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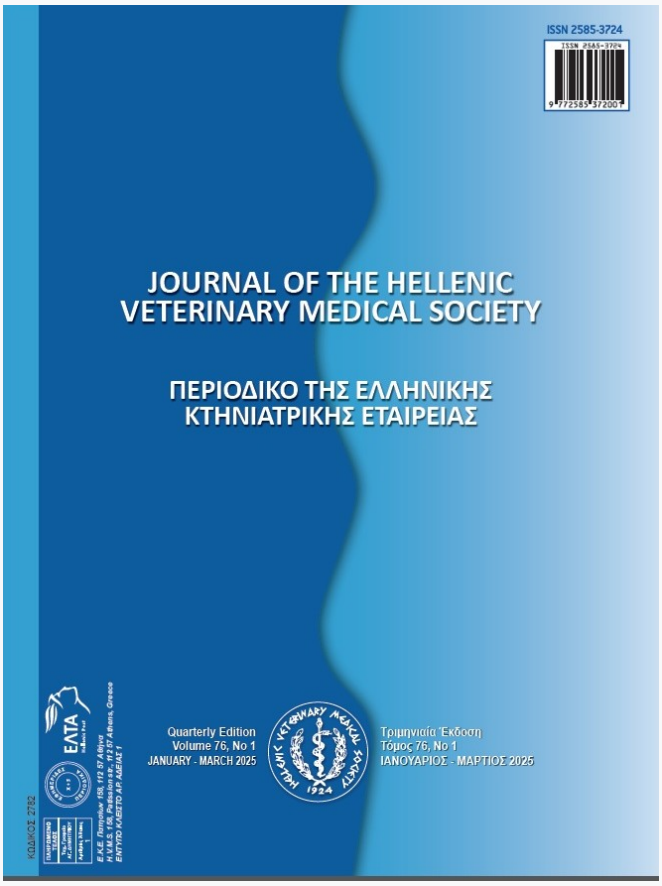

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Research article
Ερευνητικό άρθρο

Investigating the integration of Alternative Methods to Animal Use in Hellenic Biomedical Research and Educational Institutions

D. Chatziprokopiou[1,*], M.A. Tsoutsou[2], E. Fragkiadaki[1,3], A. Tsingotjidou[4]

[1]*Master of Arts "Animal Welfare, Ethics and the Law", School of Philosophy, National and Kapodistrian University of Athens, Athens, Greece*

[2]*Experimental, Educational & Research Center ELPEN, Athens, Greece*

[3]*Department of Animal Models for Biomedical Research, Hellenic Pasteur Institute, Athens, Greece*

[4]*Laboratory of Anatomy, Histology and Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

ABSTRACT: Replacement is the primary of the Three Rs (3Rs standing for Replacement, Reduction, Refinement) which are the established humane experimentation principles, incorporated in the EU member states legislation for the protection of animals used for scientific purposes. Replacement is defined as the alternative methods or New Approach Methodologies (NAM) that decline or substitute the use of animals. Our aim was to investigate for the first time the integration of replacement methods in laboratory animal facilities serving Biomedical Research and Education in Greece, after over 10 years of harmonized implementation of Directive 2010/63/EU. An anonymous questionnaire survey was addressed to personnel responsible for managing 56 licensed facilities. Information about the characteristics of participating facilities; the use of animal alternatives and the perceptions around available alternatives were collected. A 50% response rate was yielded for analysis. The majority of the facilities were public non-profit organizations, running animal protocols for both research and training purposes. To our pleasant surprise, most people responsible for managing licensed Hellenic animal facilities reported the usage of alternative methods, indicating a moderate and low degree of replacement of animal models. 62.5% of the facilities had partially substituted animal models, with cell cultures, Systematic Review and/or Meta-Analysis, and other non-animal methods being the major adopted methods. Rodents and farm animals were the predominant species to be replaced, whereas fishes were used to replace rodents and other laboratory animals. To our knowledge, these are the first data regarding animals' replacement in Greece, especially in a transition period where deep learning and fluidics are about to change the animal research landscape. The main finding of our survey was the established perception that scientific reproducibility, time efficacy of research outcomes, and moral integrity are adequately guaranteed by adopting alternative methods to animal use. However, until more validated alternatives are present and widely accessible, the use of animals in biomedical research and education in Greece is still imperative and ongoing.

Key words: animal use; alternatives; biomedical research; education; Greece

Corresponding Author:
Chatziprokopiou D, Master of Arts "Animal Welfare, Ethics and the Law", School of Philosophy, National and Kapodistrian University of Athens, Athens, Greece
E-mail address: chatziprokopiou.despoina@gmail.com

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INTRODUCTION

Alternative methods to animals' use are considered an ethical act due to the intrinsic value of animals' lives, a legal obligation due to the Replacement principle adopted in Directive 2010/63/EU, a financially sustainable solution against the expensive quality standards in vivaria and a direct translational research approach for human conditions overcoming the uncertainties introduced by animal species differences (Pound and Ritskes-Hoitinga, 2018).

