



**ANIMPACT - AN ETHICAL, LEGAL AND PRACTICAL
PERSPECTIVE ON THE IMPACT OF A NEW REGULATORY
FRAMEWORK FOR THE SCIENTIFIC USE OF ANIMALS ON
RESEARCH AND INNOVATION**

Final Publishable Summary Report

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EXECUTIVE SUMMARY

European legislation regulating animal experimentation is based on the need to balance the two important interests at stake: advancing research and protecting animal welfare. Both aspects of this balance were important for the revision of EU legislation that resulted in Directive 2010/63/EU, a revision for which harmonization and a level playing field across the European research area in terms of animal welfare and conditions for research were major drivers. Whereas legislation and technical harmonization are essential, there are important aspects where the Directive only give very general indications, allowing very different solutions in practice. In addition, legislation is not the only mechanism which regulate how animals are used in research – internal norms and rules in different areas of science are also important. The project ANIMPACT - *An ethical, legal and practical perspective on the impact of a new regulatory framework for the scientific use of animals on research and innovation* have addressed external and internal regulation mechanisms regulating animal research in Europe.

In the review of external regulation, ANIMPACT focused on legal analysis of the Directive, empirical analysis of the project evaluation process and analysis of the perception of important stakeholders. The legal analysis indicates certain issues where rather different solutions may result from the national transposition. This includes where the Directive introduces and gives important roles to entities that many Member States did not have (e.g. institutional animal-welfare bodies and the national committees) and where it relies on concepts for which there is no universal standard (e.g. the 3Rs). Of special interest is the project evaluation and authorization, where the Directive specifies which aspects should be considered in the project evaluation process but not who should perform this evaluation or how the process should be organized. A detailed mapping shows that different Member States have very different approaches. On the geographic or administrative level there are systems where evaluation is either national or regional or institutional as well as several different combinations of national and institutional. As regards composition of review committees, scientific and veterinary / animal welfare expertise is present in all committees, whereas there is great variation in other types of expertise. Whether or not different interests are represented and how they are balanced within committees also vary. Among stakeholders directly affected by the Directive (bench scientists, pharma industry and research funders) all see a clear need for regulation of animal use and to a large degree share the intentions behind the directive. There are critical concerns – to some degree differing across countries – about the functioning of the actual regulation and a widespread concern about unproductive paperwork.

In the study of internal regulation, ANIMPACT focused on mechanisms within the scientific community and scientists' own choices. Looking at a specific field at the forefront of the development of discipline-specific guidelines for animal-based research, the results suggest that the guidelines reflect the standard within the field rather than directly influencing it. There was no obvious difference between research published before or after the publication of the guidelines, but the research field with guidelines was notably better in one key animal welfare area – disease severity in relation to experimental endpoint - than a comparable field with no guidelines. A survey to editors of the main journals which publish research with animals showed that they consider measures to improve animal welfare important but are split on whether they consider journals to be responsible for ensuring good animal welfare in research. A survey to scientists about what determines their choice of animal models show that scientific considerations are extremely important, followed by ethical considerations and in third place legislation. In contrast, public opinion was ranked very low in importance.

PROJECT CONTEXT AND OBJECTIVES

European legislation regulating animal experimentation is based on the need to balance the two important interests at stake: advancing research and protecting animal welfare. Both aspects of this balance were important for the revision resulting in Directive 2010/63/EU. The previous directive (86/609/EEC) was limited in scope and did not necessarily protect animal welfare effectively (especially as animal welfare became a more established European Union value), whereas the fact that some Member States had extensive national legislation and others a mere transposition of Directive 86/609/EEC meant large and undesirable discrepancies. One of the main objectives of revising this legislation was to create a greater harmonization across the European research area in terms of animal welfare and conditions for research.

There is an unprecedented effort by the European Commission in promoting a common understanding and implementation of the new legislation, and the ongoing discussion in the laboratory animal science community with working groups on different aspects of the Directive is evidence of a strong stakeholder engagement in a shared legislation. On the other hand, even with a far-reaching technical harmonization, there are important aspects where the Directive only give very general indications and therefore Member States may find very different solutions. A notable example is project evaluation and authorization.

By analyzing how ethics review and project authorization operate and are perceived by stakeholders in animal research, as well as mapping the mechanisms which scientists operate themselves when deciding over animal research, ANIMPACT aimed to produce information about major and previously uncharted aspects of regulation and decision-making mechanisms in animal experimentation.

In doing so ANIMPACT took its starting point in a broad understanding of regulation and decision-making, as illustrated in Figure 1. We understand that how animal research is regulated is not only determined by legislation, which is perhaps what one most often associates with regulation, but also through a set of mechanisms that operate within the research community.

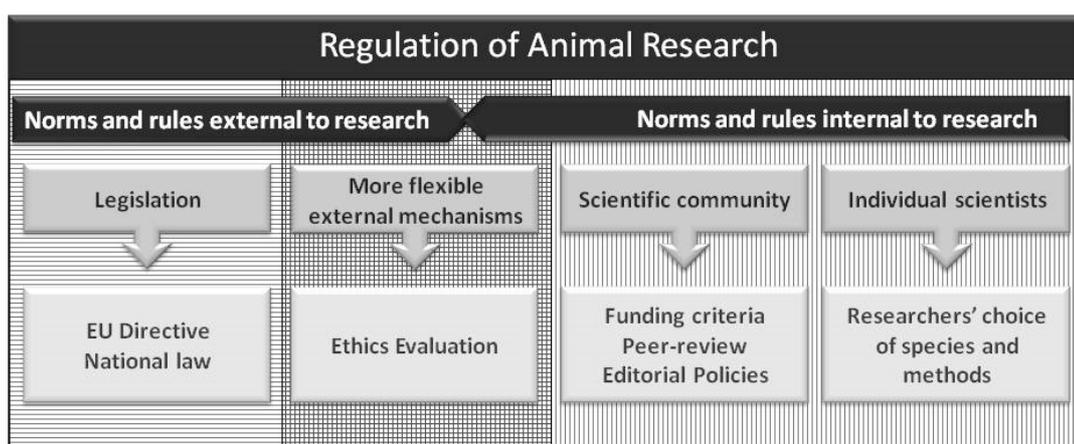


Figure 1. Overview of how regulation of animal research is understood within the ANIMPACT project.

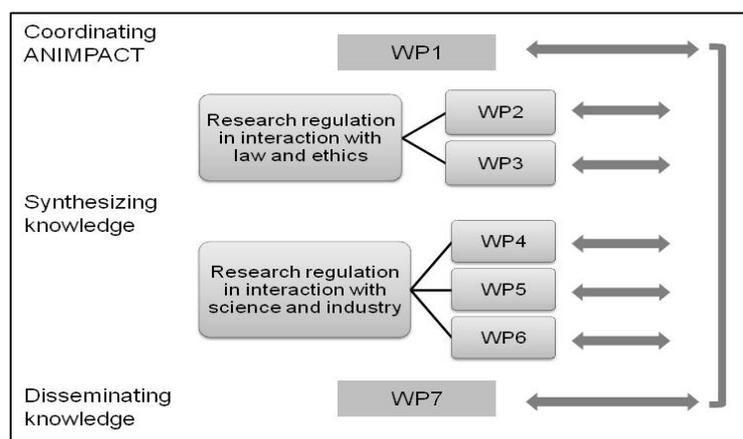


Figure 2. Overall organization of the ANIMPACT project

Within ANIMPACT, five work packages were focused on *synthesizing knowledge*, one on dissemination and one on management (Figure 2).

This report is organized according to the set-up of the five information-synthesizing work packages:

- WP2 The Directive, the broader legal landscape and the licensing process
- WP3 The Directive, the ethical landscape and the practice of ethical review
- WP4 Internal regulation in research: Safeguarding animal welfare through the 3Rs
- WP5 Fish, mice or monkeys? The influence of different factors on the choice of model species
- WP6 External regulation and competitiveness: practice and perception of key stakeholders in animal research

The context and objectives of each of these work packages is presented in the following.

[The Directive, the broader legal landscape and the licensing process](#)

Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes was one of the first European Directives to set standards for animal protection. It was however comparatively limited in scope, and by the time the revision process was initiated in 2002, the Directive was widely considered to be out-dated and no longer efficient in setting the desired high and harmonised standards for the European research area. A major problem was the great discrepancies and differences between Member States, ranging from a mere transposition of the Directive at a very basic level to extensive and potentially more restrictive national legislation.

Directive 2010/63/EU introduces substantial changes compared to 86/609/EEC. It aims to protect animal welfare as a value of the European Union and to eliminate disparities in legislation and practice between Member States. Important new features include extended scope, enhanced focus on the 3Rs and alternative methods, mandatory project evaluation, severity classification and retrospective assessment, as well as revised guidelines for accommodation and care, which are now mandatory. The ongoing transposition process for 2010/63/EU is being carefully monitored by the Commission, through meetings with national contact points generating consensus documents for a common interpretation and implementation of the Directive, and Expert Working Groups working at

a more technical level to implement sections of the Directive. Directive 2010/63/EU should, therefore, be more effective in ensuring appropriate animal welfare in scientific research across the Member States. It has been the task of work package 2 to map regulatory mechanisms external to research through the study of legal norms and the licensing process in different Member States.

