multi-organ toxicities, toxicities that arise from drug metabolism, and diseases that involve multiple organs. Single and multi-organ models are at various stages of development ranging from academic proof-of-concepts through to products which are commercially available through companies such as Emulate, MIMETAS and TissUse (16,17,18). A number of OoC companies have secured millions of dollars of private investment and have started to generate recurring sales and a consistent revenue stream (1,18,20). OoC companies are collaborating with end-users from pharmaceutical companies and the US Food and Drug Administration (FDA) (21,22,23).

7. Despite significant progress in the development and application of OoC over the last ten years the field is still in its relative infancy. Respondents to our survey indicated that the field is immature in terms of its technology readiness, agreeing with data published by Allwardt *et al*., 2020 (2). There are challenges that must be overcome before the technology can be widely adopted into routine use. These include further characterisation and qualification/validation to demonstrate the technology is fit-for-purpose for its context of use, is predictive and adds value over conventional 2D *in vitro* and *in vivo* approaches.

Use of the technology

8. Data from our survey and the literature indicates that the majority of end-users are from the pharmaceutical and academic sectors (72% of respondents to our survey) and are using the technology for drug discovery and development, disease modelling and basic research (1,2,3).

9. End-users who completed our survey were predominantly at the stage of evaluating the technology and most were assessing single OoC systems including liver, blood vessels, lung, gut and cancer for applications including toxicology, disease modelling, efficacy, mechanistic/investigative, ADME and personalised medicine. Multi-organ systems undergoing evaluation mainly involved combinations of up to three organ systems, with most including a liver component.

10. Respondents indicated that they expected the technology to be deployed in the future for decision making from basic research through to preclinical and clinical drug development. Specific areas highlighted included:

   - For investigating the mechanisms of a compound-induced effect. For example, an unexpected result (efficacy or safety) detected in the clinic.
   - To inform *in vivo* study design.

- To screen out compounds with undesirable properties early prior to *in vivo* studies.
- To progress molecules to the clinic without using *in vivo* efficacy models.
- For personalised medicine.
- For assessing chemical safety for endocrine disruptors.

11. There are a number of a examples from our survey and the literature where OoCs have started to be adopted. In the pharmaceutical sector the technology is being used throughout the drug discovery and development process for internal portfolio decision making, for example, a liver-pancreas model is being applied at the target identification/validation stage in drug discovery, and a bone marrow model is being used for preclinical safety in drug development (3).

3Rs impacts

12. Results from our survey indicate that the technology is starting to deliver some impact on the 3Rs. For example, in one respondent's organisation, the technology has replaced up to 300 animals per year used for basic research. The technology has the potential to significantly impact the 3Rs in the future. Examples of future 3Rs impact from our survey included to replace mice used in immunology studies, and to reduce, replace and refine rodent and non-rodents used in preclinical mechanistic studies. One respondent estimated that the technology has the potential to replace up to 500 rats per year in rodent mechanistic studies within their organisation.

13. The technology has significant 3Rs potential, but key opinion leaders in the field have stated that it is unlikely to directly replace the routine use of animals in the near future, particularly for regulatory purposes, and is primarily a complementary approach to *in vivo* studies to build confidence in the technology (1,3). In the short to medium-term this includes to screen out compounds prior to *in vivo* tests, to investigate effects detected in animals and the clinic, and to refine animal experiments by informing dose selection so that animals are not exposed to unnecessarily high doses. There may also be utility for species-specific chips to assess the human relevance of effects detected in animals. The long-term goal is to exploit the technology to directly replace animal studies. This may require a suite of *in vitro* tests together with *in silico* approaches, capable of recapitulating the complexities of whole organs and tissues *in vitro*. Regulatory acceptance of these approaches will be essential in their wider uptake to maximise 3Rs impacts (24).

Opportunities of the technology

14. The most commonly cited application for OoC technology in the literature and from our survey is in pharmaceutical drug discovery and development. Safety or lack of efficacy are major causes of attrition during drug development due to poorly predictive *in vitro* and animal models (27). Large numbers of animals are used throughout the drug discovery and development pipeline to assess pharmacokinetics, efficacy and safety. There is a need for predictive translatable human relevant models to reduce drug attrition and animal use, save time and costs, thereby improving and accelerating the delivery of safe and efficacious medicines to patients. There has been a shift in pharmaceutical portfolios to modalities beyond

small molecules such as monoclonal antibodies where *in vivo* studies are often carried out in non-human primates, and to emerging therapeutic areas such as cell and gene therapies, providing opportunity for the implementation of OoCs where the regulatory landscape for preclinical testing is still evolving.

15. Areas of opportunities for OoC technology identified by respondents to our survey align with those reported in the literature (1,3,8,14,25,26,28) and the key areas are outlined below:

- Personalised medicine represents an unmet need and provides new opportunities to assess patient specific drug safety and efficacy, taking into account important genetic, ethnic and gender differences (25,29). Patient specific primary cells or induced pluripotent stem cells (iPSCs) can be used to populate the chips. The advantage of iPSCs is that multiple organs/tissues can be derived from the same patient. Gene editing techniques (e.g. CRISPR) can be employed to create genetic diseases from cells derived from iPSCs. Better representation of the patient population could help reduce attrition rates of promising drugs. However, there are challenges around accessing patient samples as well as health data. Patient-derived iPSCs or primary cells and organoids from patients with genetic diseases or healthy individuals can be obtained from biobanks (e.g. the [European Bank For Induced Pluripotent Stem Cells](#) (EBiSC)), but there are barriers such as logistics, infrastructure and patient consent that need to be overcome.

- The development of human relevant models for both common and rare diseases and in areas of toxicity for which no adequate animal model exists represent a significant opportunity of OoCs. In 2020 in the UK, the most common areas where animals (particularly mice) were used in scientific procedures for basic and applied research focused on the immune system, the nervous system, cancer and infectious disorders (30). There is interest in OoC models for cancer to model the tumour microenvironment and metastasis, study drug efficacy and provide personalised cancer treatments (1). Cancer was one of the top five OoC models that respondents to our survey were evaluating for disease modelling, efficacy, mechanistic/investigative, personalised medicine and toxicology applications. Many companies have major investment in developing oncology drugs. Animal models are poorly predictive, with drugs that have shown efficacy in animals failing in the clinic. There is a clear need for more predictive models for oncology drug discovery and development, and in basic research. A human relevant model could also add significant value in immunology and this was an area identified by respondents to our survey where OoC technology has the potential to replace mice. For example, for testing molecules targeting immune-related targets and pathways where there are well known differences in the immune system between animals and humans. For toxicology, Mastrangeli *et al*., 2019 highlighted predictive models for gametogenesis and testicular toxicity, an infrequent but severe cause of drug attrition for which no satisfactory preclinical model exists, as a valuable niche market for OoC technology (1).

- The COVID-19 pandemic has resulted in a significant number of animal models being used to understand disease biology and develop treatments and vaccines. There are opportunities for OoC models of the lung and other key organ systems affected by COVID-19 to be applied to reduce

reliance on animal models used in COVID-19 studies, as well as for future infectious diseases. The [MPSCoRe working group](#) has been established to help coordinate global efforts in this area (31).

- Linking up organ systems provides utility for OoC in a number of areas such as the development of ADME models to better model pharmacokinetic (PK) profiles, and for measuring temporal PK and pharmacodynamic relationships (drug concentration to drug effect) for safety and efficacy. In addition, it also provides opportunities to study diseases involving multiple organs, and in combining efficacy and safety models to allow safety questions requiring specific disease states to be investigated.

- There is a need for automation, as well as the integration of online sensors to enable readouts in real time, and the use of computational modelling, artificial intelligence (AI) and machine learning (ML) to help mine the large datasets that are generated in these models, to enable the technology to be routinely used within an industrial setting (3,32). For example, AI/ML could be applied to imaging analysis of data generated in cancer-on-a-chip models (33).

- There is utility for species-specific chips to assess the human relevance of effects detected in animals and help build up confidence in the translatability of the chips. Jang *et al*., 2019 highlighted the value of species-specific liver chips as a tool for assessing the human relevance of drug-induced liver toxicities seen in animals (34).

- Beyond pharma, the technology is being explored by other sectors (e.g. chemical and cosmetic industries) for toxicological hazard identification and risk assessment. Opportunities highlighted by respondents to our survey included for environmental chemical mixtures toxicity assessment, repeat dose and long-term exposure studies for chemicals and cosmetics and for assessing chemical safety for endocrine disruptors.

Barriers to adoption of the technology

16. Challenges remain that must be overcome before OoC technology can be widely adopted. The most significant barriers to adoption identified from our survey listed in rank order included:

- Cost.
- Lack of core components (e.g. endocrine and immune) in current systems.
- Data generated is not accepted by regulators.
- Limited qualification/validation to demonstrate that the technology is fit-for-purpose.

17. Respondents were asked to provide any other significant barriers to adoption, which included the need for standardisation and access to funding. Respondents were provided with a list of criteria and asked to rate them in terms of their importance in facilitating the adoption of the technology, from not important to very important. The top five criteria considered to be very important in facilitating adoption of the technology listed in rank order were:

- Robust characterisation and qualification/validation to demonstrate the technology is fit-for-purpose.

- Publication of case studies to demonstrate utility and added value over conventional models.
- Regulatory acceptance.
- Establishment of a global network to share the latest developments, knowledge and experience.
- Establishment of independent testing centres to evaluate and validate the technology.

18. Further details on the challenges identified from our survey and literature (1,2,3,8,28), as well as how the community is working towards overcoming these to facilitate adoption are highlighted below.

19. The technologies need to be fully characterised and qualified/validated, but not necessarily in the sense of formal validation to replace animal models for regulatory purposes, but rather in generating scientific evidence to demonstrate that a particular technology is fit-for-purpose in addressing a specific question, and to show robustness and reproducibility. Standardisation is critical to support the characterisation and qualification/validation of the technology to accelerate uptake, especially if the technology is to be used for regulatory decision making in the future. A number of consortia have published roadmaps, with standardisation being a key building block to adoption (1,2,3). The Joint Research Centre of the European Commission (JRC) and the European Standarisation Organisations CEN and CENELEC held a 'Putting Science into Standards Workshop' focusing on OoC technologies in April 2021, providing the opportunity to bring together stakeholders in the field to share their views on future developments and stakeholder requirements, and provide recommendations to CEN-CENELEC on next steps. The Innovation and Quality consortium MPS affiliate has published a series of papers outlining the pharmaceutical industry perspectives and considerations for developing, evaluating and characterising MPS models to support drug discovery and development (35), and GSK has published recommended guidelines for developing, qualifying and implementing complex *in vitro* models, including OoC, for drug discovery (26). The NIH has funded the establishment of two Tissue Chip Testing Centres to independently test and validate the OoC technology developed through its Tissue Chip for Drug Screening programme.