In 1959 Russell and Burch published the concept of 3Rs (Replacement, Reduction, Refinement) and defined Replacement as "any scientific method employing non-sentient material that may replace methods using conscious living vertebrates". They also made a distinction between two types of Replacement, namely absolute and partial. In the case of absolute Replacement, animals are not required at any stage, while for partial Replacement, animals are still required (i.e. for obtaining cells for *in vitro* methods) but are not exposed to any distress. The term partial Replacement has also been used to indicate that a non-animal test may not fully replace an animal study, but rather replace only certain aspects of it. In such cases, it would take a combination of non-animal methods to fully replace animal use. If a non-animal model can replace animal experimentation (for example, for skin irritation), this is called a full or one-on-one Replacement (ETPLAS).

In Directive 2010/63/EU, on the protection of animals used for scientific purposes, Article 4.1 defines Replacement as follows: *"Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure."* This implies avoiding the use of whole living animals, whether or not these animal species are covered by the scope of the Directive. With this assumption, organisms like invertebrates, plants, or early-stage embryonic forms of vertebrates that have immature nervous systems or do not experience pain and distress as intensely as the Directive's regulated organisms, are not considered free of ethical implications.

Animals are still used for basic and translational biomedical research, regulatory testing of products as well as for higher education purposes aiming at the acquisition of specific technical competencies (i.e., surgical). On the other hand, their partial or absolute replacement is prioritized by the European Commission. In this direction, it has created the EU Reference Laboratory for Validation of Alternatives to Animal Testing (EURL-ECVAM) as a unit of its Joint Research Center (JRC) Directorate General. The main focus of this service is to promote the dissemination of developed validated alternative methods and to enhance the dialogue among main stakeholders (industry, research, education, regulators, animal rights associations).

Today NAM stands for New Approach Methodologies and includes any *in vitro*, *in silico,* or *in chemico* (chemistry-based) method, as well as the strategies to implement them, with the scope to provide information about chemical safety assessment of products that need to fulfill regulatory requirements (Stucki *et al*., 2022; Westmoreland *et al.*, 2022; Zuang *et al.*, 2022).

Current non-animal approaches for biomedical research involve complex *in vitro* models and engineered tissues (namely the use of pluripotent stem cells in 2D structures, 3D microtissues or bioprinting such as spheroids, organoids and microfluidic systems like organs-on-a-chip), *ex vivo* tissues, use of clinical and epidemiological data, systematic reviews and metanalysis of published data (Ritskes-Hoitinga and van Luijk, 2019), computer modeling and artificial intelligence (Dash and Proctor, 2019; Kostomitsopoulos, 2018; Li *et al.*, 2023; Otero *et al.*, 2022; Perez Santin *et al.*, 2021; Vermeulen *et al.*, 2017; Zuang *et al.*, 2023). EURL-ECVAM has reviewed research literature available between 2013-2018 and over a few hundred validated, non-animal models have emerged per disease category including respiratory tract diseases, breast cancer, neurodegenerative disorders, immuno-oncology, immunogenicity testing for advanced medicinal therapy products, cardiovascular diseases, and autoimmune diseases.

Alternatives to animals' use in biomedical education include conventional audiovisual media (videos, graphics, animations), life-like mannequins, animal as well as patient-simulators (Papalois, 2017; Humpenöder *et al*., 2021; NORINA Database), animal cadaveric tissues, immersive technologies like 180^{o} or 360^{o} virtual reality (VR) and, training or augmented reality (AR) with gamification elements (Lemos *et al.*, 2023; Neuwirth and Ros, 2021; Patronek and Rauch, 2007; Tang *et al.*, 2020; Whitfield, 2023).

The replacement of animal use in education remains challenging for many educators (Patronek and Raunch, 2007; Zemavova *et a.*., 2021; Poulou *et al.*, 2022). The two main reasons for continued animal

use are stated to be: a) the necessity to use a living animal for enhancing direct somatosensory perception described as "proper" learning, and b) the lack of an adequate alternative. On the other hand, a systematic review of limited scale, comparative studies in USA and EU universities reported that the alternative method used was not perceived as inferior or less efficient by the students, instead it rather was free of any moral or emotional distress (Patroned and Raunch, 2007; Lawson *et al.*, 2022).

Limitations of alternatives' use in education depend on the method. In some cases, this method could be outdated, or quite expensive to operate since it may also require well-trained experts, while some people may experience simulator sickness and nausea i.e. when VR technology is used (Whitfield, 2023). Moreover, the alternative method may dictate an overall reformation of the studies curriculum with significant administrative consequences. Nevertheless, the combination of alternative methods and investment or resources that aim at sustainability may be the future of next-generation education.

Even so, the assessment of competencies remains an issue to be resolved. The Learning Outcomes define the minimum knowledge and skills trainees should possess upon completion of the required modules related to biomedical and laboratory animal science fields (Dontas *et al*., 2023; ETPLAS). On the other hand, the assessment of practical competence is mainly based on Objective Structured Clinical Examination (OSCE), and recent efforts are documented for Objective Structured Laboratory Animal Science Examination (OSLASE) (Costa *et al.*, 2022,) and Direct Observation of Practical/ Procedural Skills (DOPS; ETPLAS). Training programs should incorporate non-animal methods in their curriculum and respectively adapt the evaluation procedures for trainees' skills. This demands for the development of a transition strategy in order to move from existing established methods to alternative ones. In this case, the Conscientious Objection Policies to harmful animal use in education at medical and veterinary faculties will not be necessary due to the adapted alternative studies program (Zemanova, 2023).

