

Russel & Burch emphasize possible alternatives to animal experimentation, defined by the so called "**3 R**":

**Replacement:** substituting animal experimentation with alternative methods.

**Refinement:** improving experimental conditions;

**Reduction:** reducing animal experimentation and the number of animals.

### **Replacement**

It has been indicated that in some sectors complete replacement of animals is not feasible, as currently no alternative method allows for the assessment of the safety and efficacy of drugs/products. However, it is possible to limit the use of animal testing by applying the following strategies:

- \* Evaluate existing data in literature or collected in databases;
- \* Apply in silico analysis and use statistical models;
- \* Conduct in vitro investigations, which can provide important preliminary indications;
- \* Use invertebrate animal models, microorganisms, plants, eggs, fish, animals with natural diseases comparable to those in humans.

### **Refinement**

- \* Avoid any possible cause of discomfort and suffering;
- \* Limit the duration of experiments when predetermined objectives are met in the early stages (earliest endpoint);
- \* Evaluate the sensitivity of the selected animal species and dose to be used before experimentation on animals (pilot study);
- \* Use anesthetics and analgesics;
- \* Perform accurate clinical observations during the experimental phases;
- \* Employ qualified personnel.

### **Reduction**

- \* Conduct in silico investigations;
- \* Design the experimental study carefully to obtain the most information possible;
- \* Properly conduct literature analysis to avoid repeating experiments already conducted and achieve similar goals;
- \* Identify the minimum number of animals needed to obtain reliable results determined through statistical analysis (Power analysis);
- \* Perform preliminary pilot studies on a few animals to highlight any variables and reduce variability.

At an **educational level**, reduction can be achieved by using videos, mechanical or computerized models.

### **The "4R": Responsibility**

Responsibility entails full awareness that:

- \* Any procedure or control not carried out (or carried out superficially) can cause pain and stress in animals;
- \* Any unnecessary suffering inflicted on a single animal alters the response we want to observe in an entirely unpredictable way;
- \* Each animal is part of an experiment involving a much larger number: an animal in poor condition alters an entire experiment.

### **TRAINING: Ministerial Decree of August 5, 2021**

Identifies, according to Article 23, paragraph 2 of Legislative Decree 26/2014, the methods of acquiring, maintaining, and demonstrating an adequate level of education and training for personnel required by each breeder, supplier, or user engaged in the following functions:

- a) conducting procedures on animals;
- b) designing procedures and projects;
- c) caring for animals;
- d) euthanizing animals.

### **TRAINING: Directorate Decree of March 18, 2022**

Identifies, according to Article 5, paragraph 2, Article 6, paragraph 2 and Article 7, paragraph 6 of the Ministerial Decree of August 5th, 2021:

- a) criteria and the accreditation procedure for training courses;
- b) the number of credits required to fulfill training and continuous professional development obligations;
- c) the management of the skills booklet;
- d) the recognition of CME credits for the veterinarian's continuous professional development;
- e) the inclusion of training titles and certificates of continuous professional development for the designated veterinarian, project manager, animal care manager, and scientific member in VETINFO.

**Training courses must be performed in Italian and on Italian legislation., in order to be considered valid for training.**

### **Reference Centers of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) in Brescia**

- \* National Reference Center for Animal Welfare: provides technical-scientific support to the Health Ministry and the Competent Authority in the field of Animal Welfare;
- \* National Reference Center for alternative methods, welfare, and care of laboratory animals.

Both entities are crucial for the pursuit of activities in accordance with the principles of the "3R". The activities pursued by the National Reference Centers are indicated on dedicated pages on the IZSLER website ([www.izsler.it](http://www.izsler.it)) and are annually updated as required by the Ministero della Salute.



## **Animal use for scientific purposes**



**Research on animals** must be authorized by the Health Ministry in compliance with **Legislative Decree 26/2014** (resulting from the provision of Directive 2010/63/EU on the protection of animals used for scientific purposes): according to art.1, paragraph 2 the use of animals for scientific or educational purposes **is allowed only when**, in order to achieve the desired result, **no other methods or scientifically valid experimentation strategy reasonably and practically applicable that does not involve the use of live animals can be used.**

**Law no. 413 of 12/10/93 - Rules on conscientious objection to animal testing**

It is possible to declare one's conscientious objection to any act related to animal testing. **This declaration has no discriminatory effects on students and/or workers.**

Every public or private facilities are obliged to inform all students and/or workers of their right to exercise conscientious objection.

**UNITA' DI STABULAZIONE DI ATENEO**

It is the Animal Facility where animals used in scientific procedures are bred, hosted, or used within the research activity.