The Directive, the ethical landscape and the practice of ethical review

Directive 2010/63/EU provides specific technical detail for example in respect of animal housing or euthanasia methods. It also provides general recommendations for research practice, in particular regarding how to protect the welfare of animals. In a small number of aspects it may also set more extensive limits to research, for example, limiting the use of non-human primates. However, European and Member State legislation cannot regulate in detail which individual projects should be licensed or how research should be designed and carried out: the variety of research protocols and rapid development of science requires a more flexible decision-making mechanism. As a consequence, the real decision-making on treatment of animals in research projects takes place in the project evaluation process at the local or national level. The licensing procedure - together with the decisions made by scientists in the planning of experiments, and, in many jurisdictions, evaluation by ethics or animal care and use committees - is therefore a major determinant of the types, methodologies and practices of the research that are actually developed.

Directive 2010/63/EU specifies which aspects (e. g. predicted benefit, 3Rs compliance, severity, harm-benefit analysis) should be considered in the project evaluation process (Article 38) but not who should perform this evaluation or how the process should be organized. In work package 3, work has focused on providing a picture of the actual workings of the evaluation process across EU countries. At the start of ANIMPACT, an overall perspective of the formal and informal procedural aspects of the review process of animal experiments in EU-27 was missing. Work package 3 has focused on providing that perspective.

Internal regulation in research: Safeguarding animal welfare through the 3Rs

The integration of research practice with regulation intended to protect animal welfare occurs at many levels and in varying ways from discipline to discipline, institution to institution and country to country. Since biomedical science is a clearly interdisciplinary, inter-organisational and international pursuit, differences in culture and regulation affects both co-operation and competition between different groups and the way that animals are treated, which raises not only welfare concerns, but also concerns about the comparability, and hence the reliability of studies conducted by different groups. In this respect, the standardisation of practice in science should align both the goal of improving animal welfare and that of improving the reliability and reproducibility of science.

Efforts to achieve such standardisation also occurs at many levels, from the informal, local codes of individual laboratories, through formal institutional guidelines, to national and international regulation backed by legislation. However, standards are also maintained both formally and informally, by the peer review process that operates to screen both funding allocation and publication, and by guidelines of operation that are self-imposed by specific fields or disciplines.

The overall aim of work package 4 was to explore how such internal norms may influence the ethical treatment of animals in research through researchers' decisions when planning and reporting their research, and when engaging in the peer review process (as reviewers, journal editors or panel members of funding agencies).

Fish, mice or monkeys? The influence of different factors on the choice of model species

Animals are no longer considered as being only instruments for the different practices and aims of humans. A consequence of this rather new attitude, in the scenario of human/non-human relations, is the rise of ethical concerns on the use of animals in scientific research and worries for their welfare. In this context, different species are given different considerations. For example, non-human primates (NHP) occupy a special position in research and its regulation for a variety of reasons, one of these reasons being the evolutionary proximity to humans which makes them more likely to suffer in cognitively sophisticated manner. Another case is the position occupied by species traditionally thought as pets, such as cats and dogs, which are objects of special concerns for the general public. Furthermore, the use of different species can be differentially affected by economic factors (for example, rats are more costly to house than mice). All of the factors outlined above could influence the choice of a particular species to generate particular animal models. In this WorkPackage (WP) we were interested in exploring external and internal factors (in terms of pressures, motivation and attitudes) which characterise the scientific practice of animal experimentation, questioning researchers on the topic of choice of species for a particular model to be studied.

External regulation and competitiveness: practice and perception of key stakeholders in animal research

The successful implementation of Directive 2010/63/EU depends on how it is received by key actors with a practical or a strategic role in relation to research and development involving animal research. Therefore, we in WP6 have studied the following three groups of actors: bench scientists, pharma industry and funders

Our aim was to find out what key stakeholders, whose work is directly influenced by Directive 2010/63/EU, consider a good regulatory regime and to determine how the directive and regulations build upon it compare with that regime. In relation to this aim it is important to consider that some scientists and other stakeholders primarily are aware of their own national regulation and may only indirectly be aware of the Directive.

We proceeded by means of qualitative methods, i.e. individual interviews. We interviewed 26 bench scientists, with two exceptions, all from universities. Most of these were biomedical scientists but also a few from basic or animal science. They worked in three EU countries: Portugal (8), UK (9), and Denmark (9); and were, with two exceptions, native. There were 12 females and 14 males. Their age ranged from 35 to 64 years with an average of 50 years – so there was in our group of interviewees an overweight of senior people. They worked with a range of species. The following

indicates the distribution concerning the main species with which they worked: Mice (16), pigs (3), fish (3), goats and sheep (2), and rats (1)

We also interviewed six representatives of pharma companies, and three representatives of funders. They were all from UK and Denmark, but we still have ongoing efforts to get one interview with a funder and one interview with a representative of a pharma company from Portugal.

MAIN RESULTS

The Directive, the broader legal landscape and the licensing process

WP2 has primarily undertaken legal exegesis concerning the Directive 2010/63/EU and Member States' legal transposition of the Directive into domestic Law (**Task 2.1**), in combination with empirical work to ask how has the paper transposition worked in practice (**Tasks 2.2-2.5**).

2.1 Issues from a legal analysis of the Directive

The Directive presents a number of compromises that are particularly challenging in terms of achieving the harmonisation and animal welfare aims of the Directive.

i) **Animal Welfare is a strong, but not paramount motivator behind the Directive.** The early Recitals indicate the importance of animal welfare as a European value because of "the capacity of animals to sense and express pain, suffering, distress and lasting harm", and the need to recognise the deepening scientific understanding of those capacities (Rec. 6) (deepening since the 1986 Directive). This motivates a raising of standards across the European Union, whilst at the same time recognising cultural differences between Member States (MS) in the treatment of animals. Further, there is a compromise that, the Directive is predicated on the paradigm that animal use in science is necessary both for human and animal health. However, it claims that "this Directive represents an important step towards achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so" (Rec. 10). In this, there is an explicit tension between different issues within the Directive itself.

ii) **Animal welfare has to be balanced with broader free market ideas, and the needs of the scientific community.** Equally, although less prominent in the Directive, the competence for legislating after the Treaty of Lisbon is Article 114 of the Treaty on the Functioning of the European Union (TFEU), the motive force to create a single market - to remove obstacles to the free movement of people, goods, services and capital, and to remove anti-competitive practices. This is not necessarily at odds with animal welfare. Article 13 (TFEU) states: "In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage." Thus, this does not give competence to legislate for animal welfare in itself, but where there is EU activity it must pay 'full regard' to the 'welfare requirements' of animals, as sentient beings. Thus, Art. 114 (TFEU) is in the background, concerned with ensuring a harmonisation of standards in the use of animals in science and education for issues of developing competitive research and technology industries in the EU, animal welfare sets the level of tone of those standards. Animal welfare is not the paramount consideration; the necessities of science for broader human and animal welfare require the continued use at this time, and the Directive sets the parameters for that use.

iii) **The 3Rs and harmonisation.** The construction of the Directive ensures a regulation of all points in the use of animals through the requirements for a complex arrangement of licensing. The breeding of animals for use in science and education, the welfare of the animals during that use, and either the euthanasia or release of the animals from the science and education is licensed through the licensing of people and facilities. Likewise, the projects themselves are licensed. Replacement and reduction are essentially scientific considerations - is there a viable alternative available to the

use of the particular animal model, and is the number of animals used and the harm burden for each animal acceptable, are matters primarily for the science of the project -; refinement is also concerned with the individual harm burden, but also is a matter for the conditions under which the animals are bred, kept and leave science or education. What is clear from the other work packages and the scientific literature, is that there is not a universal standard for the 3Rs, and this lack of a standard will affect the way the 3Rs operate through the Directive. The issues of refinement are most clearly defined in the Directive, considering cage sizes (Annex III), methods of euthanising animals (Annex IV), etc., and these point to a clear standard of animal husbandry and starting point for debate. Reduction is a matter for ensuring statistical robustness of results, and replacement depends on the acceptability of alternatives in the scientific community (see, for example, Rec. 13)(and as is seen elsewhere in ANIMPACT, in standards set by project review committees, reviewers in peer-review journals, or clinical trials committees). Harmonisation will be strongly affected by how much agreement there is on those standards.

iv) **Animal-Welfare Bodies, The Competent Authority and The National Committee** The Directive establishes several entities with critical roles for how the Directive will effectively operate within the Member States. The Animal-Welfare bodies established through Art. 26 are not centralised in the MSs, rather they are individual to the particular 'business/ institution' involved. The animal-welfare body has particular responsibilities about ensuring animal welfare in the business/ institution, and duties to keep records. The Competent Authority is charged with certain duties under the Directive, particularly the authorisation of projects (Arts. 36–45) and the (unannounced) inspection of breeders, suppliers and users (Art. 34). The Competent Authorities are jurisdictional, and regulated primarily by the MS of the jurisdiction. Finally, each MS is required under Art. 49 to create a "National Committee for the Protection of Animals Used for Scientific Purposes" to advise Competent Authorities and Animal-Welfare Bodies and "ensure sharing of best practice". These three entities will critically impact how the Directive will work in practice, their functioning is strongly interdependent and their effectiveness will be as good as the effectiveness of the competent authority, and the will of the MS.