In Greece, there is no official database for the level and type of NAMs used in national Institutions. The aim of our study was to investigate the integration of animals alternatives in Biomedical Research and Education institutions in Greece following the completion of 10 years since the initial implementation of Directive 2010/63/EU and national PD 56/2013.

MATERIALS AND METHODS

A questionnaire survey was performed with people responsible for managing licensed animal facilities serving biomedical research and education purposes in Greece. The list of licensed facilities used was created in 2022 for a Master's Thesis regarding the Openness and Transparency of animal research in Greece (Tsoutsou, 2022). Following confirmation of relevant contact data validity and updating any changes, the questionnaire created for our study purposes was sent to 56 Institutions in March 2023.

The questionnaire included 11 closed-ended questions in the form of single-choice, multiple-choice as well as graded scales (Appendix 1). The answers to the graded scale questions were modeled according to a four-point Likert-type scale so that the participant could clearly state the desired tendency and avoid a neutral position. Moreover, the "I do not know" option was also included. Questions were categorized into 3 groups targeting information about the characteristics of the facility (questions 1-4), the assessment of alternative methods' integration per facility (questions 5-8), and the managers' perception of available alternatives (questions 9-11). Not all questions needed to be answered, and there was no option to revisit a question once it had been answered. The questionnaire was provided in the Greek language while the estimated time for completion was 10 minutes.

The questionnaire survey was designed electronically using the LimeSurvey application (https://www.limesurvey.org/) and was approved by the Ethical Committee of the Aristotle University of Thessaloniki (Protocol Number of Approval: 113930/2023). It was then distributed using the Gmail platform, to individual, personalized, email addressed of the managers of animal facilities (1 questionnaire per facility). The allowed timeframe for submitting the completed questionnaires was 10 days.

The survey was completely anonymous and no personal information- such as email addresses- was collected. Data analysis was performed using Excel (MS Office).

RESULTS

Out of the 56 distributed questionnaires, a total of 32 questionnaires were received back, representing a response rate of 57.1%. Among those, 25 question-

naires were fully completed, while 7 were partially completed. Within the group of partially completed responses, 3 out of the 7 had an overall completion rate of 66% (indicating that 2 out of the 3 constituent units were completed). The remaining 4 out of the 7 had a completion rate of less than 10% and were excluded from the subsequent analysis. As a result, the dataset subjected to analysis was comprised of 28 completed responses, yielding a survey response rate of 50%.

Characteristics of the Participated Facilities

Facilities in the study were initially categorized based on their legal entity and profit type. According to Figure 1, the majority- 82.1% of the total sample-fell within the Public Sector, while 14.3% were affiliated with the Private Sector. A 3.6% of the facilities fell under the category 'Other'. Furthermore, the facilities were categorized based on the nature of the organization. A substantial proportion, in particular 92.9% of the participating organizations, were classified as non-profit entities, with the remaining 7.1% identified as for-profit entities.

The objective of our survey was to solicit responses from facilities engaged in animal use. Our primary aim was to ascertain the underlying objectives of animal use within each facility. We established that 32.1% of all surveyed institutions employed animals exclusively for research purposes (Figure 2). Notably, a 67.9% majority of organizations, indicated that the use of animals within their respective operations was driven by dual objectives, encompassing both educational and research purposes.

Among the participating Hellenic facilities, it was observed (Figure 3) that a significant proportion, that is the 64.3%, acknowledged the use of non-animal model alternative methods. In contrast, 32.1% of the surveyed institutions indicated that they exclusively relied on traditional animal-based approaches, without employing any alternatives. Additionally, a relatively small 3.6% either refrained from responding or expressed uncertainty regarding their use of alternative methods.

In our survey, we sought to examine the extent of individuals' engagement in various scientific roles within their respective fields. According to Figure 4, our findings indicate that the majority of our participants held two distinct scientific roles, while approximately 15% of respondents concurrently occupied three roles within the same domain.

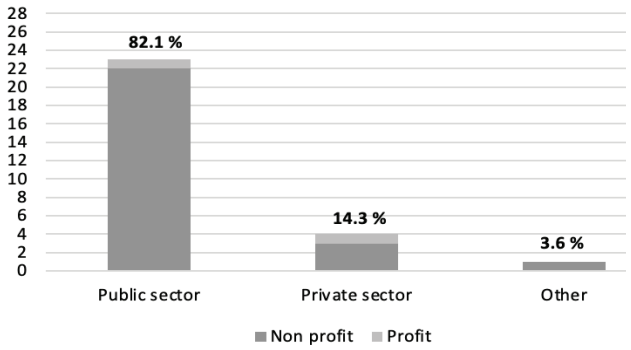

Figure 1: Facilities' structure based on legal entity and profit type.

