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Animal experimentation in Italy. Legislation and the authorisation of research protocols

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Summary. - In Italy, the European Directive 86/609/EEC ("The Council Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes") has been given effect with Legislative Decree 116/92, whose main objective is to guarantee the welfare of animals used in research and to prevent unnecessary experiments on animals from being carried out. The regulatory authority for controlling the use of laboratory animals in Italy is the Ministry of Health, which requires that researchers requesting authorisation to perform experiments on animals submit not only a copy of the experimental protocol but also a detailed application form that focuses on how the animals will be used. In the evaluation process, the Istituto Superiore di Sanità (Italy's National Institute of Health), in particular, the Service for Biotechnology and Animal Welfare, plays a key decision-making role. The evaluation is conducted by experts in the given area of research and by a veterinarian specifically trained in evaluating experimental protocols involving animal use. In the present work, the evaluation process is explained and a point-by-point description of the application form is provided.

Key words: animal experimentation, laboratory animal welfare, European legislation, Italian legislation.

Riassunto *(Valutazione dei protocolli di ricerca: legislazione e riduzione del numero degli animali)*. - Il decreto legislativo 116/92, che rappresenta il recepimento italiano della direttiva europea 86/609/EEC, è entrato in vigore al fine di tutelare il benessere degli animali da laboratorio e di evitare l'esecuzione di esperimenti inutili sugli animali. L'autorità regolatoria è rappresentata in Italia dal Ministero della Salute, al quale i ricercatori devono inviare i protocolli di ricerca che prevedono l'impiego di animali. Il Ministero della Salute acquisisce, quindi, il parere tecnico-scientifico dell'Istituto Superiore di Sanità, prima di emettere il decreto che autorizza il ricercatore ad eseguire gli esperimenti. Tale attività di controllo, eseguita avvalendosi di esperti negli specifici settori scientifici della ricerca e di medici veterinari, si è dimostrata estremamente utile ad assicurare il benessere degli animali da laboratorio e ridurne l'impiego a fini sperimentali.

Parole chiave: sperimentazione animale, benessere degli animali da laboratorio, legislazione europea, legislazione italiana.

Introduction

In 1986, the growing concern for the welfare of laboratory animals, together with the lack of specific legislation for guaranteeing this welfare, led the European Union to approve Directive 86/609/EEC [1]: "The Council Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes". The Directive has the objective of improving the treatment of laboratory animals, covering various issues related to animal welfare, such as housing, handling, transport, and feeding, and it includes a list of purposes for which laboratory animals can be used. Since the Directive is binding for all Member States, it has been given effect in each State's national legislation, which, in some cases, is stricter than the Directive itself.

In Italy, the Directive, six years after its approval, was given effect with Legislative Decree 116/92 (*Decreto legislativo* 116/92) [2]. As in other Member States, the Directive has been adopted by the regulatory authority responsible for controlling the use of laboratory animals, which in Italy is represented by the Ministry of Health. When submitting an experimental protocol to the Ministry of Health, the Principal Investigator of the study must complete an application form (Table 1). Below is provided a point-by-point description of this application and its importance in ensuring animal welfare.

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Table 1. - Application for the use of laboratory animals for research purposes (Legislative Decree 116/92)

Principal Investigator _____

☐ Communication (Article 7)
☐ Authorisation (Articles 8 and 9)

Expected duration of the research _____

Animal species and strains _____ No. _____ Supplier _____

Where will the animals be housed? _____

Title of research protocol _____

1. Please explain the rationale behind the study and its importance _____

2. Please describe the experimental procedures to which the animals will be subjected and the reasons for choosing this species and the number of animals specified (the least number of animals possible for meeting the goals of the research must be used) _____

3. Would it be possible to conduct this research using methods that do not involve animals? ☐ YES ☐ NO
If not, explain why _____

4. Please specify the type of local and/or general anaesthesia, and, if necessary, the means of performing euthanasia.
_____

If the animals will not be anaesthetised, explain why _____

5. Will the animals be used for purposes other than blood sampling and inoculation?
☐ NO ☐ YES If yes, describe the procedures