v) **The need for a "Working Group" (analogous to the "Article 29 Working Group" in Data Protection).** A critical issue is the lack of a formal space for inter-MS debate on best practice. In the period, particularly from 2002–2009, the EC created expert committees that reported on specific issues relating to the reforms¹, and there are many Commission led activities for reporting and discussing interpretations and practice. However, a measure that could well be considered in the 2017 Art. 58 Review, would be a Working Group similar in form and power to the "Article 29 Working Group" under the Data Protection Directive 95/46/EC. The Article 29 Working Group is an independent, permanent committee with "advisory status", and it consists of a representative from each of the MS designated Data Protection Supervisory Authority or Authorities, and EC representatives. Members discuss areas outlined in Art. 30 (95/46/EC) of difficulty in the operation of the Directive found by MS, and it has been very active in publishing reports and recommendations; it is a mechanism to work towards harmonisation within the discretion afforded in the open-texture of the Directive. One could imagine such a Working Group having a powerful role in seeking scientific and procedural consensus, or at least understanding, in relation to Directive 2010/63/EU. An immediately obvious membership will be from representatives of each National Committee. There may be a need to broaden the representation of different expertise and also different stakeholders. A balance has to be struck between range of voices to be heard and efficient working practice, but a

¹ http://ec.europa.eu/environment/chemicals/lab_animals/scientific_committees_en.htm

possible method could be to empower such a Working Group to commission background papers and research on, for example, different reporting criteria and the efficiency of reporting requirements, or on the definition of aspects of the 3Rs.

2.2-2.5. The Implementation of the Directive in Member States' Domestic Law

WP2 undertook desk-based analysis of the transposition of the Directive into a number of MSs' domestic Law (UK, NL, Germany, Ireland, Spain, and Belgium). A number of ways of gaining meaningful information about the practical nature of the transposition were attempted - small pilots using questionnaire survey with open questions to researchers and to the public (in a place where the use of companion animals in research had been stopped in the light of public opinion), and interviews. At the level of transposition into MS Law, each of the MS studied had a full transposition of the Directive. This is in as much as the aims of the Directive, the principles of the 3Rs underpinning the decisions about the use of animals, the licensing, approval and inspection systems are in place, as are animal-welfare bodies, competent authorities, and national committees have been created. Further, the requirements of animal husbandry and the methods of euthanasia for the different animals appear in the Law. Some countries, for example the UK and the NL have extensive codes of guidance accompanying the Law, but this is the level where difference begins to be seen. From the legal analysis of the Directive, however, this is not a surprising finding. The open-texture of the Directive, discussed above, means that a spectrum of perceptions of animal welfare can be accommodated in a 'full' transposition of the Law; each MS can have a transposition that on its face fully accepts the principles of the Directive, but that does not guarantee a harmonisation of standards in animal welfare.

Discussions about local practice with researchers from different MS indicate, tentatively, that there are regional, cultural differences in the interpretation of the underpinning concept of animal welfare. Interviews with researchers in a German pharmaceutical company begin to show this. The German legislation, the German Animal Welfare Act is a very thorough and complete transposition of the Directive. Indeed, one could say that it goes further than the EU regime as it deals with animal welfare in more than scientific and educational settings, with stated aims for that comprehensive animal welfare being "to protect the lives and well-being of animals, based on the responsibility of human beings for their fellow creatures. No one may cause pain, suffering or harm without good reasons." Leaving aside the lawyers "good reasons" as an implicit discretion opportunity, this is arguably a stronger statement than even the principles of the Directive. And, indeed, the desire to pursue animal welfare was found in the responses of the participants. However, it was reported that, as the operation of the licensing, approval and inspection was with the regions rather than centrally controlled, there were discernible differences in the application of the Law. However, there were moves in Germany to extend the transparency of inspection by not only having inspections by the statutory authorities, but also from international non-profit organisations. This was reported to be accepted by pharma as enhancing their transparency. Germany, with its strongly two-level government, perhaps makes the regional differences more apparent. But this will be explored in further cross-jurisdictional webinars, using a fictional protocol and case study to discuss what would be acceptable within the 3Rs (and the Law) within the different jurisdictions represented.

4.4 One particularly interesting legal issue was raised by an interviewee from the Netherlands. There is a current difficulty in that jurisdiction as freedom of information Laws are being used to gain disclosures of personal and other sensitive data about scientific research using animals. It is clear that the Dutch approach to freedom of information is much stricter than other EU jurisdictions - that information should be made available as transparency in the approval process is necessary for public confidence. This is, however, arguably against the spirit of harmonisation in the Directive; the

gathering of personal and other sensitive data places researchers in a position of greater vulnerability because of the approval requirements of the Directive than colleagues in other MS. Here, the EC might consider an amendment to the Directive in line with the provisions of transparency it uses in its Clinical Trials Regulation 536/2014 (Art. 81.4). There it is made explicit that (in relation to data held on the EU Clinical Trials Portal), whereas the basic presumption is for the transparency - availability - of data on the portal, commercially sensitive data and personal data shall not be available and confidentiality in relation to such data is justified. Of course, it may be the case that the Dutch legislators and courts wish to extend transparency into personal and other sensitive data in relation to animal trials, and that would be a matter for the margin of appreciation. However, as a harmonising measure, the effect of a lack of clarity on the point in the Directive might be considered.

The Directive, the ethical landscape and the practice of ethical review

This part of the project explored the process for project evaluation and authorization. The work package was split into 4 tasks: **Task 3.1** Map ethical bodies and ethical review systems for animal research in EU by expanding and updating the FELASA WG Report to cover EU-27 as of 2013. **Task 3.2** Select case studies with the aim of finding four cases which represent the spectrum of different approaches found at EU level, **Task 3.3** In-depth analysis of selected case countries based on interviews with committee members. **Task 3.4** To experimentally study the diversity in animal ethics review committee deliberation by presenting standard case protocols to committee members in several Member States and review committees and ask how they would evaluate the case if they received it through their committee.

3.1 Mapping ethical bodies and ethical review systems for animal research in EU

For this purpose, we collected data through a combined approach, in which first a web search was combined with individual informants in each Member States (MS) to generate a first set of country-specific information. This was then sent to the respective competent authority (MS authorities for Directive 2010/63/EU/ National contact points as per article 59 of the Directive), asking them to confirm, complement or correct the existing information. For the first version of the report we received a response from competent authorities (CA) from 20 Member States. A request for renewed confirmation and update of the information is being processed in order to leave the website with the most updated information. The main results are summarized here, whereas a [complete report is available on the ANIMPACT website](#).

Changes introduced by Directive 2020/63/EU In most Member States, the transposition of the Directive has not introduced major changes to the review and authorisation procedure for projects with animals.

Main actors: The regulation of animal experiments falls under the Ministry of Agriculture in most MS.

Organization of the project evaluation and the authorisation process: There is considerable variation in organization as regards at which level the evaluation and the authorisation takes place. In many MS there is a combination of several approaches. A detailed overview of the situation across the EU is provided in Figure 3.

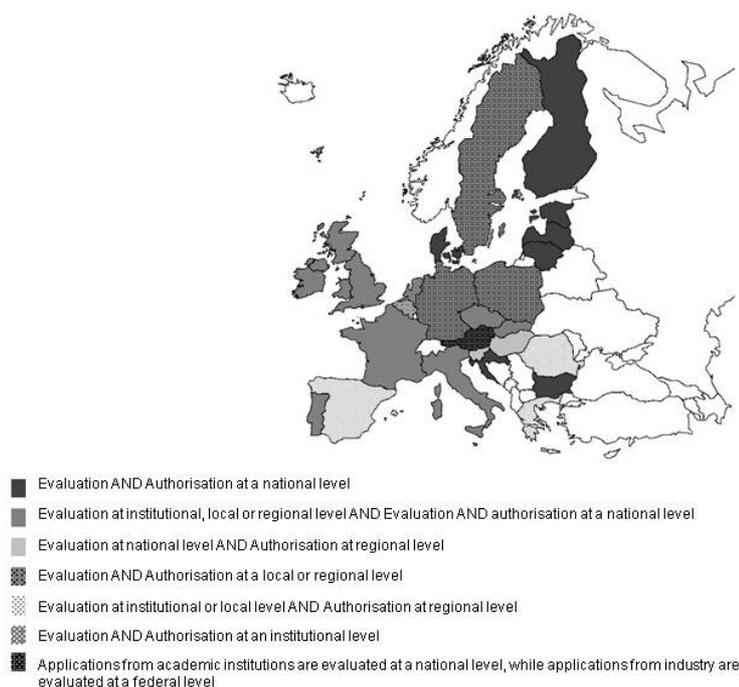


Figure 3. Overview of the geographic and administrative organization of the process for evaluation and authorization of animal experiments

Composition of evaluation committees: The information reported here refers to the recommended or required composition and not the actual composition of different committees. The different types of expertise and representation identified are listed below. Scientific and animal welfare / veterinary expertise is generally present across MS, but the involvement of other types of expertise and representation varies greatly between MS.