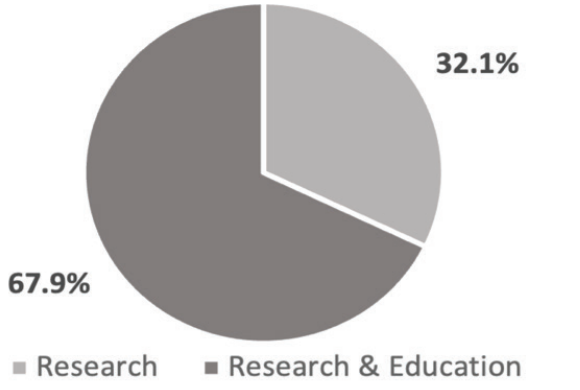

Figure 2: Purpose(s) of projects using animals in each facility.

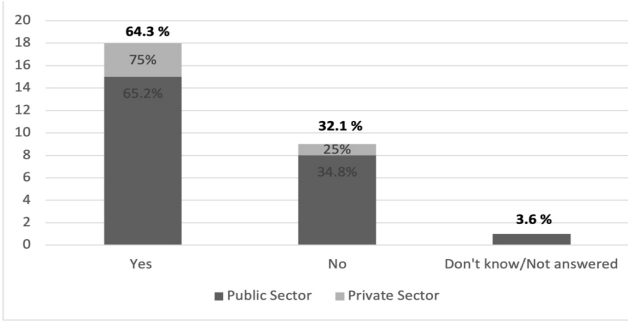

Figure 3: Usage of non-animal model alternative methods.

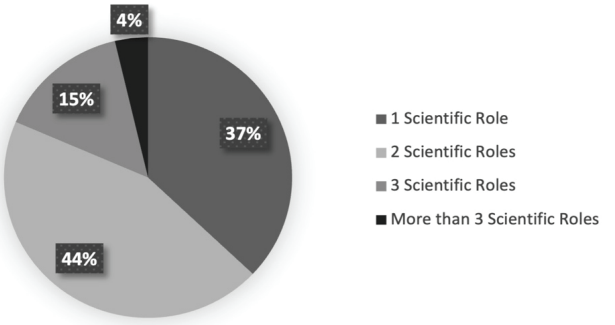

Figure 4: Participants' roles in the facilities.

Assessment of alternative methods' integration per facility

Mouse (*Mus musculus*) (67.9%) and Rat (*Rattus norvegicus*) (46.4%) are the two most widely used species in Hellenic facilities, for both research and education projects. Other animal species also used are fishes (32.1%), rabbits (*Oryctolagus cuniculus*) (28.6%), swine (*Sus scrofa domesticus*) (17.9%), birds (7.1%), and other farm animals (7.1%) (Figure 5).

As presented in Table 1, among the institutions indicating the adoption of non-animal alternative methods, it was evident that cell culture (66%), along with Systematic Review and/or Meta-Analysis (44%), emerged as the most prominently favored choices.

Table 1 illustrates the diverse array of non-animal alternatives employed by Hellenic facilities. A small percentage of managers (3.6%) either indicated uncertainty or chose not to respond to this question (Appendix 1; Question 5) while later (Appendix 1; Question 7) selected options about alternative methods. These participants reported the use of systematic review and/or meta-analysis, cell culture, media and graphics, simulators, and epidemiological studies within their facilities (Figure 6). Furthermore, it is important to note that the questionnaire did not offer the option to revisit and modify previous responses, rendering it impossible for participants to revise their answers. The level of replacement per animal species was documented as 60-74% in facilities that used small rodents, around 50% when farm animals, fishes, and swine were used, and finally around 25% in case of rabbit use (Table 2).

Perceptions about the use of alternative methods

Regarding the extent to which animal use has been substituted by non-animal alternative methods in the past five years, according to Figure 7, it is noteworthy that 62.5% of the participants indicated a moderate degree of substitution, while the remaining 37.5% reported 'To a little extent'. Notably, as outlined in the aforementioned Figure no respondents selected 'To a great extent' or 'Not at all' as their response. Application of alternative methods was reported to be achieved mainly through attempts made exclusively by the facility's staff (71,4%), while others established relevant collaborations with local or international facilities (Figure 8).

When participants were asked to express their views regarding the factors motivating the use of animal alternatives, ethical considerations emerged as the primary driver. Additionally, as presented on Figure 9, scientists took into account the improved reproducibility associated with non-animal methods as well as the fact that such methods are significantly less time-consuming.