20. Regulatory acceptance of data generated in these models is often seen as a barrier to developing and adopting OoC technologies. However, regulators are actively engaged in programmes to understand how the technology can be used for regulatory decision making. The FDA has been involved in the NIH and DARPA programmes from the start and is evaluating commercially available platforms in-house (22). The European Medicines Agency held a workshop in 2017 to look at the challenges and opportunities for use of MPS.

21. There are many programmes and initiatives and a more joined-up approach globally is required to avoid duplication of effort in the field and to share experiences and impacts. One of the recommendations from a workshop sponsored by the Centre for Alternatives to Animal Testing held in June 2019 was the formation of an international MPS society (3). A series of conferences have been organised (the world summit on microphysiological systems) to bring together the OoC community to build a roadmap for the technology and a global network. The first meeting was held in June 2021 and the plan is to establish an international society to facilitate stakeholder communication and promote international standardisation and harmonisation of the technology.

22. Current OoC models do not recapitulate the full structural and functional complexities of organs and tissues *in vivo* and lack core components (e.g. immune and endocrine). Models are often only composed of a few cell types or able to measure one functionality. One example is models of the kidney which are composed of proximal tubule cells and endothelial cells. However, the full level of complexity may not always be required and brings with it its own challenges such as being over-complicated, labour intensive, expensive and it may be difficult to interpret the large amounts of data generated.

23. The cell source used remains a challenge and is critical in producing robust and reproducible models. While primary cells are phenotypically mature, they are difficult to obtain, limited in quantity, de-differentiate over time and not all cell types can be obtained from the same donor. Cell lines are available in large quantities, are cheap and easy to use, but often lack key structural and functional properties. Fluid flow has been shown to improve the function of primary cells and cell lines cultured in OoCs compared to static cultures (36,37). Patient specific iPSCs provide an unlimited supply of cells and can be used to generate cell types from the same genetic background. However, iPSC-derived cells are often immature in their phenotype and there is a need to develop conditions to mature the cells as well as robust differentiation protocols. Organoids formed from tissue-derived stem cells consisting of mature cell types of an organ are another potential cell source.

24. The models must demonstrate added value over conventional approaches (e.g. 2D *in vitro* and *in vivo* models) such as improved predictivity. Publication of case studies highlighting how and where these models can add value are needed as there are few examples specifically addressing this in the literature. An important example of this is a vessel-on-a-chip model that recapitulated the thrombotic behaviour of a monoclonal antibody therapeutic which caused deaths in patients in Phase 1 clinical trials due to pulmonary embolisms (38). This toxicity had not been observed in preclinical animal models.

25. The cost associated with the models as well as changing infrastructure and practices is seen as a major barrier, although costs are expected to decrease as the technology becomes more widely used. Current systems are often complicated to set up and use, and the technology needs to be scaleable and easy to use within an industrial setting. There is a need for automation and integration of online sensors for real time non-invasive readouts, and AI and ML to mine the large datasets generated in these models and efforts are starting to address this. Online sensors for real-time readouts are being integrated into OoC models such as TransEpithelial/Endothelial Electrical Resistance sensors for measuring barrier integrity and multielectrode array electrodes for measuring electrical activity (32). TissUse has developed the HUMIMC AutoLab that allows the automation of 24 multi-organ chips in parallel.

26. Scaling-up and manufacturing of OoCs to meet end-user demand is a challenge. Polydimethylsiloxane (PDMS) is frequently used to fabricate OoC technology. However, PDMS is not suitable for large scale production as well as readily binds drugs reducing bioavailability (39). There is a need to move away from PDMS which is starting to be addressed with OoC companies exploring other substrates such as glass and polyurethane and partnering with manufacturing companies to scale-up production (e.g. Emulate and mini-fab). Additional challenges include the issues of scaling ratios of organ sizes and cell-to-liquid ratios,

bubbles in the system, flow rate differences between organs and tissues, and the need for a common cell culture media that can support all cell types (2).

Summary

27. OoC technology is a rapidly growing field, with significant funding and partnerships established over the last ten years leading to the development of models for multiple organs and tissues. Some of these models are now being commercialised by OoC companies who are partnering with end-users, primarily from the pharmaceutical industry, to assess whether the technology is fit-for-purpose. Most end-users are currently evaluating the technology, but there are a few examples from our survey and the literature where the technology has been adopted and is being used for internal decision making. The technology has started to have some impact on animal use and has the potential to significantly reduce reliance on animal models in the future. However, the field is still in its relative infancy and there are challenges that must be overcome before the technology is widely adopted into routine use, with further work needed to fully characterise and qualify/validate the models to build up confidence in their utility. Communication between all stakeholders (including academia, developers, industry and regulators) is essential for early engagement with end-users to clarify their needs, for establishing clear criteria for characterising and qualifying/validating the models so they are fit-for-purpose, robust and reproducible, and for publication of case studies to highlight where the technology adds value and how it is being used in a real-world setting. Below, we set out our recommendations for how we will support the development and wider adoption of OoC technology to maximise their 3Rs potential.

Recommendations

28. Funding and partnerships remain critical in supporting the development and application of OoC technology. Given its potential to reduce reliance on animal models, we will continue to support the development and uptake of OoC technology across our research and innovation funding schemes and through the technology partnering platform konfer.

29. We will place greater emphasis on characterisation and qualification/validation to demonstrate the technology is fit-for-purpose, robust and reproducible, building capacity to enable wider uptake by end-users. We will continue to support these efforts, for example, through our Skills and Knowledge Transfer and Business Growth funding schemes. We will develop guidance for applicants applying to our funding schemes so that clear criteria for characterisation and qualification/validation is built into project plans.

30. We will publish case studies to highlight where the technology adds value and is being used to deliver 3Rs impacts. We will also run a technology showcase to support the uptake of OoCs which are ready for adoption with end-users and focus on engagement with regulators.

31. We are developing guidelines for improved experimental design and reporting of *in vitro* experiments to optimise the reproducibility of these studies to support their wider uptake to replace animal studies. Given that complex *in vitro* models, including OoC technologies are increasingly being used, we plan to include

these models within the scope of the guidelines to support the design and reporting of *in vitro* experiments involving these models from the outset.

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Annex 1. NC3Rs organ-on-a-chip survey results

Introduction

32. Organ-on-a-chip (OoC) technologies, also known as microphysiological systems or tissue chips, are microfluidic cell culture devices that can recapitulate the structure, function and (patho)physiology of human tissues and organs *in vitro,* and are promising tools to reduce the reliance on animal models in basic and applied research.

33. To provide insight into the current landscape and how the technology is being used across sectors, as well as 3Rs impacts, and barriers and opportunities to adoption, a survey was conducted targeted to end-users.

Methods

34. The survey was constructed using the SurveyMonkey platform and was granted ethical approval by the Social Sciences Research Ethical Review Board of the Royal Veterinary College (URN SR2020-0203). A link to the survey and explanatory text was disseminated to current, future and past users of the technology from across the life science sector through individually targeting stakeholders active in the field, through relevant partner networks (e.g. the UK Organ-on-a-Chip Technologies Network, the European organ-on-a-chip Society, the Medicines Discovery Catapult, the IQ Microphysiological Systems Affiliate) and via the NC3Rs monthly newsletter and social media platforms (Twitter and LinkedIn). Responses to the survey were collected during July and August 2020. The survey was split into different sections and questions depending on the respondent's use of the technology (currently, considering and not currently using).