1. **Scientific & Science-related expertise:** includes members with scientific expertise/background and with expertise in experimental design or experimental procedures, research techniques and statistical analysis (e.g. statistician or a person with expertise in statistics).
2. **Veterinary & Animal health and welfare:** includes the persons responsible for overseeing the health, welfare, housing and care of the animal and/or the designated veterinarian.
3. **Legal expertise:** Including lawyers, judges and members with a degree in Law.
4. **Ethics:** includes members with expertise/experts in ethics
5. **Alternatives to animal experiments:** members with expertise in alternatives to animal experiments/research or alternative methods.
6. **Other technical expertise:** technology and industry (Denmark only).
7. **Representation of special interest groups**
 - 7.1. **Animal welfare/protection:** representatives of animal protection' or welfare' non-profit organizations [NGOs]/associations or appointed by these associations to represent their interests
 - 7.2. **Patients: only in Denmark – one member appointed by a patients' association**
8. **Society representation:** includes references to "lay persons", public interest representatives or independent persons

3.2-3.3 Organization and functioning of different animal ethics review committees

For the in-depth study of organization and functioning of different animal ethics review committees, four case countries were selected based on what was known about the formal organization. Countries were selected to guarantee that the analysis focused on countries with a certain amount of experience and standards and to represent diversity within the aspects of organization that had been defined. In order to make sure that we were studying countries where there was enough experience with ethics review and where legislation was not undergoing dramatic changes as a result of Directive 2010/63/EU, we set as inclusion criteria that the country should have had extensive animal experimentation legislation, including ethics review, before the transposition of the Directive, and that the country should have considerable biomedical research and pharmaceutical industry activity. We then defined division criteria to ensure desired diversity in terms of composition of committees, level of evaluation, status of the laboratory animal issue on the national agenda and political geography.

The selected countries and a brief characterization of each as regards the division criteria is presented in Table 1. Note that the terms “technocratic” and “participatory” are used to refer to committees with a strong role of experts (technocratic) versus committees where lay persons or representatives of special interests play an important role (participatory).

ITALY	TECHNOCRATIC ETHICS REVIEW SYSTEM, COMBINING NATIONAL AND INSTITUTIONAL REVIEW MUCH PUBLIC AND POLITICAL CONTROVERSY OVER ANIMAL RESEARCH PRECEDING TRANSPOSITION SOUTHERN EUROPE
UK	PREDOMINANTLY TECHNOCRATIC ETHICS REVIEW SYSTEM, COMBINING NATIONAL AND INSTITUTIONAL REVIEW HIGH PUBLIC CONTROVERSY OVER ANIMAL RESEARCH NORTH-WESTERN EUROPE
SWEDEN	PARTICIPATORY ETHICS REVIEW SYSTEM, REGIONAL LITTLE PUBLIC CONTROVERSY OVER ANIMAL RESEARCH NORTHERN EUROPE
HUNGARY	SEEMINGLY TECHNOCRATIC SYSTEM, COMBINING NATIONAL AND INSTITUTIONAL EVALUATION LITTLE PUBLIC CONTROVERSY OVER ANIMAL RESEARCH NEW MEMBER STATE

Table 1. Selected Countries

For the actual interviews, two committees per country were selected with the exception of Hungary where there is only one national committee. For each committee, the chair person and several members were interviewed.

The analysis of the huge amount of data generated through these interviews is still ongoing, but the preliminary results point out some interesting similarities and differences between the committees. The four case countries have committees with quite different compositions – this was in fact a criterion for including them in the study. Despite this, across most of the committees, the interviewees are happy with the composition of their committee and few of them see strong reasons for expanding membership to include the type of expertise or representation that is present in committees in some of the other countries. For example, this UK interviewee does not think a representative of an animal protection NGO (which is standard membership in Swedish committees) would “be very helpful actually because I think that (...) they would like to say well you know we don’t

want animal experiments without actually understanding why you do these animal experiments". An Italian committee secretary argues that *"we don't need a lawyer because that is one of my duty, I have to perfectly know the law and so I have to be able to manage this point and I believe that we really don't need someone from a patient association and we don't need someone from the animal welfare rights groups because they are too involved within the discussion and the patients groups are obviously driven by interest (...) opposite side but they are at extreme points, we just need people that can scientifically understand the relevance of the results and that can really understand the suffering of the animals (...) an idea of (...) stress or distress or pain in the animals.*

None of the committee members had been specially trained for the task and most of them did not know the reason behind their recruitment, but they generally referred to the relevance of their own background and experience for the work in the committee.

In terms of how the committees work vis-à-vis applicants, there is a very big difference between the institutional committees and the committees which are more remote from the applicants. In the institutional committees, and especially in the UK, the applicants are often invited to the committee meetings to present their work and answer questions from the committee. This is perceived by the interviewees as being a constructive way of working, and particularly useful in the case of complex projects, such as the one described by a UK interviewee: *"A very complex license because there are a lot of different steps in the protocol which are optional. So an animal may go through step 5 and step 8, may go through step 2 and... so it's about trying to understand how that works. And that wasn't quite clear in the document. Again when the applicant spoke about it it became very clear what they tried to achieve."* In contrast, the interaction between committee and applicants in the case of more remote national or regional committees typically takes place in writing and result in the application being held up. The flipside of the proximity is the conflict of interest, expressed here by one of the Italian interviewees *"very important to have an external member. It is very difficult for two scientists working at the same university to be completely honest about their opinion on the other's research. If one is like me, not a member of that university, it is easier to tell a scientist that the scientific value of his research is low"*.

3.4 Diversity in animal ethics review committee deliberation

To address whether diversity in review system affects how deliberation works, we used interviews with committee members in six countries, selected to represent a variety in review systems (Estonia, The Netherlands, Portugal, Ireland, Sweden and Switzerland). The interviewees were presented with four different case protocols, specifically designed to represent different combinations of harms and benefits. This included a) a case with genetically modified mice undergoing a severe procedure to develop a new drug with relatively limited impact on a severe human disease with fatal outcome, b) research involving capture and captive breeding of wild rabbits to evaluate a possible reintroduction to increase genetic diversity c) a case with mice undergoing moderate procedures to develop a cheaper drug for a condition for which some drugs already exist and c) a case with dogs undergoing mild procedures and subsequently given for adoption in order to study basic aspects of a disease. They were asked to deliberate over these cases, and were also asked to comment on how their committee would handle each case.

The preliminary results indicate that the diversity present in the cases is indeed taken into account in the deliberation process. Of course, the observation that the committees seem to take into consideration the same issue does not tell us whether the same conclusions will also be reached. The latter question proved challenging to address using the experimental approach where only brief versions of case protocols can be used (evaluating a full protocol takes several hours) and most

interviewees referred to the need for more information before they could make a decision. Figure 4 shows an overview of the aspects that committee members consider when they evaluate experiments.

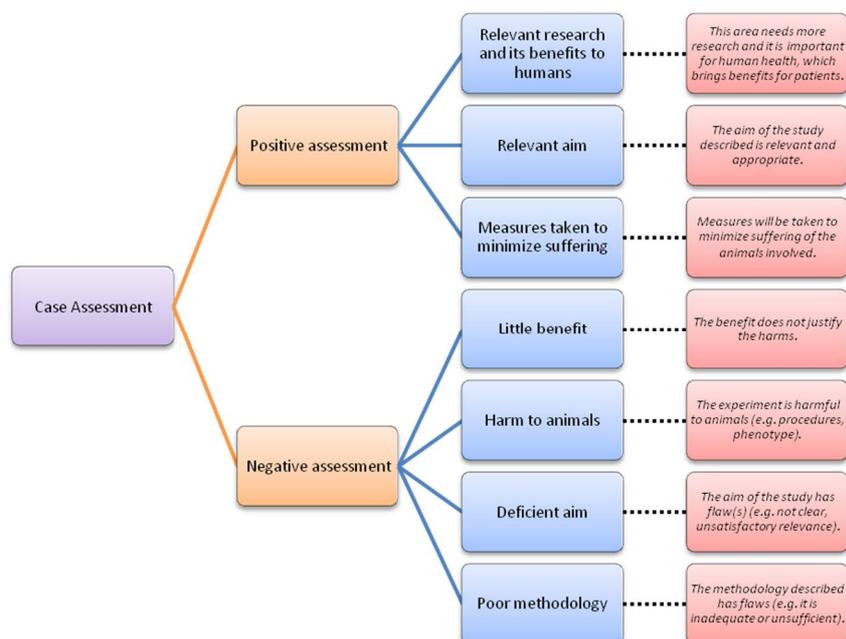


Figure 4. Overview of issues that come up when members of animal ethics committees deliberate over experimental case studies in interviews

Internal regulation in research: Safeguarding animal welfare through the 3Rs

This part of the project explored how internal norms in the scientific community may influence the ethical treatment of animals in research through researchers' decisions when planning and reporting their research, and when engaging in the peer review process (as reviewers, journal editors or panel members of funding agencies). The WP was split into 3 tasks: **Task 4.1** Retrospective assessment of animal welfare and the implementation of Refinement in research through a systematic review of published studies of animal models of neurological diseases. **Task 4.2** Survey of medical journals to evaluate how animal welfare and the implementation of the 3Rs are reflected in the peer review process. **Task 4.3** Survey of funding agencies to evaluate how animal welfare and the implementation of the 3Rs are reflected in the peer review process.