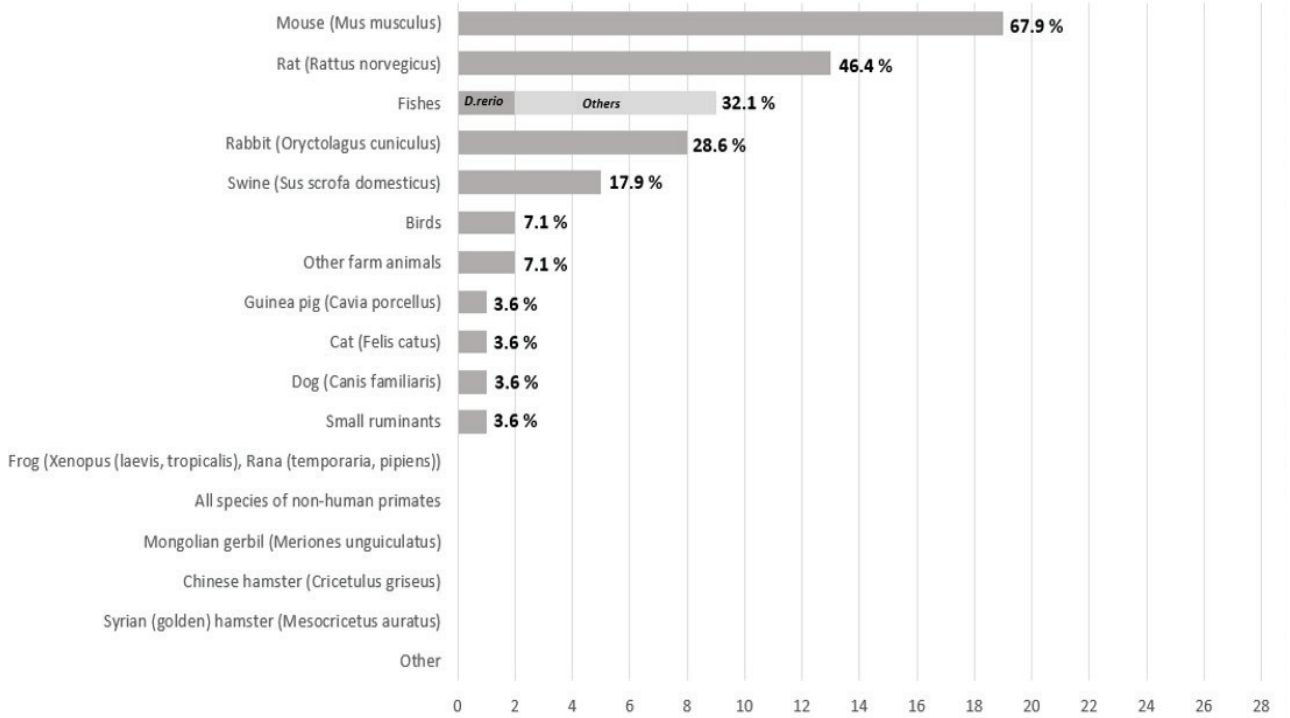

Figure 5: Animal species used in the facilities.

Alternative Methods (AM)	Number of facilities using specific AM/ Total facilities using AM	Percentage %
Cell culture (animal or human cells)	12/18	66.6
Systematic Review and/or Meta-Analysis	8/18	44.4
Media and Graphics	6/18	33.3
Tissue culture (animal or human tissues)		
Simulators use for educational purposes	4/18	22.2
Omic technologies		
Organoids/ spheroids		
Biobank		
Life-like Mannequin		
Clinical data utilization		
Insects	3/18	16.6
Epidemiological studies	2/18	11.1
Organ-on-a-chip, Microfluidics, Multi-organ chip		
Artificial Intelligence		
Animal cadaveric tissues	1/18	5.55
Nematode		
Foetal forms of mammals as from the last third of their normal development		
Chick embryo tissue culture		
Human cadaveric tissues		
Tissue engineering/ Computanional models		
Virtual Reality		
Organ culture (animal or human organs)	0/18	0
3D Bioprinting		

Table 1: Percentage of specific alternative methods used in surveyed facilities.

Animal Model	Number of facilities that replaced the model/ Total facilities that used the model	Percentage of replacement
Mouse (*Mus musculus*)	14/19	73.70%
Rat (*Rattus norvegicus*)	8/13	61.50%
Other farm animals	1/2	50%
Fish [Zebra fish (*Danio rerio*) + other]	4/9	44.40%
Swine (*Sus scrofa domesticus*)	2/5	40%
Rabbit (*Oryctolagus cuniculus*)	2/8	25%

Table 2: Animal models and their percentage of replacement pre facility.

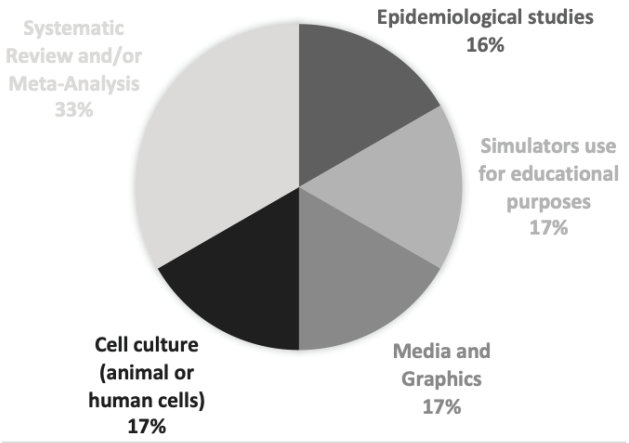

Figure 6: Alternatives methods selected from participants that retrospectively indicated no-use of alternatives in their facilities.