Results

Responses and demographic information

35. There were 53 responses to the survey from respondents in 11 countries, with the majority based in the United Kingdom and Europe (Figure 1A). Respondents mainly worked in the academic and pharmaceutical sectors (Figure 1B). The breakdown of respondents according to the position held within their organisation can be found in Figure 1C.
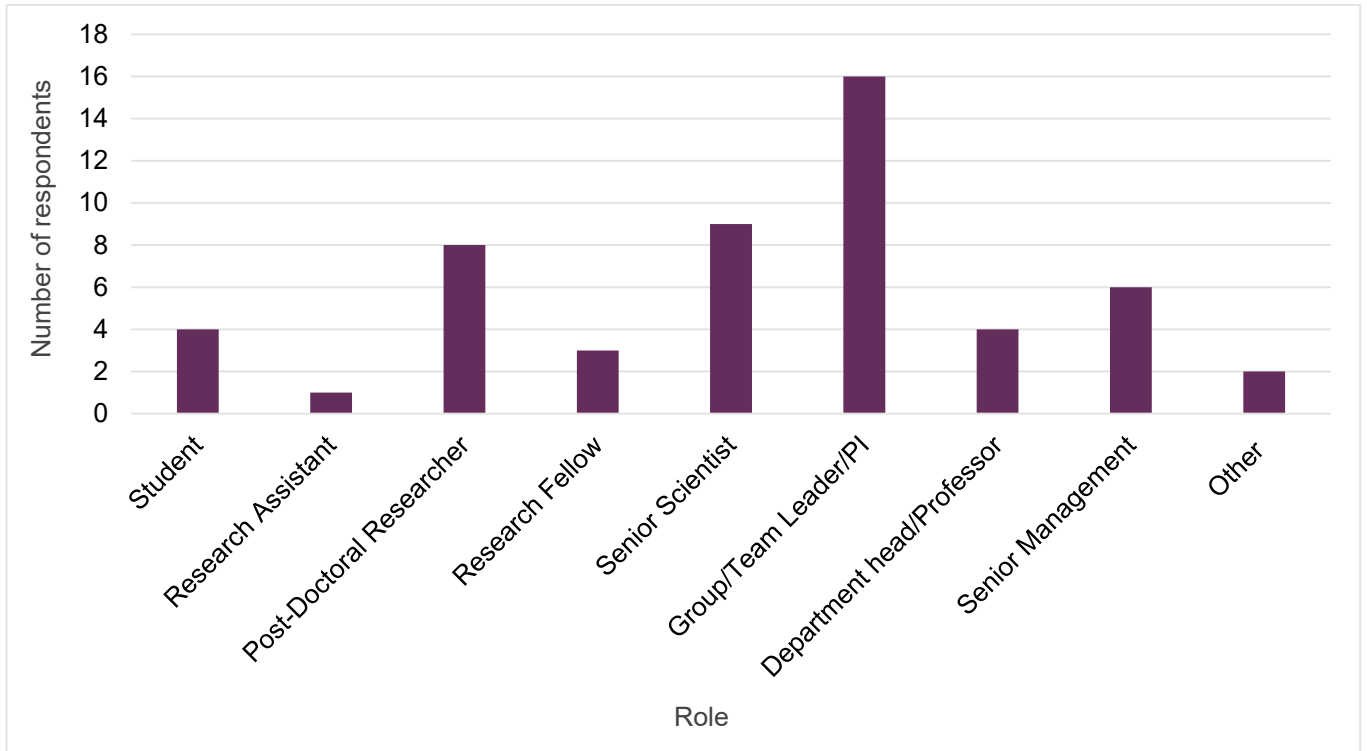
A)
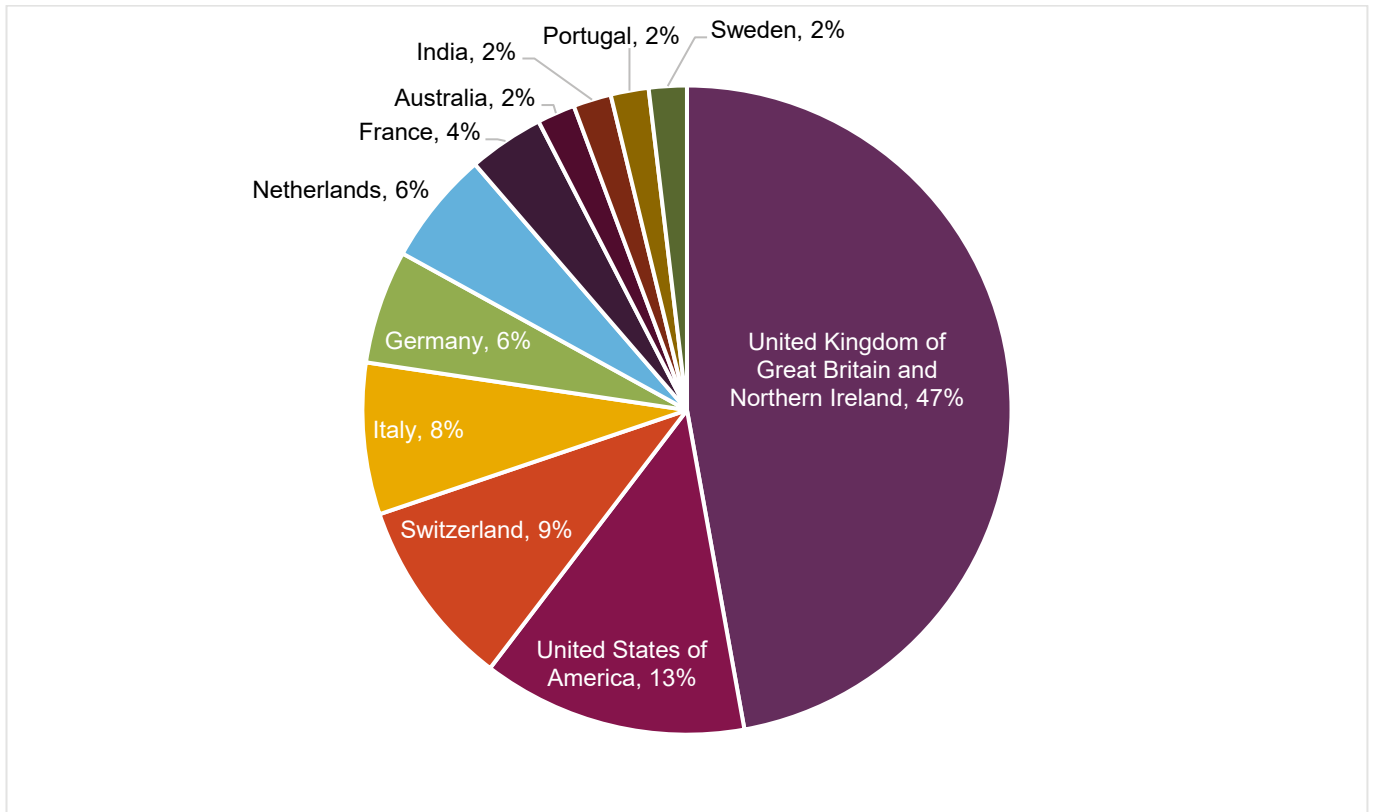
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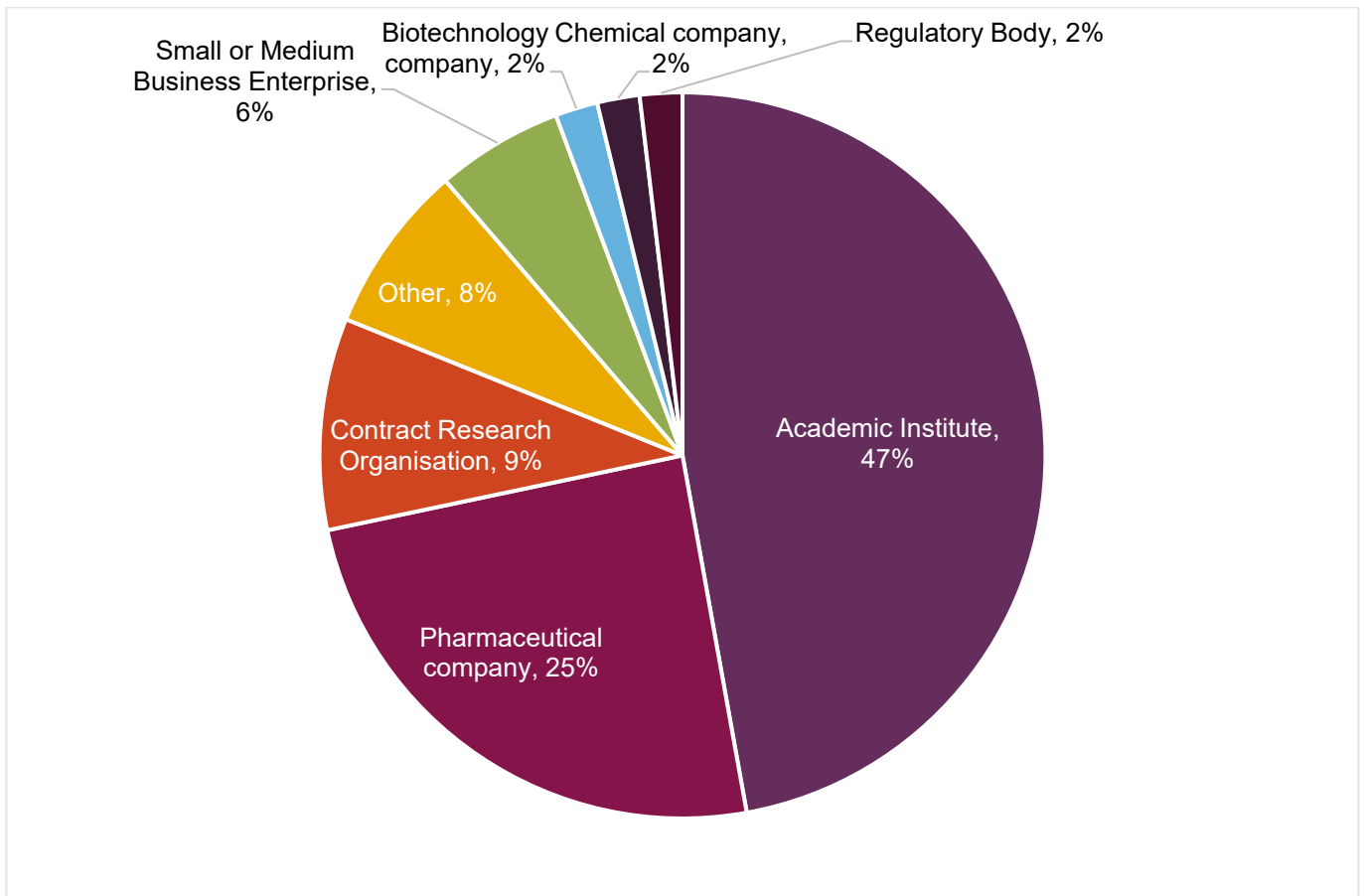
C)

Figure 1. Respondents demographic information. A) Respondents location, B) Respondents sector and C) Respondents role. N=53 respondents.