Task 4.1: Retrospective assessment of animal welfare and the implementation of Refinement in research through a systematic review of published studies of animal models of neurological diseases

Systematic reviews of animal use in both neuroscience and other fields of research indicate that self-reported regulatory compliance – including of ethical approval of protocols – has steadily increased over the last decade, but that significant progress could still be made to minimize and prevent avoidable suffering of laboratory animals. Of related concern are reports that a number of published animal studies fail to uphold basic standards regarding experimental design – e.g. random assignment of animals to treatment groups, blinding of observers – or use too few animals often leading to irreproducible results of limited translational value.

The objective of this task was to examine the implementation of Refinement in a field of research in which standardisation of methodology and quality of reporting have been of sufficient concern to the research community that it has published guidelines for the use of animals in studies in this field. The intention was to survey the standards of methodological reporting, to assess severity and refinement measures and to determine whether these factors have changed in response to the introduction of self-imposed guidelines.

The field chosen was that of mouse studies of Amyotrophic Lateral Sclerosis (ALS) which published guidelines for preclinical research with animals in 2007 and 2010. Aside from this representing a field evidently intent on achieving internal standards, ALS was chosen because the disease is complex and there are strong arguments that experiments in the whole organism are necessary to properly model its etiology. Moreover, the field has a widely accepted 'model' (a transgenic mouse that mimics many aspects of the human disease) that is used in a large number of studies.

We initially surveyed the scientific literature in this field in 2005, 2009, 2011 and 2013 and analysed the reporting in some 382 full-text articles spread across those years. A key finding consistent with previous studies of other fields, is that reporting of regulatory compliance increases significantly over this time period such that over 90% of papers published in 2013. Notably, this incorporated a 40% increase in studies reporting protocol approval by a 3rd party (e.g. ethics committee, competent authority).

Over the same period, however, there was no change in the severity of the experiments carried out and no apparent correlation between the reporting of protocol approval and severity rating. On the other hand, the study revealed that the standards of reporting of methodology in 'preclinical studies' – those that aim to test the therapeutic potential of drugs prior to translation to humans – were significantly greater than in 'proof-of-concept' studies (studies in which the primary goal is to decipher the mechanism of the disease, rather than a preparation for therapeutic application in humans), although it was not possible to conclude, with the initial dataset, that this is related to the introduction of guidelines by the ALS community.

Scientific journals can play an important role in promoting best practice in animal research, not only by demanding regulatory compliance, but also by making sure such regulations are followed, or by having their own policies on the ethical treatment of animals. We surveyed the eight most represented journals in our sample (together accounting for 35% of the papers). Although there was significant variation in the reporting of regulatory compliance – papers in two journals always reported compliance, while in another journal this was as low as 61% - there was no significant correlation of this with either the reporting of relevant research parameters or of severity levels.

Thus, our study showed widespread reporting of regulatory compliance and significantly better reporting of methodologies in preclinical studies, but these could not be correlated to the introduction of research community guidelines, nor were there obvious changes in the actual severity of experimental procedures over the period of study. It is important to note, however, that a key aim of the ALS community guidelines were standardisation of protocols to an existing experimental end point, so this is perhaps not surprising. The study is being extended to include 2007 and 2015 to enable us better to conclude whether or not the reporting of methodology has improved over time.

Task 4.2 Survey of medical journals to evaluate how animal welfare and the implementation of the 3Rs are reflected in the peer review process.

To begin to address more broadly the role of journals in the implementation of the 3Rs, we conducted a survey of journal editors across the biomedical sciences to determine how factors that relate to animal welfare are taken into account during the reviewing of animal studies submitted for publication.

As a first step a questionnaire plan was constructed aiming to determine, among other factors: how reviewers are selected (what expertise is expected); whether standard questions are asked; what guidance is provided (and based on what principles); whether animal welfare is a key factor in determining publication; what aspects of experimental design are considered; whether there are concern about specific model animals; what level of ethical compliance is expected (and whether this was checked); how often are issues relating to welfare arise; how important reporting of animal welfare is considered; and where responsibility lies for ensuring the welfare of animals in scientific experimentation. In addition to standard demographic information, the questionnaire also aimed to collect information about the background and experience of editors with welfare issues and information about the scope of the journal, the extent to which it publishes animal studies and in what context (preclinical vs basic biological science etc) and its standing in the field (Impact Factor).

To test the appropriateness of the questions, they were posed in semi-structured face-to-face interviews, at first with colleagues and then with a small group of journal editors. On the basis of this feedback, the questions were modified and the overall length of the questionnaire was shortened so that the survey could be completed online in approximately 15-20 minutes.

In parallel, relevant journals were selected by searching EuropePMC.org with the terms 'Biomedical AND (METHODS:"Animal") AND ((METHODS:"permission") OR (METHODS:"guidelines"))'. This revealed ~17000 articles published since 1994 in ~1700 different journals. These journals were then ranked by the numbers of papers published. This ranking revealed that over half of the papers were published in just 62 journals with two journals accounting for ~18% of all the publications. Although the list of journals was by no means exhaustive and the number of papers published in this timeframe that used animals is almost certain to higher, the dataset appeared to include the majority of the key journals across biomedical research in which animal research typically is published.

A contact list of the 'editors-in-chief' (or equivalent) of the top ~800 journals was established, representing those journals that had published 2 or more papers satisfying the search criteria, and editors were approached by email to respond to the online questionnaire. The invitation indicated that the overall objective was 'to examine the effects of animal welfare regulation on the scientific process', the specific aim of the survey was 'to understand the views and practices of editors of peer reviewed journals publishing scientific research studies using animals'.

Responses were received from 119 journals. With the objective of encouraging responses and promoting frank answers to questions, the questionnaires maintained the anonymity of both the journal and the editor. Although this means it is difficult to know precisely how representative this is of all journals publishing animal studies, information about the impact factor of the journal suggests the proportions of journals in different impact factor classes was similar in the sample to that in the 800 journals polled (including 12% with IF ≥ 6 compared to 15% in the total sample). Moreover, the majority of respondent journals review >10 papers/month on animal experimentation, while as many as 24% review >30 papers/month. Together this suggests that these are likely to include journals with significant influence on the publishing of animal studies.

The largest proportion of responding journals were based in the USA (46%) with a further 41% based in Europe (UK, CH, NL, DE and IT). The large majority (97.5%) cover the life sciences only, while 87% cover both clinical and basic research. Eighty seven percent of responses came from Editors-in-

Chief, 65% trained in basic science, 30% in clinical science. While 59% had 10 or more years of experience working with animals, 21% had none.

We cannot report here exhaustively on all of the questions posed – these will be presented in a peer-reviewed publication currently in preparation), but we will give some highlights and examples of what the survey suggests.

The first is that animal welfare is taken seriously by the majority of journals that responded. Among several factors that suggest this, the first is that >94% of responding journals require a statement from authors indicating that ethical approval was obtained for the study. Though perhaps not especially surprising, this mirrors our findings in the ALS survey described in Task 4.1 and those of other recent publications. Perhaps more surprising was that 12% of these journals always check the veracity of statements of ethical approval and nearly 70% check when there are concerns. **From the point of view of future policy making it is of interest that 85% of respondents said they would ‘probably’ or ‘definitely’ use an online database of ethical approvals to check veracity if this facility were available.**

Further indicative that animal welfare is taken seriously is that >82% of respondents consider measures taken to reduce suffering and discomfort as ‘important’ or ‘very important’. While this may not be surprising among editors voluntarily responding to such a survey, more surprising was that >25% of respondents claimed to have rejected manuscripts ‘Sometimes’ or ‘Often’ because of insufficiencies in the measures taken to protect animal welfare.

When it comes to what help and guidance is provided to authors and reviewers, practice is more varied. While authors of manuscripts are provided with specific guidance, the use of which is mandatory in over 75% of journals, by contrast guidance is mandatorily provided in only ~30% of cases and not at all in 20% of journals.

The guidance and policies used by journals in either case is largely based on the ARRIVE guidelines, although in the US guidance from the ‘US Institutional Animal Care and Use Committees’ and ‘NIH Guide for the Care and Use of Laboratory Animals’ was prevalent. **Among EU-based journals, 35% indicated that the journals policies had changed in response to Directive 2010/63/EU.**

Not surprisingly, the key expertise sought by journals in selecting reviewers for manuscripts is in the subject area of the manuscript (in 95% of cases this was ‘always’ considered). Perhaps surprisingly expertise in animal research ethics was ranked joint second with expertise in relevant methodology (‘always’ considered in ~40% of journals), although there was a clear dichotomy in the responses with over 25% indicating that animal ethics expertise was ‘never’ considered in reviewer selection.

There was a similar dichotomy of response to the question ‘Who should be responsible for ensuring welfare of animals in scientific experimentation?’ Respondents ‘strongly agree’ that responsibility lies: with the scientists doing the research (>90%); with Ethics committees (>75%); with institutional or national agencies (>70%); or with funding agencies (>40%); whereas <20% ‘strongly agree’ that journals should have that responsibility and >30% strongly disagree that this is where responsibility lies.