6. Please specify the level of suffering to which the animals will be exposed.
☐ No suffering (level I)
☐ Some suffering (for a short period of time) (level II)
☐ Extreme and unbearable suffering in conscious animals (level III)
☐ Enormous deprivation, mutilation or trauma (level IV)

7. Pathogens that will be used in the experiments _____

8. Will potentially dangerous substances be used?
☐ NO ☐ YES If yes, please specify _____

9. Will radioactive substances be used?
☐ NO ☐ YES If yes, please specify _____

10. Will cancerogenic substances be used?
☐ NO ☐ YES If yes, please specify _____

11. What specific measures and precautions will be necessary in handling and housing the animals? _____

12. What will be done with the materials used for the experiment after their use? ☐ Autoclaved ☐ Incinerated

13. Will any procedures be performed outside of the animal facility?
☐ NO ☐ YES If yes, specify where _____

14. Please provide a bibliography of literature pertinent to the experiment _____

15. Principal Investigator _____

University Degree _____

Experience working with animals _____

(continues)

Table 1. - *(continued)*

16. Please list of all the persons who will be working with the animals

Name	Specific duties in the research	Education	Experience in this area of research

17. Please enclose the *curriculum vitae* of the Principal Investigator and any pertinent scientific publications.

Principal Investigator

_____
(signature)

Welfare Veterinarian

_____
(signature)

Communication or authorisation

Differently from Directive 86/609/EEC, Legislative Decree 116/92 states that the specific procedure for authorising an experimental protocol depends on the species used, the level of suffering to which the animals will be subjected, and the purpose of the research (Articles 7, 8, and 9). Based on these factors, the Principal Investigator must specify on the application form whether the experimental protocol will be evaluated as requiring "communication" or "authorisation". "Communication" is required when the experiments are to be performed on vertebrates other than those specified in Article 8 (i.e. species other than dogs, cats, non-human primates, and species protected by the Washington Convention), yet only when the procedures do not cause pain, suffering, or lasting harm, and when they are not part of teaching activities. In these cases, only the Ministry of Health is involved in the evaluation process. The term "communication" refers to the fact that the researcher is merely "communicating" to the Ministry that the research is going to be performed, although this term can be considered as somewhat of a misnomer, in that the protocol must in any case undergo evaluation.

When the experiments are to be performed on the species specified in Article 8 or on any vertebrate for teaching purposes, or when the procedures can cause suffering, stress, or lasting harm, the evaluation process is referred to as "authorisation". In these cases, the Ministry of Health is required to contact the Istituto Superiore di Sanità (ISS; Italy's National Institute of Health), which plays a key decision-making role in the evaluation process. Within the ISS, the department responsible for coordinating this activity is the Service for Biotechnology and Animal Welfare. The first step in the process consists of the evaluation of the relevance and feasibility of the experiment by experts in the field, both within the ISS and externally. The protocol is also evaluated by a veterinarian working at the Service for Biotechnology and Animal Welfare, who is responsible for ensuring that Article 5 of Directive 86/609/EEC and the annexed guidelines on animal welfare are followed and that all of the procedures on the animals are correctly performed.

This article reads as follows:

"Member States shall ensure that, as far as the general care and accommodation of animals is concerned:

(a) all experimental animals shall be provided with housing, an environment, at least some freedom of movement, food, water and care which are appropriate to their health and well-being;

(b) any restriction on the extent to which an experimental animal can satisfy its physiological and ethological needs shall be limited to the absolute minimum;

(c) the environmental conditions in which experimental animals are bred, kept or used must be checked daily;

(d) the well-being and state of health of experimental animals shall be observed by a competent person to prevent pain or avoidable suffering, distress or lasting harm;

(e) arrangements are made to ensure that any defect or suffering discovered is eliminated as quickly as possible."

Although the Principal Investigator must state whether the research protocol is to be considered as requiring "communication" or "authorisation", this

must be confirmed by the Ministry of Health, which, in cases of uncertainty, follows the procedure for "authorisation".

Expected duration of the research

The duration of the research, which must also be specified, according to Legislative Decree 116/92, cannot last for more than three years. A one-year extension can be requested: in this case, protocols are sent to the Ministry of Health and then, regardless of whether they are being considered for "authorisation" or "communication", to the ISS, which evaluates the need for an extension, placing particular attention on requests for the use of additional animals. The results from the first three years of activity must generally be provided.