Respondents use of the technology

36. Respondents were asked to select their current use of the technology (Figure 2). The majority were using (47%) or considering using (40%) the technology, with the remaining respondents not currently using the technology (13%).
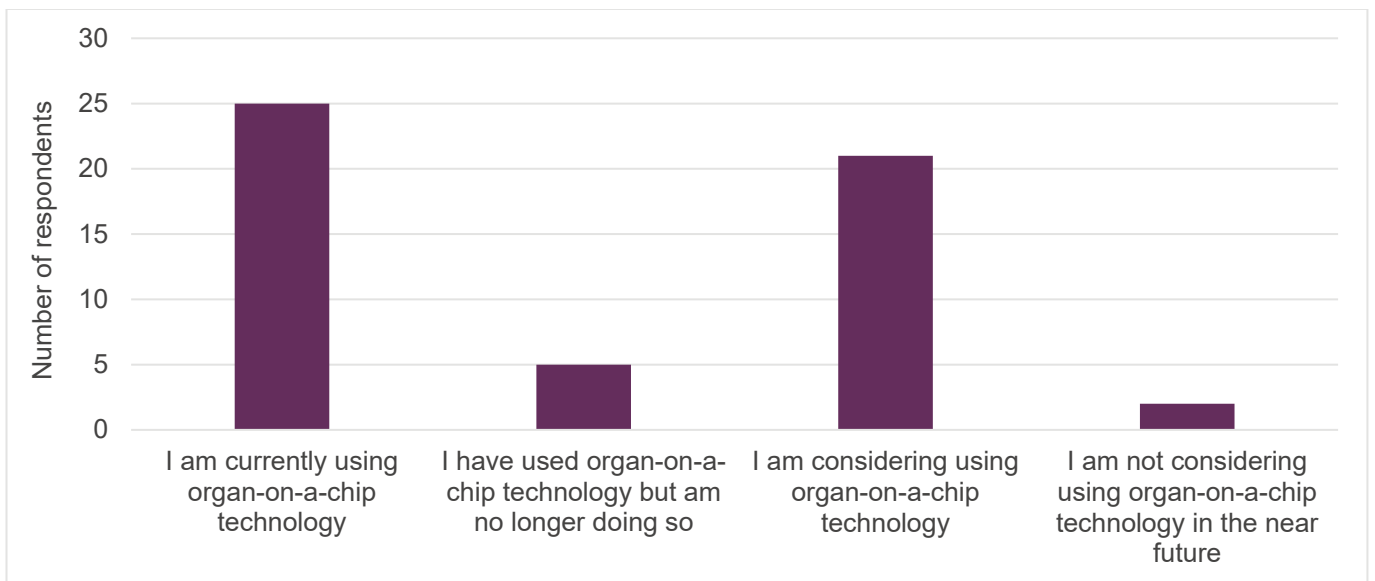

Figure 2. Respondents use of the technology. N=53 respondents.

Respondents not currently using the technology

37. Respondents were asked to select the reason(s) why they were not currently using the technology, selecting all that apply, which included (Figure 3):

   - Complex to use and labour intensive.
   - Current models not being fit-for-purpose.
   - Low throughput and not scaleable for routine use.
   - Not supported by their organisation.
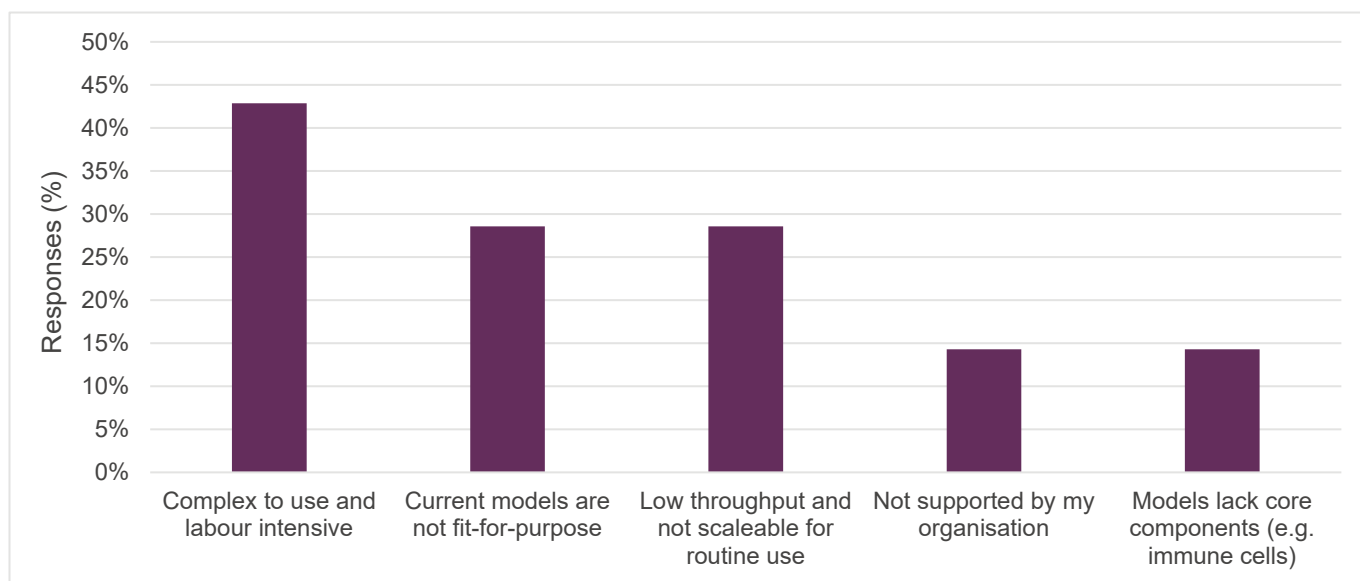   - Models lack core components (e.g. immune cells).

Figure 3. Responses (%) to why respondents were currently not using the technology (N=7 respondents).

Respondents considering using the technology

38. 16 out of 21 respondents were considering using single organ systems which included the gut/digestive system, the nervous system, the liver, the lung/respiratory system and the blood-brain barrier largely for toxicology applications, but also for disease modelling, mechanistic/investigative, ADME (absorption, distribution, metabolism and excretion) and personalised medicine (Figure 4). 16 out of 21 respondents were considering using multi-organ systems (two or more different organs or tissues).
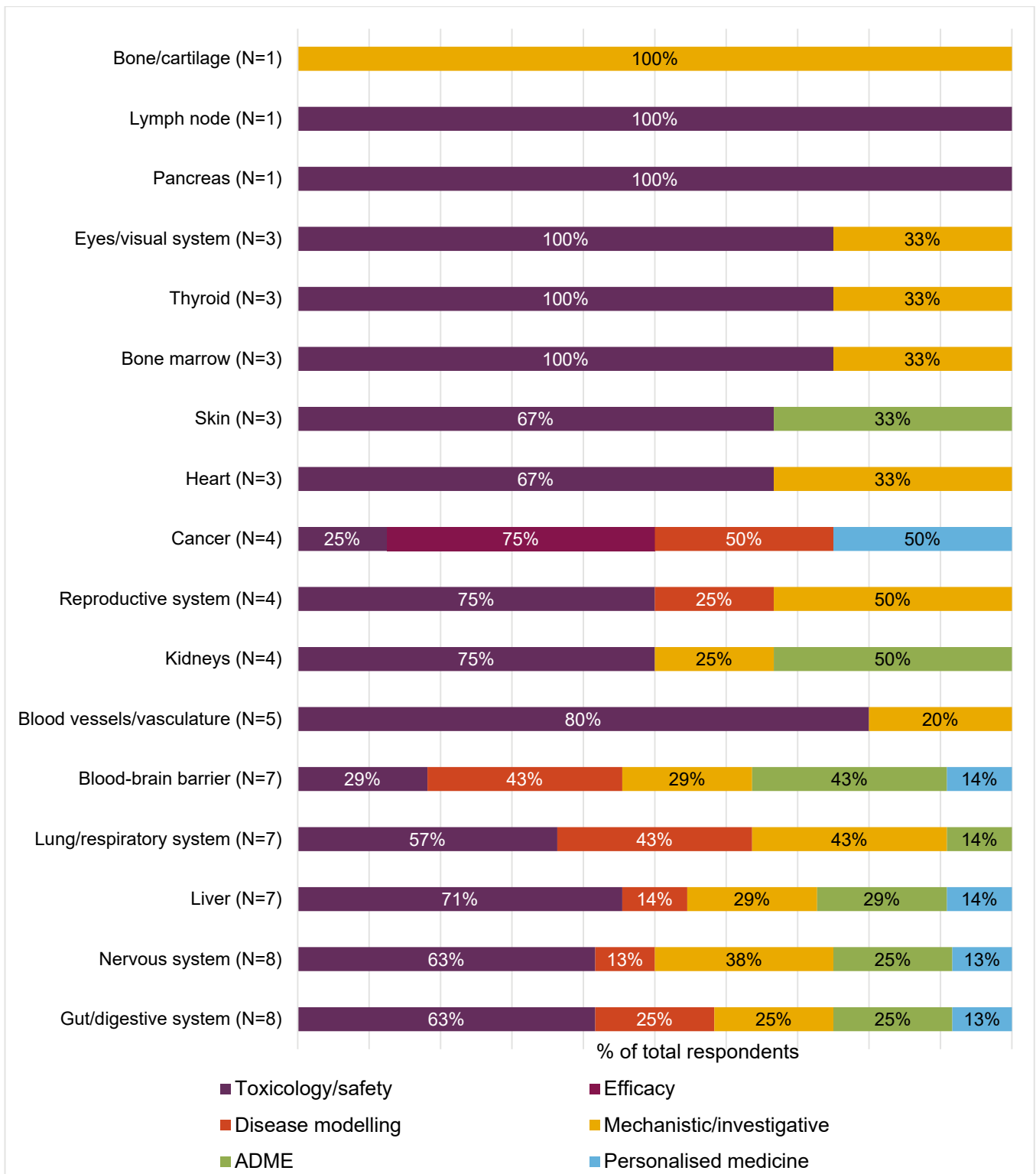
Figure 4. Organ systems respondents were considering using and their applications. The number of respondents who selected a particular organ is shown in brackets. The number of respondents who selected a particular application is plotted as a % of the total respondents for each organ (e.g. five out of eight (63%) respondents selected that they were considering using the gut/digestive system for toxicology applications).

