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Application of 3R principles in small animal GLP testing of biomaterials

Abstract: On the protection of animals used for scientific purposes, the EU Parliament adopted Directive 2010/63/EU. The essential factor is the 3R principle: Replacement, Reduction and Refinement. In 2013, the third amendment to the German Animal Welfare Act was revised and adapted to the European Directive.

The majority of animals in science are used in basic research, as well as in translational and applied research. In medical research, animal experimentation is conducted to clarify previously unknown life processes and basic biological relationships, in order to improve diagnostics and treatment of human diseases.

Before an animal experiment can be performed, it must be reported to and approved by the responsible authorities. The planned research project must be justified scientifically, and it must be demonstrated that the personnel and spatial/technical prerequisites are in place to successfully complete the project. If all conditions are met, the approval can be granted, but may be subject to conditions.

The guiding principle of essentiality also affects the procedure of the experiments: The number of animals used and the pain, suffering and damage caused to these animals must be limited to what is absolutely necessary. In this context, the 3R principle has to be applied.

To obtain reliable results, it is essential that the laboratory animals are in normal physiological conditions and free of pain and fear. Scientific interest and animal welfare are therefore not in opposition, but rather mutually dependent.

In our GLP (Good Laboratory Practice) laboratory we test new drug release systems for different biomedical applications in rabbits after careful selection of the animal model. Stress during animal experiments must be avoided as

far as possible. Providing pain-killers and ensuring the best possible husbandry and care conditions are crucial for the animal's wellbeing and absence of pain and anxiety. In the present work we report our different experience in a GLP-certified biomaterial test laboratory.

Keywords: animal ethics, animal rights, medical research, good laboratory practice, biomedical applications, ophthalmology.

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1 Introduction

In recent years, a number of new demands and legal regulations have catalysed and reignited the discussion on ethics in animal-based research.

In 2003, the 7th amendment to the European Union's Cosmetics Directive (76/768/EEC) was adopted. This document stipulated an end to animal testing in the EU for cosmetic products by 2009, regardless of the availability of substitutive models by then. Ever since, there has been a joint effort of the scientific community in Europe and across the world to develop and validate alternative methods to avoid using animals wherever it may suit [1]. First described in 1959 by Russel & Burch [2], the 3R Principles (Replacement, Reduction and Refinement) has been considered a plausible factor to guide more ethical use of animals in testing.

The Third Amendment to the Animal Welfare Act was introduced in July 2013, which transposes into national law the Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes. It ensures a higher level of animal protection and raises the minimum standards, taking into consideration the latest scientific findings and developments in keeping with the 3R principles. Germany implemented this directive on 4 July 2013 with an amendment to the Animal Welfare Act.

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2 Principles of the Three Rs

The aim of the principles of the Three Rs (Replacement, Reduction and Refinement) is to avoid animal experiments where ever possible, to reduce their number and to limit the harm caused to animals during the experiments to the absolute minimum. A consistent and responsible implementation of the Three Rs principle may alleviate accommodates ethical concerns against the use of animals, while also improving the quality of the test results.

Replacement means substituting animal experiments with alternative methods wherever possible, or avoiding them completely. If the research question permits, simple organisms, such as bacteria or invertebrates, cell and tissue cultures, computer models, or other alternative methods may be used instead.

Reduction aims to reduce the number of animals to obtain information at a given amount and precision. Basically, statistical and methodological improvements help to reduce the number of laboratory animals. Furthermore, even centrally registering and recording results from animal experiments can reduce the demand for testing.

Refinement refers to any approach which furthers the well-being of the animals and which serves to avoid or reduce the actual or potential pain, distress and other negative effects experienced by the animal at any point during its live. This includes sourcing and transporting, as well as housing and keeping conditions. In terms of actual experimentation, it refers to experimental design and techniques, how the animals are cared for and handled at all times before, during and after each procedure, as well as how they are sacrificed or subjected to another fate at the end. It is also essential, that potential further refinements should be continually explored throughout the research program [3].