Animal species and strains and their suppliers

The application must include not only the animal species and strains but also their suppliers, which, according to both European and Italian legislation, must be authorised to breed animals for research purposes. This helps to ensure animal welfare, given that the suppliers must meet high breeding standards. However, this applies only to those species most commonly used in research (listed in Annex 1 of Legislative Decree 116/92): vertebrate species not commonly used for research, for which there do not exist farms that breed them for experimental purposes (e.g., pigs, cows, and sheep), must be acquired from zootecnical breeders, whose activities are regulated by different legislation.

Application for the use of laboratory animals for research purposes

The animal research application form has to be submitted by the Principal Investigator to the Ministry of Health. It is extremely important that the animal research application form is completely and exhaustively filled item by item, so that referees from ISS can properly analyse it. Hereafter all the items of the application form will be discussed.

Item 1. - "Please explain the rationale behind the study and its importance."

The objective of the research and the necessary background must be described as clearly and completely as possible, so that it can be determined whether or not the protocol complies with the following:

- the rationale of the study, the hypothesis to be tested, and the methodology must be appropriate for obtaining valid results: these aspects are particularly important, in that the research, even if fully complying to Legislative Decree 116/92, may not contribute to any scientific progress;

- the objective of the research must be among those purposes listed in Article 3 of Directive 86/609/EEC (in Italy given effect with Article 3 of Legislative Decree 116/92), specifically:

"(a) the development, manufacture, quality, effectiveness and safety testing of drugs, foodstuffs and other substances or products: (i) for the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants; (ii) for assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

(b) the protection of the natural environment in the interests of the health or welfare of man or animal".

Evaluating compliance with the above criteria is fundamental in avoiding unnecessary research, which ultimately contributes to reducing animal use. To this end, as indicated in Legislative Decree 116/92, it is important that this evaluation be performed by experts in the specific area of research.

Item 2. - "Please describe the experimental procedures to which the animals will be subjected and the reasons for choosing this species and the number of animals specified (the least number of animals possible for meeting the goals of the research must be used)."

In addition to describing the experimental procedures and justifying the choice of species, it must be stated that the least number of animals possible for obtaining statistically acceptable data will be used. The choice of the species and the number of animals must comply with Paragraph 3 of Article 7 of Directive 86/609/EEC, which has been given effect with Paragraph 2 of Article 4 of Legislative Decree 116/92, which reads as follows:

"When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected."

Item 3. - "Would it be possible to conduct this research using methods that do not involve animals? If not, explain why."

As required by both the European and Italian legislation, it must be stated that the research cannot be performed with methods that do not involve animal use. To this regard, in evaluating the protocol, it is important to choose the most qualified experts in the specific area of research, so as to avoid the unnecessary use of animals and thus reduce their use in research.

Item 4. - "Please specify the type of local and/or general anaesthesia, and, if necessary, the means of performing euthanasia. If the animals will not be anaesthetised, explain why."

The veterinarian from the Service for Biotechnology and Animal Welfare must verify that procedures causing pain will not be performed without anaesthesia. Only in those cases in which it can be demonstrated that the use of anaesthesia will interfere with the research can anaesthesia be excluded. The veterinarian must also confirm that the specific type of anaesthesia and the method for performing euthanasia are the most suitable for the given species.

Items 5 and 6. - In item 5, which can be considered as complementary to item 2, it must be stated whether the animals will be used for purposes other than blood sampling and inoculation; if so, the procedures must be specified. In item 6, the level of suffering to which the animals will be exposed must be specified (four levels, ranging from no suffering to extreme suffering with mutilation and/or trauma). In evaluating the protocol, the veterinarian from the Service for Biotechnology and Animal Welfare must pay particularly close attention to this point, given that the experimental use of all vertebrate species, except those specified in Article 8 of Legislative Decree 116/92, if entailing level I suffering (i.e. no suffering), will be considered as only requiring communication, whereas authorisation is required for the other three levels of suffering.