Respondents using the technology

39. Respondents were asked how they were using the technology, selecting all options that apply. The majority of respondents were evaluating the technology in-house or through an external collaboration or partnership, with some selecting that the technology had been adopted for decision making (Figure 5).
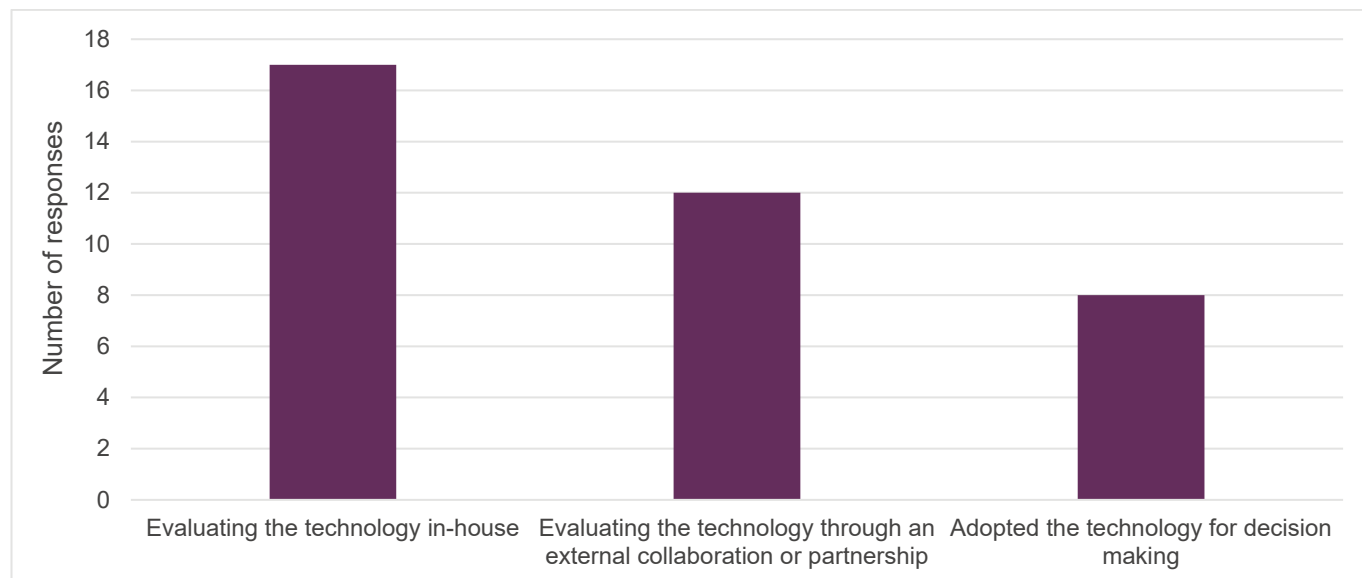

Figure 5. Number of responses to how respondents were using the technology. N=25 respondents.

40. 21 respondents were evaluating single organ systems which included liver, blood vessels/vasculature, lung/respiratory system, gut/digestive system and cancer for toxicology, efficacy, disease modelling, mechanistic/investigative, ADME and personalised medicine applications (Figure 6).

41. Eight respondents were evaluating multi-organ systems composed of combinations of up to three organ systems, with most including a liver component for a variety of applications including ADME, disease modelling, efficacy, mechanistic/investigative and toxicology/safety (Table 1).
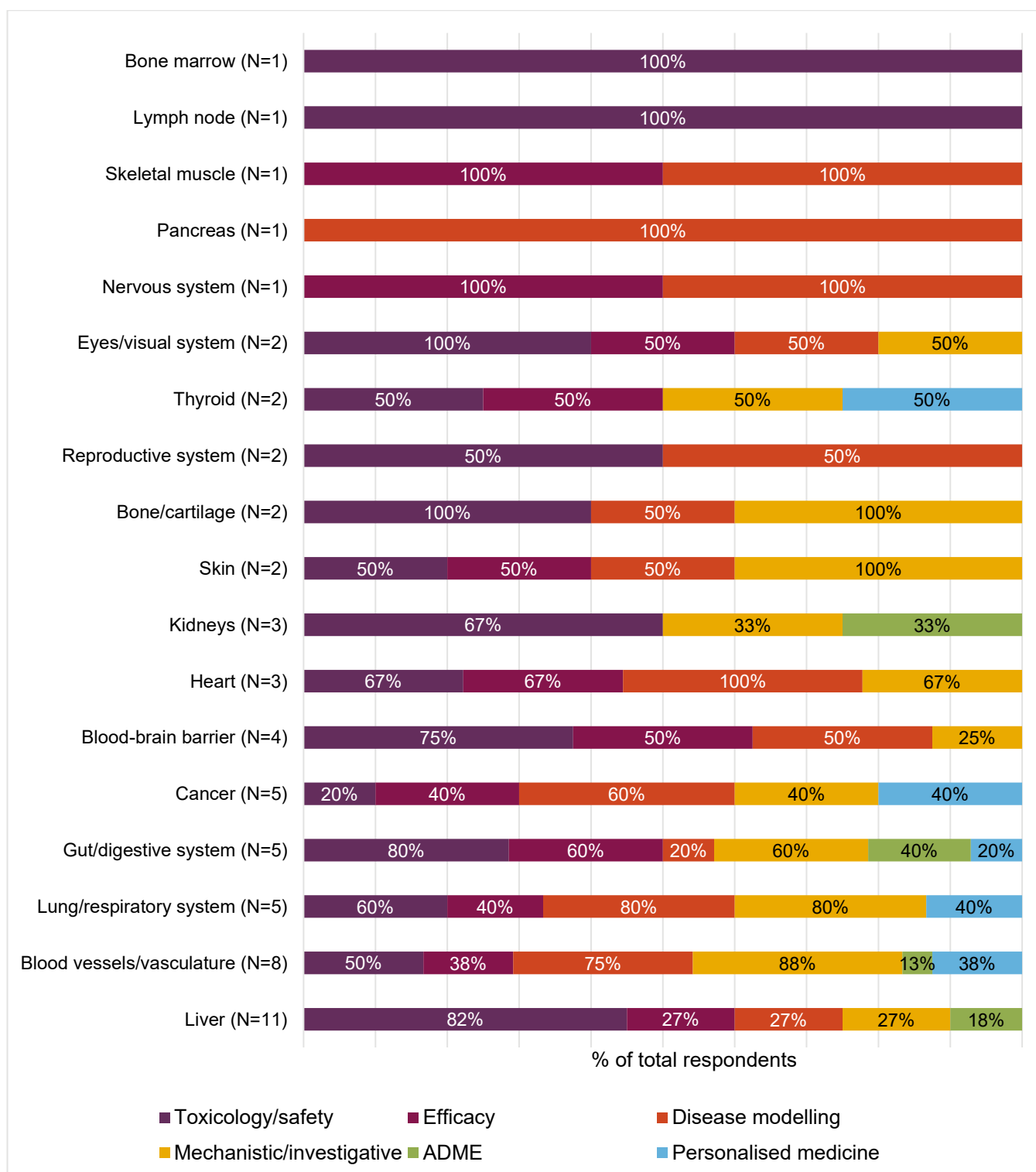
Figure 6. Organ systems respondents were evaluating and their applications. The number of respondents who selected a particular organ is shown in brackets. The number of respondents who selected a particular application is plotted as a % of the total respondents for each organ (e.g. nine out of 11 (82%) respondents selected that they were evaluating liver for toxicology applications).

Application	Combination of organs
ADME	Thyroid and liver
Disease modelling	Adipose tissue and liver
Disease modelling	Liver, circulatory and adipose for diabetes
Disease modelling	Skin and oral mucosa
Efficacy	Skin and cancer
Efficacy	Skin and oral mucosa
Mechanistic/investigative	Adipose tissue and liver
Mechanistic/investigative	Liver, circulatory and adipose for diabetes
Mechanistic/investigative	Liver, gut and immune system
Mechanistic/investigative	Skin and oral mucosa
Toxicology/safety	Heart and liver
Toxicology/safety	Liver, gut and immune system
Toxicology/safety	Skin and cancer
Toxicology/safety	Skin and oral mucosa
Toxicology/safety	Thyroid and liver
Toxicology/safety	Tumour and liver

Table 1. Multi-organ systems and application(s) respondents were evaluating (N=7 respondents).

42. Respondents were asked where they saw the technology they were evaluating being deployed and used for decision making in the future. Responses included for basic research, through to preclinical and clinical drug development, and for applications within the food, chemical and cosmetics industries (Figure 7). Specific areas highlighted included:

- To investigate the mechanisms of a compound-induced effect.
- To inform *in vivo* study design.
- To screen out compounds with undesirable properties early prior to *in vivo* studies.
- To progress molecules to the clinic without using *in vivo* efficacy models.
- For personalised medicine.
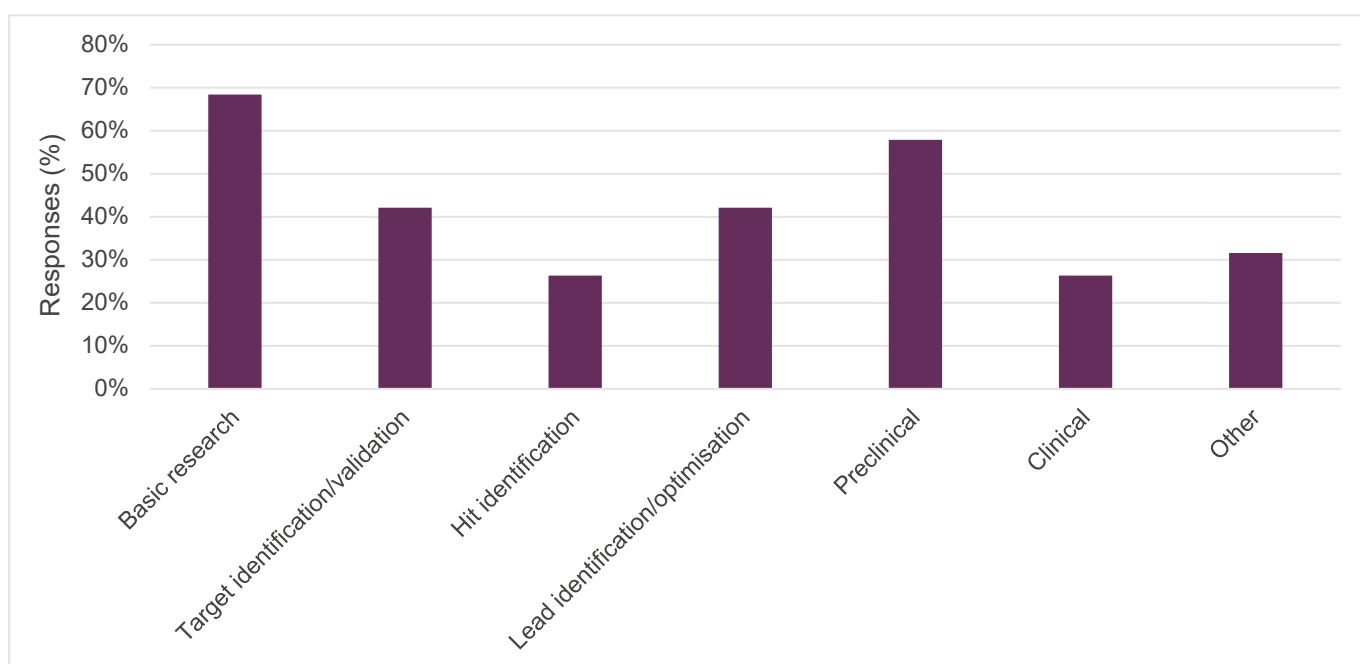- For assessing chemical safety for endocrine disruptors.