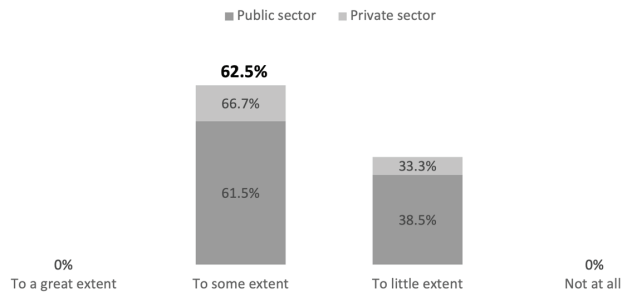

Figure 7: Perceived extent of animal replacement in the facility within a 5-year period.

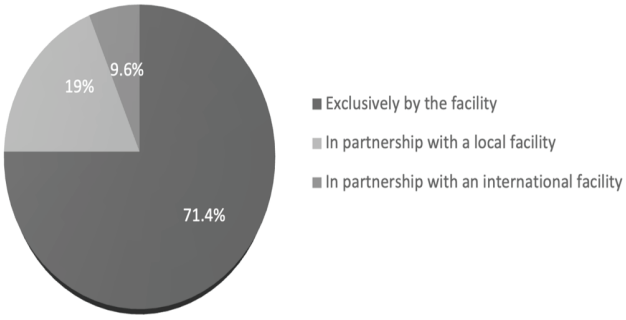

Figure 8: Perceived autonomy in applying alternative methods.

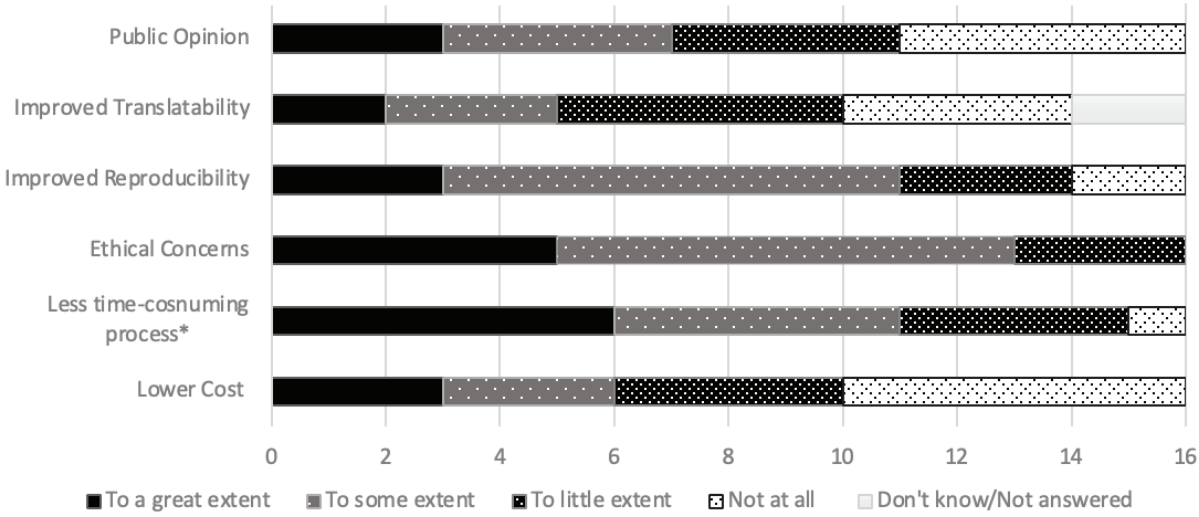

Figure 9: Perceived reasons for the replacement of animal models.

DISCUSSION

Approximately half of the country's licensed animal facilities managers offered their feedback regarding the integration of alternative methods within their facilities providing some evidence about researchers' current attitude towards replacement. In order for a questionnaire to have meaningful results, it needs to have a response rate of at least 30%. The fact that the present study had a 50% response rate is positive and shows a tendency of the national animal research community to openly discuss the relevant issues. Openness in animal research promotes public support (Mendez *et al.,*2022) however scientists and all personnel involved in animal experiments need to

achieve accurate representations of their work.

Institutions that completely replaced animals and no longer possess animal establishments or those that use forms of animals that are not regulated by the EU or national legislation were not included in the present survey. This acted as a limiting factor for the accurate assessment of applied replacement levels. Further research is necessary in order to obtain data not only from establishments that have replaced animal use but also those that use exclusively non-animal techniques.

Our findings suggest that the predominant users of animal models are national, public, non-profit organizations that serve biomedical research and education purposes. In particular, we found that 95.65% of all public sector and a 75% of all private sector facilities were registered as non-profit entities (Figure 1). Around 68% of institutions, maintained animal models for both research and educational purposes, and 32% exclusively for research (Figure 2). Rodents and fishes were the most prevalent models used in surveyed facilities (Figure 5) which is in accordance with the survey of Kiani *et al.*, 2022. We should also mention that non-human primates, amphibians, gerbils, and hamsters were absent. Along these lines, as highlighted by Lewis B. Kinter *et al.*(2021), a significant transformation in research practices is widely observed. This transformation is manifested by a noteworthy shift of the research burden away from traditional, larger, and more publicly sensitive species such as dogs, cats, and non-human primates, towards smaller, less publicly sensitive animals like mice, rats, and fishes.