The survey lends itself to cross-relational analysis that may allow us to dissect some of the dichotomies of response seen. For example, we seen a clear relationship between the policies adopted by journals on animal welfare and the interests and education of the editors. For example, editors that ‘never’ attend meetings or lectures on the principles of the 3Rs are more likely to come from journals that do not provide checklists for reviewers or that are not discriminating about which animal models are accepted for publication.

Finally, our survey provides information complementary to that provided in work package 5 in the form of a quantitative analysis of the attitudes of journal editors to the use of different species of model animals. It is notable, in this regard, that although there is predictably less concern about the use of rodents than of companion animals and non-human primates a substantial proportion of editors still have 'no concerns regarding' these models. It will be interesting to explore in greater depth how this relates to other responses in the survey and whether there is any obvious correlation with the findings of WP5.

Overall, our preliminary analysis indicates broad awareness of issues relating to the 3Rs and animal use among journal editors and a clear reflection of this in the policies of the journals that responded. Although it is clear that we have to be cautious in interpreting the responses of a relatively small self-selected group, our analysis so far suggests that the survey will provide useful insights into the effects that the attitudes of editors and the policies of journals may have on the implementation of 3Rs principles in animal research.

Task 4.3 Survey of funding agencies to evaluate how animal welfare and the implementation of the 3Rs are reflected in the peer review process.

The tasks above have focussed on the role of internal regulatory mechanisms that operate when executing and publishing animal research. The final task is intended to look at the mechanisms operating during the planning of research, particularly at the consideration given to 3Rs implementation during peer review of funding applications. Our original intention had been to conduct a survey similar to that conducted above for the publication peer review process, ie based on a questionnaire sent to a large number of funding agencies. As a preamble to this, one of us attended the 'Symposium on Reproducibility and Reliability of Biomedical Research' organised by the Academy of Medical Sciences in conjunction with the Wellcome Trust, and the Biotechnology and Biological Sciences and Medical Research Councils (BBSRC and MRC) of the UK in April 2015. Wide consultation at this meeting and subsequently suggested that, although there was evidently considerable interest in our project, its goals might be better served by conducting face-to-face interviews with key figures from the funding agencies of a defined set of EU countries.

At the same time, discussions among the work package members came to the conclusion that there were significant areas of overlap of interest between WP4 and WP6, which aims to explore how the practice of bench researchers and those involved in commercial research impacts on the implementation of 3Rs in research. With this and the feedback from funders themselves in mind, it was agreed that the purposes of both WPs would be better served by combining forces to conduct face-to-face interviews with key stakeholders and bench scientists in 3 EU countries (UK, DK and PT). A first set of interviews were conducted through the summer of 2016 with bench scientists and representatives of funding agencies and pharmaceutical companies. The data has been analyzed in collaboration with WP6 and preliminary results were presented at the WP6 webinar in February 2017. Briefly, these interviews suggest that although the funders consider the 3Rs and regulation of animal experimentation important, they generally rely on the ethical and regulatory aspects of animal experimentation to be taken care of by others (e.g. the competent authority or the institution at which the funded work is placed). One of the funders is more proactive and has a system for 3Rs review built into the project review system, although it is unclear what role the outcome of the 3Rs review has on the final decision to fund a project. Especially funders with international activity value regulatory efforts which can help them to ensure minimum standards for research done in other countries.

Fish, Mice or Monkeys? The influence of different factors on the choice of model species

This part of the project explored external and internal factors (in terms of pressures, motivation and attitudes) which characterise the scientific practice of animal experimentation, questioning researchers on the topic of choice of species for a particular model to be studied.

The idea was that the results would reflect the complexity of animal experimentation, not likely to be fruitfully discussed within the framework of simplistic pro/against dichotomy. But before approaching this task, it was considered to be very important to analyse the current scenario of the use of animals in biomedical and toxicological research in the different Member States (MS).

Therefore, the original aims of WP5, indicated as Tasks, were: **Task 5.1:** Map use of different species in the different MS, in relation to different fields of research; **Task 5.2:** analyse whether the Directive 2010/63/EU imposes technical and/or normative limitations to the use of particular species as model of choice, and how this could influence research decisions; **Task 5.3:** analyse the pressure exercised by the public opinion on research decisions, and look for significant differences among MS.

Task 5.1: Map use of different species in the different MS, in relation to different fields of research

The aim of this task was to map the use of different species in the different MS, in relation to different field of research, and to identify possible specific trends in the use of particular species in particular MS and in relation to a particular field of application.

According to the last report published at the time of completion of this Task (2011), rodents (especially mice, rats and guinea pigs) represented, and still are in the following years, the preferred species of choice used for experimental and other scientific purposes in the EU, followed by fish, birds and rabbits. Almost half of the animals were used in biological studies of fundamental nature. There has been a decrease in the total number of animals used from 2005 to 2011, but it was not homogeneously distributed among the different species. The biggest increase was represented by the use of mice, along the six years considered, whereas the number of rats used decreased. From 2005 to 2011 there has been a consistent increase of the number of animals used for basic research. Although still high, the number of animals used for research and development (and for production and quality control of products and devices) for human and veterinary medicine has been dropping since 2005.

In 2011, France, Germany and Great Britain together utilized more than the half of the animals used in Europe. However, when analysing the number of animal used in relation to GPB and the expenditure for R&D activities, these three countries did not result to be the major users, whereas Hungary, Estonia and Czech Republic occupied the first three positions. Among the MS using more than 100.000 animals in 2011, countries with atypical distribution in the use of particular species were identified. For example, four countries resulted "atypical" in their proportion of species used, in relation to the European average: Czech Republic, Hungary, Ireland, and The Netherlands. For example, in Czech Republic, of the total number of animals used in 2011, mice represented only the 21% of the total, while birds accounted for 48% of the animals used. This is a very different proportion from the general trend in Europe, where rodents are still on the top of the choices made by researchers. On the contrary, Ireland used nearly exclusively mice (90% of the total of animals). Hungary and The Netherlands used a higher number of birds than observed in other MS, and The

Netherlands reported 4.3% use of Ungulates of the total animals, which was higher than 90% of the other countries (EU average: 1.3%).

It is interesting to notice that most of the MS appear to follow general common trends in the use of animals, for example, using rodents more than any other group of animals. The fact that these animals are, for different reasons, very appropriate for laboratory research is a common thinking, shared by different countries with different scientific traditions and approach to research. But what it is of special interest here are the particular cases. Whereas some countries, for example Great Britain and Italy, continue to show numbers which reflect European average (e.g., use of rodents for basic research), others show peculiarities, such as the relatively high number of bird species used in the Czech Republic, or the percentage of fish used in Poland. In particular, in the case of Czech Republic, the ringing of birds for research purposes has to be regulated by the law, and this is the reason why these numbers appear for that particular country. We must acknowledge and discuss national differences, and learn about the different local attitudes. This process can be surely rewarding and is an interesting learning process. The effort by the EC to harmonise research on animals runs parallel to this process, sometimes interfering and limiting these heterogeneities, some other times even inspiring them.

Based on the above, 10 MS has been selected as case studies, to further enquire possible differences in the use of animal models. These were chosen among the ones which make the most use of experimental animals and to represent the spectrum of different uses of animals in research (in relation to particular species and particular fields of application), as well as differences in public attitudes towards animal research (MS where there is high and those where there is a low acceptance of animal experimentation). Selected countries were Czech Republic, Finland, Germany, Hungary, Ireland, Italy, Netherlands, Spain, Sweden, and Great Britain. In the selection of these countries we followed the following criteria:

Inclusion criteria

- 1) MS which used more than 100,000 animals in 2011;
- 2i) MS selected by the other WPs of the Consortium, **and/or**
- 2ii) MS with 2012/2013 statistical data on the use of animals in procedures available.

Division criteria

- 3) Number of animals used relative to R&D expenditure: above/below the European average
- 4) Proportion of species used in relation to the EU average: typical/atypical;
- 5) Proportion of animals used for different purposes in relation to the EU average: typical/atypical;
- 6) Level of acceptance of animal research: AVERAGE/agreement/DISAGREEMENT/neutral/average;
- 7) Participation to the Citizens' Initiative Stop Vivisection – Minimum number of signatories: OBTAINED/NOT OBTAINED;
- 8) Political geography (Northern/Western/Eastern/Southern Europe, New MS)

Task 5.2: analyse whether the Directive imposes technical and/or normative limitations to the use of particular species as model of choice, and how this could influence research decisions;

Task 5.3: analyse the pressure exercised by the public opinion on research decisions, and look for significant differences among MS.

The aim of these tasks was to determine the relative influence of different factors, in particular - technical and/or normative limitations imposed by the Directive, and the pressure exercised by the public opinion on researcher's decisions in the selected MS and on the work they carry out with animal models. The accomplishment of these two tasks followed a new plan, due to a new methodological strategy, which merged the analysis of different factors influencing species choice together, rather than to be the focus of specific tasks.