Figure 7. Responses (%) to where respondents saw the technology they were evaluating being deployed for decision making in the future. N=19 respondents.

43. Five respondents selected that they had adopted single organ systems and one respondent had adopted a multi-organ system. Examples of organ systems (and applications) that had been adopted for decision making included:

- Skin for toxicology, efficacy and mechanistic/investigation applications
- Liver for toxicology and efficacy applications.
- Nervous system for efficacy, disease modelling and mechanistic/investigative applications.
- Reproductive system for disease modelling.
- Thyroid for toxicology.

3Rs impacts

44. Respondents were provided with the following definitions of replacement, reduction and refinement:

- Replacement: Accelerating the development and use of models and tools which directly replace or avoid the use of animals in experiments where they would have otherwise been used. For example, OoC technology is used to screen out compounds with undesirable properties prior to *in vivo* studies or the technology is used to investigate the mechanisms of a compound-induced effect instead of performing the experiments in an *in vivo* or *ex vivo* animal investigative study.

- Reduction: Methods that minimise the number of animals used per experiment through appropriately designed and analysed experiments that are robust and reproducible. For example, use of OoC technology to inform the design of *in vivo* experiments so that not as many animals need to be used per experiment.

- Refinement: Methods which minimise animal suffering and improve welfare. For example, use of OoC technology to inform dose selection so that animals are not exposed to unnecessarily high doses.

45. Respondents who were considering or not currently using the technology were asked whether they agreed that the technology will replace, reduce and refine animal use. Respondents who were currently not using the technology either agreed or disagreed that the technology will replace animal use, but generally agreed that the technology will reduce and refine animal use (Figure 8). Respondents who were considering using the technology generally agreed that the technology will replace, reduce and refine animal use (Figure 9).
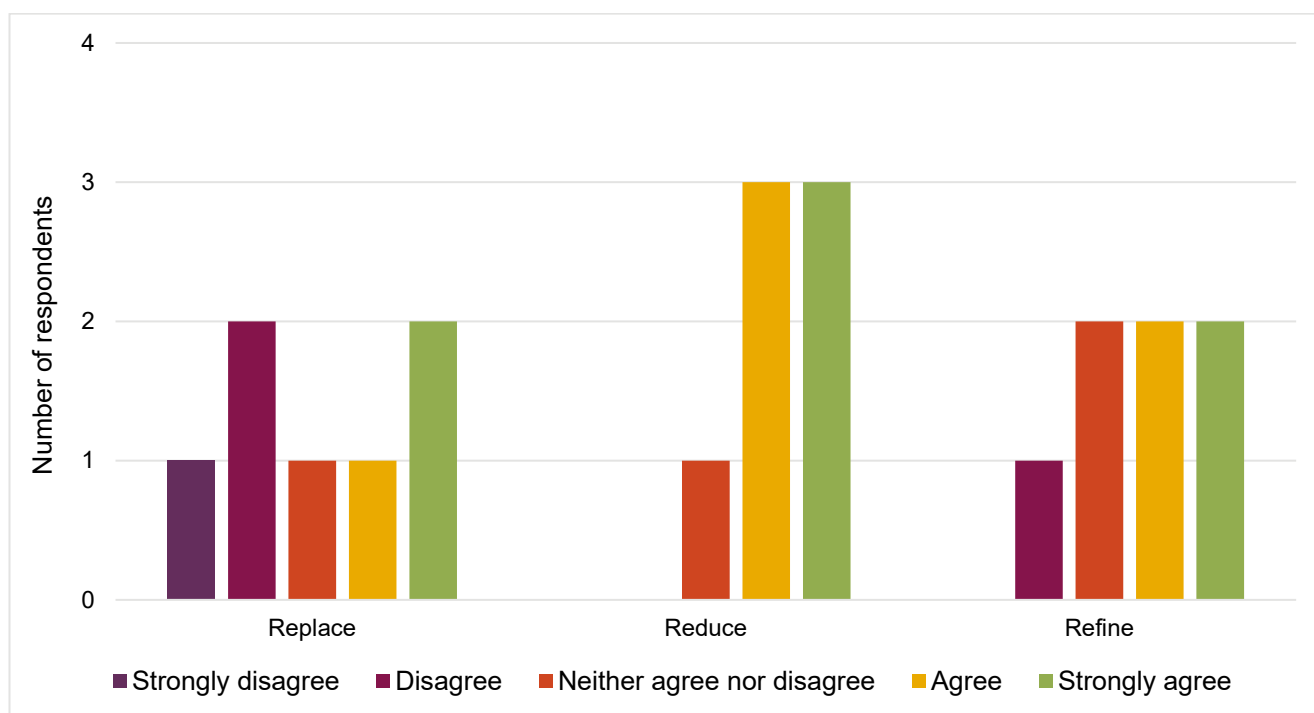
Figure 8. Respondents currently not using the technology responses to whether OoC technology will replace, reduce and refine animal use. N=7 respondents.
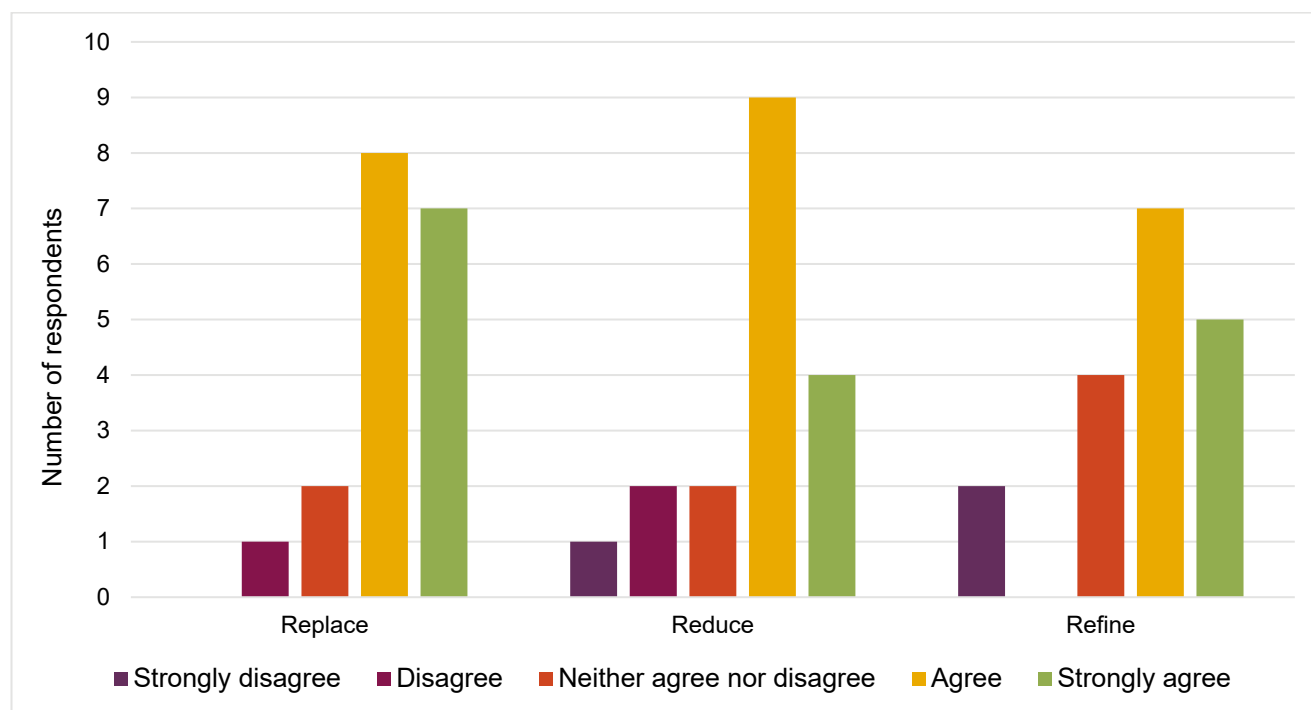

Figure 9. Respondents considering using the technology responses to whether OoC technology will replace, reduce and refine animal use. N=18 respondents.

46. Respondents who were using the technology were asked whether it had replaced, reduced or refined animal use within their organisation, selecting all that apply. There were three (17%) responses for replaced, five (28%) responses for reduced and two (11%) responses for refined, indicating that the technology has started to deliver some impact on the 3Rs (Figure 10). Three respondents provided details on the animal studies that the technology had replaced, reduced or refined within their organisation, which included studies in rodents (mice and rat). For example, in one respondent's organisation, the technology has replaced up to 300 animals per year used in basic research.

47. Respondents were then asked whether the technology will replace, reduce or refine animal use within their organisation in the future. There were 6 (35%) responses for replace, 12 (71%) responses for reduce and ten (59%) responses for refine, indicating that the technology has the potential to significantly impact animal use within respondents organisations in the future, across all of the three Rs (Figure 11).