The management structure and social background of facilities' personel as well as the professional and mental well-being of the were not investigated during our study. Nevertheless, the multiple identities that a manager assumes across national facilities was an interesting finding, taking on several responsibilities i.e. those of a designated veterinarian or a caretaker (Figure 4). These multilevel obligations may be the outcome of institutions' financial constraints, the low workload of small facilities that may not justify permanent staff for every discipline, or the lack of experienced personnel to assume distinct roles within each facility.

Notably, the implementation of alternative methods was reported in two-thirds of surveyed facilities, while in only one-third of them, it was absent. The ratio of facilities using animal alternative methods was quite comparable between public and private sector, namely 65.2% in the public and 75% in the private sector respectively (Figure 3). Among the available non-animal alternative methods, particularly prominent were found to be some *in vitro* methods (cell and tissue cultures), systematic reviews and/or meta-analysis, as well as media & graphics (Table 1). Beyond any ethical considerations that might be important for choosing any alternative methods, we believe that there are more reasons promoting the high rank of these particular three aforementioned model categories. Specifically, the use of *in vitro* methods is cost-effective and highly reproducible, rendering its widespread adoption. On the other hand, our survey underlined the importance of making use of already available data through applying systematic reviews and/or meta-analysis. Systematic reviews have potential to complement ongoing work to select optimal animal models by directing researchers towards those that are most predictive, or they may direct researchers away from animal models altogether according to Pound P. and Ritskes-Hoitinga M. (2020). This way scientists can efficiently and without experimental repetition - saving animal model life, consumables, and human resources - combine data from multiple existing studies, identify knowledge gaps, and narrow down their further experimental groups (SYRCLE). In addition, tools such as Media & Graphics scored high most likely due to being easily accessible, flexible, and affordable. It is suggested that they were of particular importance for educational purposes, especially during the pandemic (2020-2022).

It also important to mention that surveyed facility managers were aware of alternative methods use. Only the 3.6% of the managers gave a controversial answer regarding alternative methods use. Such controversial answers are indicative of a lack in understanding of the validity of certain alternative methods like simulators, audiovisual aids, systematic review and/or meta-analysis and epidemiological studies (Figure 6). Sophisticated alternatives like organoids, organ-on-chip, and artificial intelligence as well as traditional methods like cell cultures and audiovisual aids were reported as currently applicable in national facilities. Rodents and farm animals were reported to be partially replaced by alternative methods; the only carnivore breeding facility is reported as replaced but it is not clear if the facility was financially unsustainable due to lack of demand (Table 2). Among the facilities that do not apply alternatives, some reported the exclusive use of fishes, regarding

it as a challenging animal model to replace. As noted by K.A. Sloman *et al.* (2019), "*While the focus on replacement has intensified in mammalian studies, there has been a growing body of fundamental research exploring fishes as potential substitutes for mammalian model organisms. However, it is important to acknowledge that in many countries, regulatory legislation applies to fishes in the same manner as it does to mammals, and the concept of replacement typically pertains solely to young pre-feeding fishes*". Nonetheless, the complete replacement of fishes in experimental research can encompass approaches such as *in vitro* cultures or computer models, which still enable biomedical research without the necessity of live fish usage, as Schaeck *et al*. (2013) indicated.

Managers supported that there was a significant replacement pace in their facilities during the last 5 years (Figure 7). Ethical considerations emerge as a prominent factor (Figure 9) profoundly impacting scientists' decision-making processes. Less time-consuming processes and enhanced reproducibility constitute two additional factors that weigh heavily on scientists' considerations when contemplating the use of animal alternatives. Beyond ethical considerations, as underscored by Meili Kang *et al.* in 2022, it is imperative to optimize and minimize the use of laboratory animals in both educational and scientific research. On the other hand, the cost of procedures appears to be a relatively less concerning factor for Greek managers. While certain animal alternatives may indeed be cost-effective compared to live animal experimentation, the primary focus remains on the efficacy of the procedure itself, with its effectiveness taking precedence over cost considerations, as elucidated by Annamaria A. Bottini in 2009.

CONCLUSIONS

To our knowledge, these are the first data regarding animals' replacement in Greece, especially during the current transition period where deep learning and fluidics are about to change the animal research landscape. To our pleasant surprise, the majority of managers reported the use of alternative methods, indicating a moderate and low degree of replacement of animal models in Hellenic facilities. The perception that scientific reproducibility, time-efficacy of research outcomes, and moral integrity are adequately guaranteed by adopting alternative methods to animal use was the major finding from our survey. However, the use of animals in biomedical research and education in Greece remains imperative and is still ongoing until more validated alternatives are available and widely accessible.