Items 7, 8, 9, and 10. - It is necessary to specify whether the animals will be exposed to any pathogens (item 7) or substances that are potentially dangerous (item 8), cancerogenic (item 9), or radioactive (item 10), which is important not only for the welfare of the animals but also for the safety of the personnel. The use of these substances must be essential for the experiment; if there exist substances that cause less pain to the animals without compromising the results, then these must be used.

Item 11. - "What specific measures and precautions will be necessary in handling and housing the animals?" This item is important for ensuring animal welfare, in that particular attention must be placed on reducing stress and suffering for experimentally ill animals that require special care or species that require specific housing conditions. This is also important for the safety of the personnel when the handling of animals could represent a potential risk.

Item 14. - "Please provide a bibliography of literature pertinent to the experiment."

This is important for justifying the importance of the research.

Item 15. - "University degree of the Principal Investigator and experience with laboratory animals." The Principal Investigator of a study involving animal use is required by law to have a degree in one of the following fields: Medicine, Veterinary Medicine, Biology, Pharmacology, Natural Sciences, or Agronomy. In this item, he/she must also certify that they have had experience in working with laboratory animals.

Item 16. - Other personnel.

The names of any other persons who will be working with the animals, as well as their specific duties in the research and their experience with laboratory animals, must be provided.

Item 17. - "Please enclose the *curriculum vitae* of the Principal Investigator and any pertinent scientific publications."

The scientific publications of the Principal Investigator must demonstrate that he/she is an expert in the specific area of research and has experience in working with animals.

The application must be signed by both the Principal Investigator and a welfare veterinarian, who will be responsible for guaranteeing animal welfare by supervising the experimental procedures. Thus in evaluating the protocol it must be confirmed that a welfare veterinarian will be present when the experiments are performed.

The duties of the welfare veterinarian, which are essential for ensuring proper animal husbandry and care, are explained in Articles 5 (d), 9 and 19 (2; b) of Directive 86/609/EEC and have been included in Legislative Decree 116/92.

Article 5. - *"(d) the well-being and state of health of experimental animals shall be observed by a competent person to prevent pain or avoidable suffering, distress or lasting harm."*

Article 9. - *" (1) At the end of any experiment, it shall be decided whether the animal shall be kept alive or killed by a humane method, subject to the condition that it shall no be kept alive if, even though it has been restored to normal health in all other respects, it is likely to remain in lasting pain or distress. (2) The decision referred to in paragraph 1 shall be taken by a competent person, preferably a veterinarian. (3) Where, at the end of an experiment: (a) an animal is to be kept alive, it shall receive the care appropriate to its state of health, be placed under the supervision of a veterinarian or other competent person and shall be kept under conditions conforming to the requirements of Article 5. The conditions laid down in this subparagraph may, however, be waived where in the opinion of a veterinarian, the animal would not suffer as a consequence of such exemption; (b) an animal is not to be kept alive or cannot benefit from the provisions of Article 5 concerning its well-being, it shall be killed by a humane method as soon as possible."*

Article 19. - *"(2) In each user establishment: (d) a veterinarian or other competent person should be charged with advisory duties in relation to the well-being of the animals."*

The one substantial difference made to these articles in Legislative Decree 116/92 is that while Directive 86/609/EEC entrusts these responsibilities to a veterinarian or "other competent person", Legislative Decree 116/92 states that only a veterinarian can be responsible for supervising animal wellbeing in experimental animal facilities.

Conclusions

According to European and Italian legislation, a correct analysis of the animal research application form is extremely important. When carefully performed by competent referees in the given research field and in veterinary science, the evaluation of animal research application form is crucial to ensure laboratory animal welfare; moreover, as previously outlined, it allows as well to reduce the number of animals used for scientific purposes, since it prevents inestigators from repeating experiments and from using an excessively high number of animals.

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REFERENCES

1. European Union. European Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities* L 358, 18/12/1986.

2. Italia. Decreto Legislativo 27 gennaio 1992, n. 116. Attuazione della Direttiva 86/609/EEC in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici. *Gazzetta Ufficiale* n. 40, 18 febbraio 1992 (Suppl. ord.)