48. Six respondents provided details on the animal studies the technology had the potential to replace, reduce or refine within their organisation in the future. Examples provided included to replace mouse models used for immunology studies, and to replace, reduce and refine rodent and non-rodents (rats and dog) used in preclinical mechanistic studies to better understand human relevance and translation. For example, in one organisation the technology has the potential to replace up to 500 rats/year used in rodent mechanistic studies.
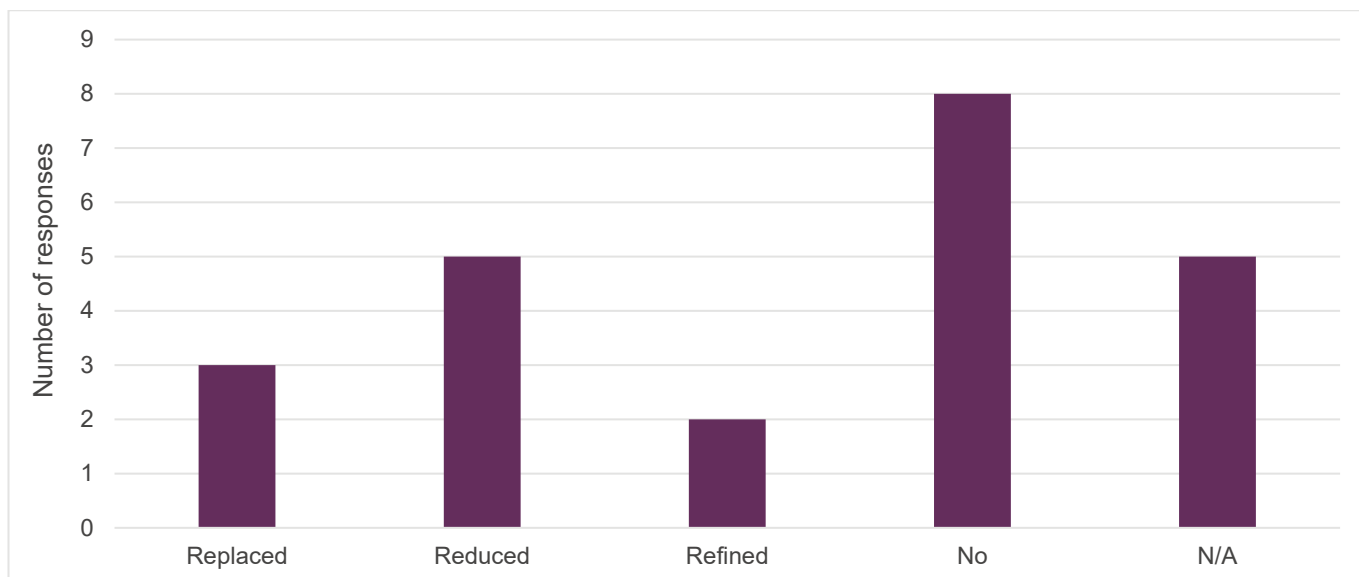
Figure 10. Respondents currently using the technology responses to whether OoC technology had replaced, reduced or refined animal use within their organisation. N=18 respondents.
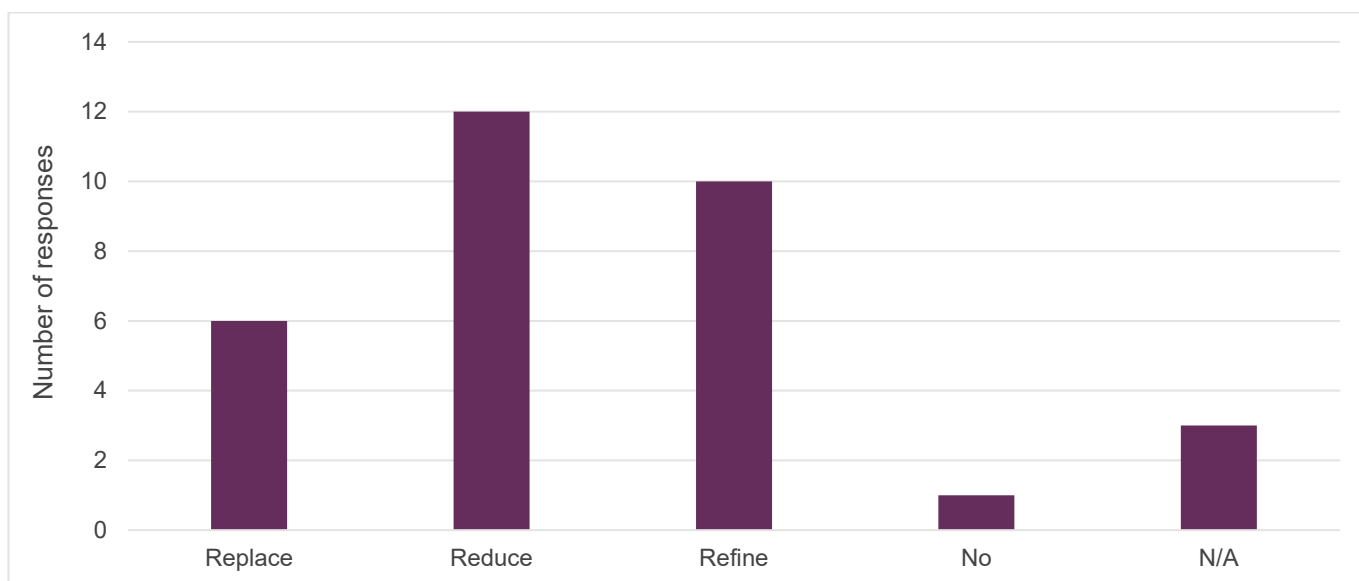

Figure 11. Respondents currently using the technology responses to whether OoC technology will replace, reduce or refine animal use within their organisation in the future. N=17 respondents.

Future opportunities

49. Respondents were asked what they considered to be the future opportunities of the technology. Examples provided included:

- In pharmaceutical drug discovery and development:
  - For better selection and optimisation of molecules prior to any *in vivo* studies that are required.
  - To study the mechanistic aspect of drugs and to understand species differences for translation.

- To investigate efficacy and safety in the same model to allow safety questions requiring specific disease states to be investigated in appropriate models.
- In DMPK for replacement of early animal PK evaluations.
- To detect complex temporal PKPD relationships for safety where current *in vitro* models are unable to fill this gap.
- For disease modelling to understand disease mechanisms.
- For personalised medicine to enable better translation using patient samples. For example, using a patient's tumour to predict response to therapy.
- To provide human relevant models to better predict human physiology.
- Replacement of mouse models in immunology.
- For environmental chemical mixtures toxicity assessment.
- For repeat dose and long-term exposure studies of drugs, chemicals and cosmetics.

Barriers to adoption

50. Respondents were provided with a number of potential barriers and asked to rate how much of a barrier they considered each to be in adopting the technology, from not a barrier to a significant barrier. The most significant barriers listed in rank order included:

- Cost.
- Current systems lack core components.
- Data generated is not accepted by regulators.
- Limited qualification/validation to demonstrate the technology is fit-for-purpose (Figure 12).

51. Respondents were asked to provide any other significant barriers which included:

- There being very few standards in the field.
- Accessing funding for validation in direct comparison with animal models.
- Difficult to access the technology and generate pilot data without significant funding in place, but also difficult to get significant funding without pilot data and ongoing access to the technology.
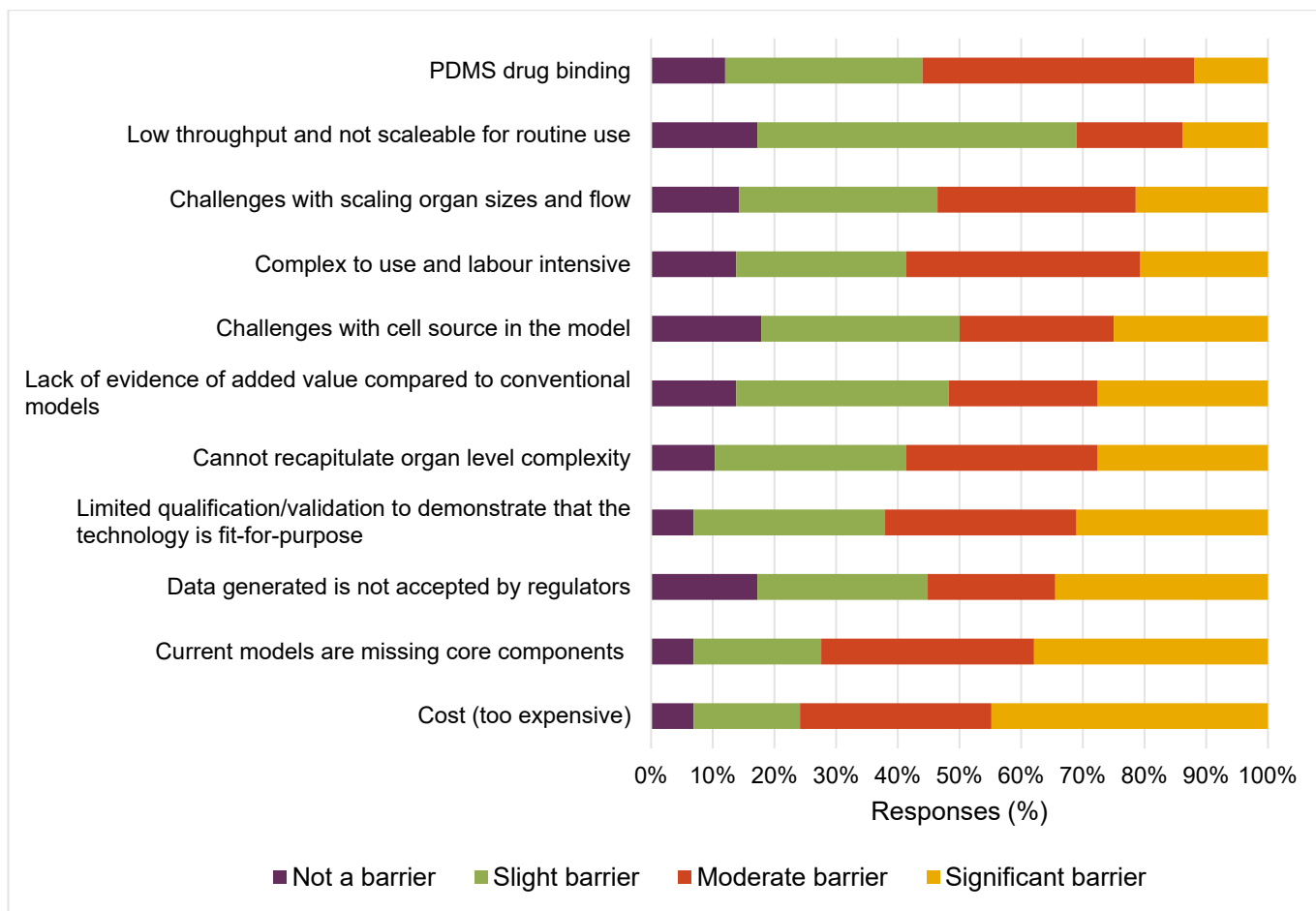
Figure 12. Respondent responses (%) to how much of a barrier they considered the options provided to be in adopting the technology. N=30 respondents.