As a first step we conducted semi-structured interviews with researchers working with animal models (*qualitative methods*) to verify whether *a priori*-determined factors were meaningful and/or whether new ones were needed to be added to our list. The interviews were carried out by an undergraduate student. Twenty-four Italian researchers (14 F, 10 M), working with rats (n=11), mice (n=7), primates (n=3), and fish (n=3) and with a broad range of practice with animals (PhDs, technicians, researchers, senior researchers) were interviewed. We collected information about their age, level of education, religion, eating habits, presence of companion animals at home. The interview scheme was designed to obtain interviewee's personal and professional information, and information about their attitude towards animals (including ethical considerations), and to determine factors influencing the choice of the model species and the work they carry out with that species of choice (e.g. effect of the new Directive, effect of the public opinion). The interviews also included a ranking (acceptability of treating different species with severe procedures) and a rating (cost-benefit of their research) to be performed during the interview. The interviews conducted with the Italian researchers were functional in confirming the factors to be looked at, and included in the questionnaires. In particular, the factors identified were: legislation public opinion, opinions of friends and relatives, evaluation of ethics committees, funding, scientific question, ethical consideration on the species' welfare, personal attitudes towards different species, cost of management and housing, trends in publishing, laboratory tradition. Furthermore, we also added questions added to better understand the attitude by researchers towards particular species.

On the basis of the results obtained with the interviews, a questionnaire was prepared to be sent out to researchers belonging to the 10 selected MS. A database of researchers was prepared. We limited our search to researchers working on 5 species to appear to us of particular interests: mice, rats, fish, cats, dogs, primates. In order not to subdivide our sample too much, we choose not to identify particular fields of research (neuroscience, behaviour...), although such categorisation would have been of interest. The names were obtained in different ways. The majority resulted from a search on PubMed, looking at studies using animal models from the selected species. Then, the websites of particular institution, where animal models are used for research, were visited, and members of staff with a significant record of publications were selected. Finally, a number of researchers were chosen based on personal acquaintance with the WP leader. The database at the end consisted of nearly 500 researchers. Although the initial effort was to try to balance the number of researchers working with the five chosen species, it was quickly evident that it was not realistic to obtain the same number of addresses for colleagues using mice and rats and the ones using cats and dogs. Furthermore, some countries do not use non-human primates (Sweden, Ireland, Finland), whereas Italy does not use cats. Therefore, the distribution of questionnaires mirrored the use of particular species in the MS, with rodents researchers outnumbering the researchers working with other species.

Out of nearly 500 questionnaires sent, 76 were received back. Therefore, due to small numbers, the results are indicative of particular trends, and the small numbers do not allow us at the moment to arrive at definitive conclusions. Unfortunately, at the moment, we are not in the position to indicate differences among the 10 MS, but only to indicate general attitudes. A scientific paper which is in preparation will present the full results; here we will focus on some interesting answers.

A first couple of questions regarded the knowledge and use of different animal models from the one currently used, or alternatives to the use of animals itself, and the reason why not to use alternatives when aware of their existence. As a matter of fact, although the majority of answers indicated both the knowledge of alternative animal models and non-sentient models, the animal model currently used was indicated as “the most suitable species to answer to the specific research question”. The importance of the research question in the choice of a particular species will return in other answer as well. The number of answers for this second point, that is, why not using a known alternative model, was smaller compared to the first question. The unwillingness to change a particular model species could be also due to laboratory tradition, and a resistance to change a model that has always provided reliable results, but the results do not support this hypothesis: the respondents indicated that if they wanted to change their model the resistance from the laboratory environment would be minimal. It must be noticed that this answer should be crossed with the age of the respondents: as a matter of fact, that the majority of answers came from Heads of Departments who, most probably, are in charge of deciding in which direction the research should go to. Related to this last question, 60% of the researchers declared that there would not be any particular species they would not work with, and the ones who said differently, mainly cited ethical and affective reasons not to do so. However, it must be noticed that for this particular question, a drop from 73 respondents to 29 was recorded.

Among the different questions provided by the questionnaire, researchers were asked to rate the weight of different factors in choosing a particular species to work with. “Scientific considerations”, in terms of appropriateness of the model, was overwhelmingly considered as “extremely important”. “Ethical considerations” appeared to be the second most important factor, followed by the influence of the Directive 2010/63/EU. These results confirm the results we obtained previously from the interviews to the Italian researchers. If we look at the other end of the spectrum, the answers to this question showed “Public opinion” and the opinion of “Friends and Relatives” practically not influential at all. If asked what aspects of their research were thought to be more influenced by public opinion, “Purpose of research” was the most frequently answer given. Other aspects, such as “Dissemination of results”, “Invasiveness of methods” and, obviously “Choice of animal model” did not receive much attention, or gave mixed results (again, the mixed results could also be a consequence of the small sample size, rather than just a mixture of opinions). However, the opinion of the public about animal welfare and human health was judged very influential on the funding bodies. In particular, 43% respondents indicated both concerns influential, 19% just animal welfare considerations.

A question more related to the attitudes of the researchers towards animals gave interesting results. Researchers were asked to rank, in terms of acceptability, the application of a severe procedure (i.e. procedure as a result of which the animals are likely to experience severe pain, suffering or distress, or suffer a severe impairment of their well-being). Some of the results were highly predictable. For example, about 56% of the answers found not acceptable the use of a chimpanzee in such a procedure (although 10% found it acceptable or very acceptable) and, more or less the same ranking was given on the use of Rhesus monkey (and for *Drosophila* was the opposite). Interesting results were obtained for other species. In particular, the acceptability of severe

procedures on rats and mouse was ranked nearly evenly across the five rank points (from “not acceptable” to “very acceptable”). This could be due to different opinions of the researchers on the ability to feel pain and sufferance of these animals, then indicating the need to better define what really “pain” and “sufferance” mean for a particular species. To use a severe procedure on a pig was considered not acceptable for 30.4% of the respondents (1.4% very acceptable), whereas octopus was considered very similar to fish in this respect (octopus: 10% very acceptable; 18.8% not acceptable. Fish: 17.4 very acceptable; 14.5% not acceptable). When asked what criteria were used to choose the particular ranking for that particular species, “Perceived ability to experience different cognitive states including sufferance” was by far the most frequent reason, followed by “Moral relevance attributed the species”.

The results from the questionnaires appear to show that a certain “self-referential” attitude of researchers working with animal models exists. The most important values are confined within the scientific method (see the relevance of the “research question” among the factors influencing species choice): the most important factor in choosing a particular species to work with is the correspondence between the model and the question asked. Furthermore, animal welfare is considered important because it enhances the quality of the data collected. Having said that, ethical issues ran very close second in the researchers’ motivations. The influence of public opinion appears to be limited for the researchers’ choice of methodology and species to work with. It could be that the gap between the world of animal research and the general public still has to be filled. The attitude of researchers towards particular species is worth further analysis. Non-human primates appear to be considered as special case, but also the use of invasive procedures on octopus raises similar concerns as with the fishes. It seems that the concept of “pain” and sufferance” in animals need to be better defined.

These conclusions which, due to the small number of answers, must remain mostly indication of particular trends, can be taken into consideration for further initiatives aimed at better understanding the relationships between researchers and animals used in scientific procedures, as well as the relationship between animal science and society.

External regulation and competitiveness: practice and perception of key stakeholders in animal research

Our findings fall within four main themes and four subthemes: 6.1) Is regulation needed, and if so, why? 6.2) Which ideas do the stakeholders have about a good and well-functioning regulatory system? 6.3) How well does the existing regulation function? 3a) What are the thoughts on minimum standards for housing and the like? 3b) What are the thoughts on the authorization system? 3c) What are the thoughts on the implementation of the 3Rs? 3d) What are the thoughts on the reporting requirements, e.g. severity assessment? 6.4) How are changes following the revision of the Directive perceived?

6.1 Is regulation needed, and if so, why?

When it comes to the issue of whether regulation is needed, and if so, why, there is wide agreement among bench scientists and pharma companies that regulation is needed for two

reasons: Firstly because it protects research and researchers. Secondly, because without regulation some researchers would go beyond what is an ethically acceptable way of treating animals. The following two quotations from scientists give examples concerning the first argument:

"[...] much better for us to have this kind of system [where experiments are approved by a committee with representatives of animal welfare organisations] because it takes away that kind of speculation, that must be terrible, [...] to sit in a situation where you don't want to tell what you do, you don't want to put signs on your building that this is the lab of something because then you know people would come in and, and burn it, or you would be attacked [...]" SCI DK

"I think it protects us, as well as the animals, that we have a system that's very clear [...] It means that we can safely say with honesty that we are doing the experiments under an umbrella of regulation that requires us to take into account animal welfare and to have [...] a valuable purpose to the experiment." SCI UK

Representatives from pharma industry and funders clearly saw the value of a level playing field and the value of bringing good standards already existing in some places to be applied across Europe. They made specific references to how common rules and practices across countries may facilitate the international operations of the interviewee's organization. These points are well covered in the following two quotations:

"... it's very important that [biomedical research] is regulated at European level [...] the wider we can get in that level of standards is great from an animal welfare point of view [...] the term that gets used is level playing field [...] let's say, if you want to collaborate with a lab in Spain, you would want to have the confidence that if they're working under the EU directive you know that their standards will be of a certain level. [...] it's really important, so that you know wherever you go in the EU there is a baseline that is good." PHARMA UK

"... for us as a funder it's comforting to know that there is legislation across the EU, so that we can be confident. In the UK we're confident in the processes and we understand the regulations that exists and that makes us confident to fund in the UK, and so if we had a similar thing across Europe then obviously that means that we're much more comfortable funding in different countries." FUND UK

6.2 Which ideas do the stakeholders have about a good and well-functioning regulatory system?

When it comes to the issue of what is good and proper regulation, the interviewed bench scientists saw the following points as important: That it is a system without a too long, bureaucratic and time consuming application process; that it is run by competent people; that it works with reasonable and not too restrictive standards; that it is flexible, that it is consistent over time; that it is proportional compared to regulation of other forms of animal use; and that there is a fair level of requirements compared to other countries.