ACKNOWLEDGMENTS

We express our gratitude to all participants for their valuable contributions to our survey .The openness of laboratory animals' managers in participating in our survey was really appreciated, considering the limited time of questionnaire's availability and their demanding multitasks.

List of supplementary material (tables, figures)

Appendix 1. Questionnaire for assessing the animals' replacement in surveyed institutions

Table 1. Percentage of specific alternative methods used in surveyed facilities

Table 2. Animal models and their percentage of replacement per facility

Figure 1. Facilities' structure based on legal entity and profit type

Figure 2. Purpose(s) of projects using animals in each facility

Figure 3. Usage of non-animal model alternative methods

Figure 4. Participants' roles in the facilities

Figure 5. Animal species used in the facilities

Figure 6. Alternative methods selected from participants that retrospectively indicated no-use of alternatives in their facilities

Figure 7. Perceived extent of animal replacement in the facility within a 5-years period

Figure 8. Perceived autonomy in applying alternative methods

Figure 9. Perceived reasons for the replacement of animal models

Appendix 1

Questionnaire Survey for Alternative Methods to Animal Use in Hellenic Research and Educational Institutions

1. Does your facility belong to:

☐ Public sector

☐ Private sector

☐ Other

1. What is the nature of your facility:

☐ For profit

☐ Non profit

☐ Other

2. What is your role in the facility?

☐ Facility Responsible

☐ Designated Veterinarian

☐ Researcher

☐ Master's/ Doctoral student

☐ Postdoctoral student

☐ Faculty member

☐ Animal caretaker/animal technician

☐ Other ……..

3. Does your facility use animals for research or training purposes?

☐ Research

☐ Education

☐ Both

4. Are alternative methods of replacing laboratory animals used in your facility?

☐ Yes

☐ No

☐ I don't know

5. What species of animals are used in your facility?

☐ Syrian (golden) hamster (Mesocricetus auratus)

☐ Chinese hamster (Cricetulus griseus)

☐ Mongolian gerbil (Meriones unguiculatus)

☐ All species of non-human primates

☐ Frog (Xenopus (laevis, tropicalis), Rana (temporaria, pipiens))

☐ Guinea pig (Cavia porcellus)

☐ Dog (Canis familiaris)

☐ Cat (Felis catus)

☐ Small ruminants

☐ Other farm animals

☐ Birds

☐ Swine (Sus scrofa domesticus)

☐ Rabbit (Oryctolagus cuniculus)

☐ Fishes

☐ Rat (Rattus norvegicus)

☐ Mouse (Mus musculus)

☐ Other

6. Which of the following non-animal alternative methods are used in your facility?

☐ Systematic Review and/or Meta-Analysis

☐ Epidemiological studies

☐ Clinical data utilization

☐ Simulators use for educational purposes

☐ Virtual Reality

☐ Life-like Mannequin

☐ Media and Graphics

☐ Biobank

☐ Computanional models

☐ Artificial Intelligence

☐ Organ-on-a-chip, Microfluidics, Multi-organ chip

☐ Tissue engineering

☐ 3D Bioprinting

☐ Cell culture (animal or human)

☐ Tissue culture (animal or human)

☐ Organ culture (animal or human)

☐ Organoids/ spheroids

☐ Omic technologies

☐ Animal cadaveric tissues

☐ Human cadaveric tissues

☐ Chick embryo tissue culture

☐ "Foetal forms of mammals as from the last third of their normal development"

☐ Insects

☐ Nematode

☐ No alternative methods are used

☐ Other

☐ I don't know

7. Which animal species were replaced (full/absolute or partial/relative replacement) by the use of alternative methods?

☐ Syrian (golden) hamster (Mesocricetus auratus)

☐ Chinese hamster (Cricetulus griseus)

☐ Mongolian gerbil (Meriones unguiculatus)

☐ All species of non-human primates

☐ Frog (Xenopus (laevis, tropicalis), Rana (temporaria, pipiens))

☐ Guinea pig (Cavia porcellus)

☐ Dog (Canis familiaris)

☐ Cat (Felis catus)

☐ Small ruminants

☐ Other farm animals

☐ Birds

☐ Swine (Sus scrofa domesticus)

☐ Rabbit (Oryctolagus cuniculus)

☐ Fishes

☐ Rat (Rattus norvegicus)

☐ Mouse (Mus musculus)

☐ Other

8. To what extent do you think that animal use has been replaced by alternative methods in your facility within the last 5 years?

☐ To a great extent

☐ To some extent

☐ To little extent

☐ Not at All

☐ I don't know

9. Alternative methods are used

☐ Exclusively by the facility

☐ In partnership with a local facility

☐ In partnership with an international facility

☐ In partnership with both a local and an international facility

☐ I don't know

10. Reasons which have led to absolute/relative Replacement of animal use in each facility

	Not at All	Very Little	Somewhat	To a Great Extent	I don't know
Public opinion	○	○	○	○	○
Improved reproducibility	○	○	○	○	○
Improved translatability	○	○	○	○	○
Lower cost	○	○	○	○	○
Less time-consuming process (licensing, implementation, etc.)	○	○	○	○	○
Ethical concerns	○	○	○	○	○

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