Overcoming the barriers to adoption

52. Respondents were provided with several options and asked to rate them in terms of their importance in facilitating the adoption of the technology, from not important to very important (Figure 14). The top five criteria considered to be very important in facilitating adoption of the technology were:

- Robust characterisation and qualification/validation to demonstrate the technology is fit-for-purpose.
- Publication of case studies to demonstrate utility and added value over conventional models.
- Regulatory acceptance.
- Establishment of independent testing centres to evaluate and validate the technology.
- Establishment of a global network to share the latest developments, knowledge and experience.

53. Respondents were asked to include any further criteria they considered important in facilitating adoption of the technology, these included standardisation being a key component for the field and the technology conferring an advantage over existing *in vitro* systems to justify their cost and complexity.
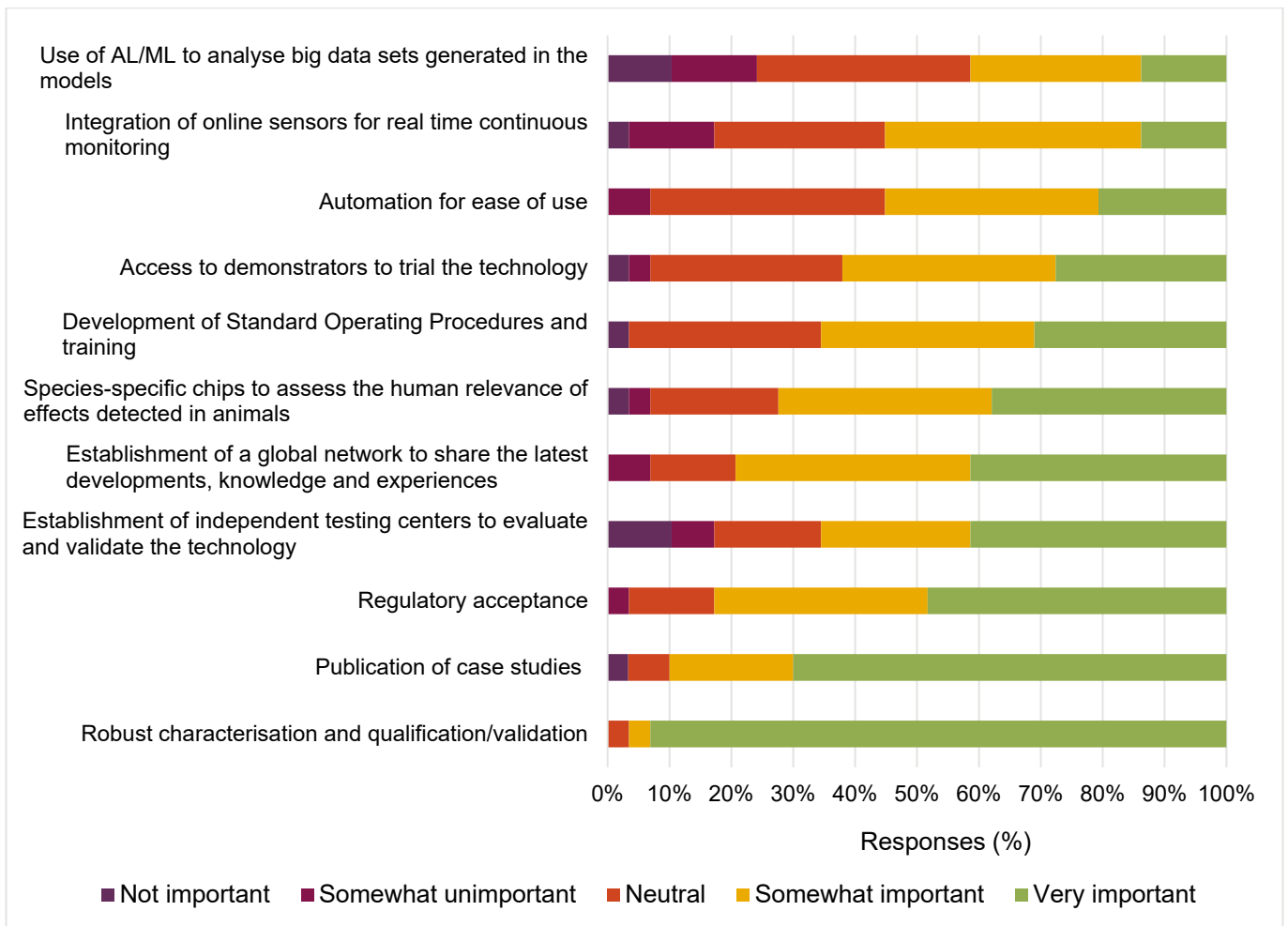
Figure 13. Respondent responses (%) to how important they considered the options provided to be in facilitating adoption of the technology. N=30 respondents.

Technology readiness

54. Respondents were asked to rate the maturity of the OoC field by assigning a modified technology readiness level (mTRL, taken from Phadke & Vyakarnam (2017), Camels, Tigers and Unicorns: Rethinking Science and Technology enabled Innovation. *World Scientific Publications*). The majority of respondents (52%) assigned mTRL3 - a working prototype or demonstrator has been built and mTRL4 - the new product, service or technology has been refined/ modified following feedback from the initial customer (Figure 14). See Annex 2 for full mTRL definitions.
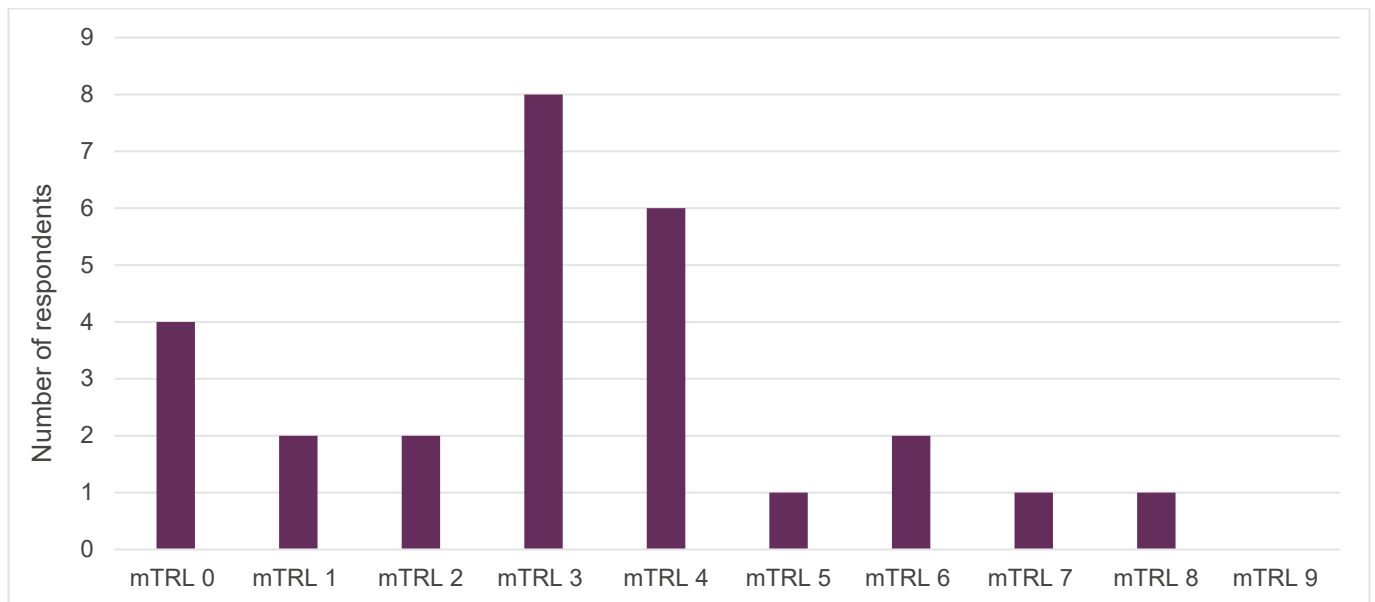
Figure 14. Modified technology readiness levels (mTRLs) assigned by respondents. N=27 respondents.

Summary

55. The survey results provide insight into the current landscape and how the technology is being used across sectors. The majority of end-users were from the academic and pharmaceutical sectors (72% of respondents) and were primarily evaluating the technology. Most respondents were evaluating single organ systems with some multi-organ systems also being assessed.

56. The technology has started to deliver some impact on the 3Rs but has the potential to significantly impact the 3Rs in the future. Respondents indicated that the field is still immature in terms of its technology readiness, and there are significant barriers that need to be overcome before it can be widely adopted into routine use. Several areas were highlighted that would help overcome these barriers to facilitate uptake including to characterise and qualify/validate the technology so it is fit-for-purpose, robust and reproducible.

Annex 2. Modified Technology Readiness Level (mTRL) definitions

Reproduced from Phadke & Vyakarnam (2017). Camels, Tigers and Unicorns: Rethinking Science and Technology enabled Innovation. *World Scientific Publications.*

- mTRL 0 - Research in progress (Fundamental research activity before any potentially useful and validated science or technology has been established).

- mTRL 1 - Validated research: Start concept definition (At the point at which the conceptual application of the technology has been defined in outline terms).

- mTRL 2 - 2 Initial concept defined (The conceptual application has now been converted to a definition of the product or service which can potentially be offered using the technology).

- mTRL 3 - Working prototype or demonstrator (A working prototype or demonstrator has been built).

- mTRL 4 - Product or service testing and concept refinement (The new product, service or technology has been refined/modified following feedback from the initial customer).

- mTRL 5 - Proven product or service (The product or service is ready at a functional level, without the collateral around the product including the method of deployment and the proposed business model).

- mTRL 6 - Deployment with early customers in real commercial environment (The product or service is now ready for use with early Customers, and so includes all the associated collateral, including a service infrastructure where relevant).

- mTRL 7 - Product or service ready for testing in real user environment (Early customer feedback has been used to define the modified product or service functionality, its required performance and critically, the chosen business model).

- mTRL 8 - Techno-commercial refinement of product or service (The refined product or service is now ready for deployment with mainstream customers).

- mTRL 9 - Ready for commercial deployment with real customers (The final product or service is now ready for commercial launch, including go-to-market collateral and proven business model. The challenge now is growing the mainstream customer base.