All these descriptions of what is a good regulatory system were going together with concerns about deficiencies in the actual system. Particularly in Portugal there were concerns among the scientists about the competent authority being slow. In the UK there were complaints about lack of consistency across Home Office inspectors. Finally, there was a general concern about lack of flexibility, long waiting times and unnecessary paperwork.

6.3 How well does the existing regulation function?

When it comes to the function of the actual regulation it is important for a start to stress that large parts of the Directive have been accepted and implemented without any serious problems. This is for example the case for minimum standards of housing and the like.

There were also several positive views expressed. For example, it was said that even though it takes time to write applications to regulatory bodies this time may be well invested, since it may lead to better research. The view was several times made that focus on ethics may be a win-win for research quality and animal welfare. Finally, it was said that the licensing process is useful when it comes to defending animal experiments to the public. These points can be exemplified by means of the following quotes:

“... it’s another level of bureaucracy, but I think in generally it leads to better projects ...” SCI UK

“... I think it is fine, we spend ... time on it, but normally this is just making the protocols more efficient. [...] We think we have been much better in planning and thinking before we start.” SCI DK

“... all aspects of the Home Office regulations of animal work, which includes the 3Rs, are a positive additive to science. And enabling you to produce science, which, first of all, has justification, and secondly is robust, and reproducible.” SCI UK

“[When we] finally get the license to do it well then my speculations about if this is OK are gone and that makes it much easier to talk with anyone about animal experiments ...” SCI DK

There were also several negative views concerning the functioning of the actual regulation. For example, it was said that there are too rigid requirements for licensing of individuals (e.g. students) helping in the lab. It was also said that too much time is wasted on filling out applications and waiting for responses. A further point relates to the fact that science by nature has a dynamic element. However, this fits badly with the regulation system with detailed license applications for 5-year projects. Finally, it was said that regulation, directly and indirectly, imposes costs which at the end of the day goes from the actual research or the care of the animals. These points may be illustrated by the following quotations:

“... originally [...] I could as a responsible investigator involve PhD students, or medical students in the carrying out of experiments, so they were allowed to help out as long I did the solution, now this no longer possible [...]” SCI DK

“...regulations] makes [my work] more expensive. [...] It is basically because things need to be checked, I mean record keeping, we have one person working in the fish facility who is basically all day putting things in a data base, that’s all she does and she has to be paid.” SCI UK

“[...] maybe after 5 years, or 4 years, there is discovered a better way to anesthetize them. More new knowledge about how to pain-kill, but then I have to use the old way because that’s what we got the license to do [...]” SCI DK

“That type of very bureaucratic regulation is not good at all. It doesn’t benefit the collaboration with researchers, all. [...] one should try to alert researchers, one should make an effort [...] one has to be careful with how one does things and bureaucracy doesn’t help.” SCI P

“I think it’s, that’s a heavy process in Denmark. [...] It’s really difficult. Awkward and that destroys also our competition because we are way behind, I can wait for a year then everybody [else] is publishing right. [...] I think in other places, they do all these experiments, I mean all my collaborators do experiments. And I never heard them complain about a one year process, I mean that’s ridiculous. [...]” SCI DK

A specific view of the funders is that they have confidence that the system for regulating animal experiments ensures that research in funded projects are compliant with the law. Although some funders say that funding is conditional on authorization of the animal research, no mention is made of mechanisms for ensuring this. In contrast, one of the funders explicitly said that this was entirely the responsibility of the institution where the work was to take place.

When it comes to the theme of the 3Rs, particularly the bench scientists had view on all the 3Rs, Reduction, Replacement, and Refinement. About Reduction, there is a widely-shared concern among bench scientists, pharma and funders about the risk of using too few animals. Thus, getting results with sufficient statistical power is an extremely important consideration for all the stakeholders. Furthermore, it is also reported that some experiments are not done because they would require too many animals. Some scientists argue that they reduce numbers to limit harm done to animals. However, others highlight the need to keep animal numbers in line with what journal reviewers require. The following quotes highlight the need to focus on power:

“In order to have confident data, you would have to have at least 20 animals in each group, for each experiment, otherwise [...] you can never get accepted [...] [20 is a more or less established number] that gives you really good statistical [power]” SCI DK

“[...] just to avoid you missing one data point and that nullifying the whole study we had on some extra animals so if our power calculations give 5 to 6, we do 8 just because it gives us that confidence that [if] something that you can’t control goes wrong, we’re not going to compromise.” SCI UK

When it comes to sharing of tissue, a specific sub point relating to reduction, scientists generally have positive views. However, they also point to limitations, scientific, economic, and structural.

When it comes to replacement the scientists interviewed presented reasons for going on using animals: That they are focusing on very complex issues; that use of animals may be required by their research questions; that experiments on animals give more valid results than alternatives; that they in their research may focus on understanding animals; and that there may in their specific area of study may be no available alternatives. They also pointed to structural arguments which work against replacement: Firstly, that the role of the scientist in question was to do animal work, and secondly, that there are pressures from journals and funders to use animals. The following quote illustrate the argument that animals give more valid results:

“[...] sx the transgenic mice we work at, I mean we want to mimic the symptoms at the patient [...], these are kids and are very, very sick and we’re trying, I mean you cannot ask that in a cell culture, or any other similar system whether they will get epileptic seizures, or are they going to [...] attacks, do they behave like this, you cannot ask that in any other system ...” SCI DK

When it came to refinement there was generally a positive attitude. However, there are also some who on occasions saw a dilemma between good science and good welfare (with welfare initiatives confounding results). Some also saw structural and economic barriers for increased levels

of refinement. However, it was also common among the scientists to see refinement as a win-win: Better science and better animal welfare. The following quotes illustrate two of these points:

“If we need to study something that only happens at the end of the disease, or if we want to know whether an intervention improves the lifespan of that model then yes, [...] we’re aware that we’re going to make these mice ultimately very sick and we’re going to have to be very careful with monitoring them. If we weren’t interested in knowing that ... then we could use animals and take them to a much earlier endpoint, then we’d still sacrifice the animals, but they wouldn’t experience the same level of distress.” SCI DK

“I’m very concerned about pain and stress. Because they are going to, confound my results. I mean if I’m measuring some kind of attentive process in a mouse, if I want the mouse to pay attention to computer screen and press buttons when it’s used into all the kinds of stimuli, I cannot have this mouse stressed, or in pain because this is going to clearly distort the cognitive processes I’m on to access, so I’m very concerned about having my mice happy. [laugh]...and satisfied with their living conditions [laughing]” SCI DK

6.4 How are changes following the revision of the Directive perceived?

When it came to perception of changes in regulation there was in general not much awareness of a change associated with the Directive. The combination of a centralized competent authority and a local committee system was viewed as an extra burden in one place (Portugal). On the other hand, several appreciated the close working relation with the local committee. Rigid requirement for education before participating in work with animals caused problems for many. In general, the EU directive is seen as a compromise – necessary but also leading to inconsistencies.

As specific area where change was seen concerns reporting requirements, and particularly reporting of severity assessments. This was clearly noted by pharma interviewees. One interviewee commented on the increase in administrative burden arising from these requirements. However, another interviewee remarks that in their organization, these requirements have resulted in more engagement in discussing severity when an animal experiment is planned.

6.5 Conclusion

The overall conclusion of the WP 6 study is that all see a clear need for regulation of animal use. They also to a large degree share the intentions behind the directive. There are critical concerns – to some degree differing across countries – about the functioning of the actual regulation. Replacement is not high on the agenda for people whose role it is to do animal experimentation. There is ambivalence concerning Reduction, and more positive view on Refinement. Finally, there is a widespread concern about unproductive paperwork.

PROJECT WEBSITE

The ANIMPACT project website is located at www.animpact.eu and was launched within a few months after the start of the project.

Videos, lists of downloadable presentations and files were created to support persons involved in formal evaluation and authorization (Authorities, Government, Animal Welfare Officers, Animal Welfare Bodies, ethics commissions) as well as other types of decision-making over animal research (such as commercial and financing stakeholders, grant agencies, evaluation board members and external peer reviewers).

The domain and access to the website has been guaranteed for an additional two years, in order to enable the team to update as the ANIMPACT output is being transformed into peer-review publications.

We expect the project to receive continued interest as the Directive is undergoing review in 2017, and we will be happy to continue to interact with regulators and other stakeholders on the matter.

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