**Environmental conditions**

Environmental parameters in the Animal Facility are determined according to the species' needs and age range:

- \* Temperature (T°) in rodent holding rooms must be maintained between 20 and 24 °C;
- \* Relative humidity (RH) in rodent holding rooms must be between 45 and 65%;
- \* Illumination maintains a photoperiod of 12h light/12h dark;
- \* Ventilation ensures 15-20 air changes per hour.

**Housing**

- \* Cages/Tanks: dimensions are proportional to the size and number of animals housed;
- \* Animals, except those which are naturally solitary, are housed in stable groups of compatible individuals. Single housing is allowed, for a limited period and only if strictly necessary, maintaining visual, auditory, olfactory, and tactile contact with other animals of the same species.

**Environmental enrichment**

- \* Environmental enrichment is essential to meet the behavioral needs of animals;
- \* Environmental enrichment includes a wide range of objects and/or measures that promote physical exercise, foraging, manipulation activities, and/or cognitive activities appropriate to the species involved.

**Designated figures according to Legislative Decree 26/2014**

• **Animal Welfare Responsible**

This is the person responsible for the welfare and care of animals and the functioning of equipment in one or more facilities.

• **Designated Veterinarian**

An expert in laboratory animal medicine, possessing specific experience and training requirements, who prescribes the methods for the welfare and therapeutic treatment of animals.

• **Research Project Manager**

The researcher holding the authorization for the project, responsible for developing procedures and projects, and responsible for administrative and scientific aspects.

**Project:** a work program with a specific scientific objective that involves one or more procedures, starting from the preparation of the first procedure until further interventions or observations are needed for the ongoing project.

**Procedure:** any invasive or non-invasive use of an animal for experimental purposes or other scientific purposes with known or unknown outcomes, or for educational purposes, that may cause the animal a level of pain, suffering, distress, or prolonged harm equivalent to or greater than that caused by needle insertion according to good veterinary practices.

**ANIMAL WELFARE BODY (OPBA)**

The **Animal Welfare Body** primary tasked is **provide advice on animal welfare issues.**

**Role of the OPBA according to Legislative Decree 26/2014**

The OPBA is composed of at least the individual(s) responsible for the welfare and care of animals, the designated veterinarian, and at least one scientific member.

Tasks of the OPBA: in general, provide advice to researchers and evaluate the correct drafting and execution of projects.

The Animal Welfare Body, as per Article 25, performs at least the following tasks:

- a) advise personnel handling animals on animal welfare issues related to their acquisition, housing, care, and use;
- b) advise personnel on the application of the principles of Replacement, Reduction, and Refinement, keep them informed of technical and scientific developments, and promote the professional development of personnel involved in animal use;
- c) define and review internal operational processes for monitoring, communication, and verification related to the welfare of animals housed or used in the facility;
- d) provide a reasoned opinion on research projects and any subsequent modifications, notifying the project manager;
- e) submit authorization requests for research projects, notifying the project manager;

f) monitor the development and outcome of research projects, considering the effects on the animals used and identifying and providing advice on elements that further contribute to the principles of Replacement, Reduction, and Refinement;

g) provide advice on rehoming programs, including adequate socialization of animals to be reintegrated.

**APPLICATION FOR PROJECT AUTHORIZATION**

- \* Allegato A - Project proposal
- \* Allegato B - Certification from an OPBA member on the competency of project participants
- \* Allegato C - Reasoned opinion from the OPBA
- \* Allegato D - Absence of conflicts of interest from each scientific member of the OPBA
- \* Allegato E - Declaration on the absence of criminal convictions from the Project Manager
- \* Allegato IX - Non-technical project summary
- \* Allegato VI - Project presentation outline

**AGENZIA DI TUTELA DELLA SALUTE DELL'INSUBRIA  
DI REGIONE LOMBARDIA (ATS Insubria)**

ATS is competent at a territorial level, conducting surveillance activities in utilizing establishments and inspection activities in breeding facilities for animals used for scientific purposes.

**MINISTERO DELLA SALUTE**

It is competent at a national level. It promotes the development and research of alternative approaches involving non-animal methods, fewer animals or less painful procedures and providing the same or higher level of information as animal testing. It is also responsible of worker trainee and update.

Significant basic and applied research is aimed at implementing the 3R principle in accordance with Russell and Burch's "The Principles of Humane Experimental Technique," published in London in 1959.

**ISTITUTO SUPERIORE DI SANITÀ (ISS)**

Provides to the Ministero della Salute technical-scientific evaluations of project for their authorization.

**CONSIGLIO SUPERIORE DI SANITÀ (CSS)**

Provides to the Ministero della Salute technical-scientific evaluations for projects involving the use of non-human primates, dogs, cats, and species at risk of extinction.