In summary, adherence to the Three Rs seeks to ensure that animals are used only when absolutely necessary, that the numbers of animals used are kept to a minimum, and that suffering is minimized [4].

3 Animal research

Animal welfare is enshrined as a federal goal in the German Basic Law and regulated in the Animal Welfare Act. The German Animal Welfare Act defines animal experimentation as interventions and manipulations in living animals if this is associated with suffering, pain and injury to the animal.

Since 2014, animals used for scientific purpose are registered according to a new Europe-wide reporting

procedure. The German Ministry of Food and Agriculture and the Federal Statistical Office annually record the total number of all animals used in Germany per year.

In contrast to three million vertebrates and cephalopods used in Germany in 2014, there are only two million vertebrates and cephalopods used in animal experiments in 2017 in accordance with §7 (2) of the Animal Welfare Act. Approximately 80% of the experimental animals used were rodents, especially mice and rats, with mice accounting for about 66% of all animals used. Less frequently used animal models were: fish (12 %), rabbits (5 %), birds (2 %) and pigs (1 %) [5]. An overview of animal species frequently used in tests according to §7 (2) of the Animal Welfare Act in 2014 to 2017 is given in **table 1**.

The majority of animals in science are used in basic research (50 %) and in translational and applied research (15 %). About 27% of all animals were used either in the manufacturing and quality control of medical devices or for toxicological safety testing. Around 8 % were used for other purposes, such as training or breeding of genetically modified animals [5].

Table 1: Animal species used in animal experiments according to §7 (2) of the Animal Welfare Act in the years from 2014 to 2017 [5].

	2014	2015	2016	2017
Mice	1,901,985	1,400,822	1,400,971	1,368,447
Rats	362,530	263,318	244,339	255,449
Guinea pigs	20,576	18,237	14,184	14,851
Rabbits	105,784	110,988	98,331	92,661
Birds	55,697	39,321	46,407	36,920
Fish	836,525	154,878	265,983	239,350
Pigs	14,347	12,305	16,727	17,347

4 Good Laboratory Practice in animal studies

Non-clinical safety studies are conducted using different protocols, including animal studies, which mostly follow the Good Laboratory Practice (GLP) regulations.

Per definition, “GLP is a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported” [6]. This system and the related principles were transferred to German law [7].

While the establishment of infrastructure for GLP compliance is labor-intensive and time-consuming, achievement and maintenance of GLP standards largely contributes to the quality of the data collected from each study. This is critical for result interpretation and also contributed to the protection of human and animal health. Furthermore, it proves the mutual international acceptance of data generated in different studies.

Current studies in our GLP laboratory involved the testing of microsurgical intraocular glaucoma drainage devices [8, 9] and polymer-based drug delivery systems [10] in a rabbit model. All animal experiments are being approved by the governmental ethical board for animal research (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany) and are being carried out in accordance with the EU Parliament Directive 2010/63/EU. The animals housed in standard cages at $22 \pm 2^\circ\text{C}$ under a 12-h light/dark cycle, with free access to water and a standard diet. All painful interventions, such as operations, are being performed under anaesthesia, including additional pain-relieving measures afterwards. At the end of the experiment, all animals are being sacrificed painlessly in deep anaesthesia. The number of animals used and the pain, suffering and damage caused to these animals is limited to what is strictly essential, following the 3R principles.

5 Conclusion

Although a growing number of scientific questions can be answered today by the use of cell cultures, computer-aided methods and other alternative methods, the use of animals for scientific purposes - among others in medical research - cannot be dispensed. Hence, animal testing's are indispensable and will remain necessary for the foreseeable future and for the progress in biomedical research and medicine. In this context, the appeal is made to researchers to reduce the number of experimental animals, to avoid unnecessary animal experiments, or if possible to replace them with other methods, as well as to adequately treat the animals.

The application of the 3R principles, for replacement, reduction and refinement of animal testing will make substantial criteria to the protection of animal wellbeing and the quality of scientific data also in the environment of GLP testing.

Author Statement

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