The 3 R Principle: Reduction, Replacement, Refinement

Created in 1959, the 3 R Principle is the basis for the ethical approach applied to animal studies in Europe and North America. Its provisions serve as a basis for all research projects involving the use of animals.

The 3 R Principle was born out of the ethical concerns of two English biologists, William Russel and Rex Burch. In their book published in 1959, *The Principles of Humane Experimental Technique*, the two scientists described this principle for the first time, aiming for the Reduction, Replacement, and Refinement of animal studies.

This principle was gradually adopted by various institutions, then introduced into the regulation, namely in Europe and in France. It contributes to the development of alternative methods to animal studies.

Reduction

This objective aims to **decrease the number of animals used** for research purposes, by:

- limiting use exclusively to studies perceived as absolutely essential
- reducing the unnecessary repetition of previous studies
- drawing up a study protocol before any studies are conducted, bearing in mind that a thoroughly prepared study often avoids the need for other animal tests

While it is essential to use the smallest number of animals possible, these efforts to reduce numbers must not, however, be detrimental to the reliability of the results. A biostatistical analysis must therefore be included in the project design.

Replacement

In order to replace animal models, it is sometimes possible to **work on cells or tissue (in vitro methods) or on digital models (in silico)**. Scientists should aim to use in vitro or in silico if their studies so allow. Although this objective can be achieved in numerous areas, particularly in safety studies in the broad sense, alternative methods are still supplementary to the use of animal models.

The search for alternative methods

At Inserm, the development of methods able to replace animal models is a research field in its own right. Hence, the Institute for Stem Cell Therapy and Exploration of Monogenic Diseases in Évry regularly works on human or animal stem cells so as to reduce the use of animals.

However, the extraordinary complexity of the living being cannot be beaten by certain in vitro or in silico. "*Disease modeling is still highly imperfect*, explains Bruno Villoutreix, Director of the *In Silico* Therapeutic Molecules Unit (MTI). *Likewise, it is not yet possible to predict the side effects of biological therapy – monoclonal antibodies, gene and cell therapy – as insufficient data are available, and the understanding of certain mechanisms is still limited. However, some side effects can be predicted for small chemical molecules which are candidate medicinal products.*"
Although *in vitro* and *in silico* methods are still supplementary methods and cannot fully replace animal studies, they still contribute partly to this goal.

Refinement

"Refinement" means optimizing studies using a methodology applied to animals: this involves **reducing**, **eliminating** or **relieving their pain or distress**, and thereby improving their well-being.

Prior to the study, refinement involves the following for instance:

- carefully selecting the animal model used
- improving transport, breeding and housing conditions
- planning the protocol to avoid stress
- training the animals to cooperate for non-invasive and painless procedures
- defining procedure endpoints (or criteria for early discontinuation)

What is an "endpoint"?

The endpoint is the point at which the suffering and/or distress sustained by an animal is stopped or minimized. Each investigator must therefore be able to identify physical and emotional suffering, quantify this using a scale, and take action when the pre-defined criteria for discontinuation have been met.

Refinement during the study itself concerns the methods and operating procedures. The following should notably be envisaged:

- non-invasive procedures (imaging, telemetry, etc.)
- adequate care before, during and after the operation
- anesthesia/pain relief
- reduction in the duration of certain studies (particularly toxicological studies)
- appropriate euthanasia procedures

Refinement after studies involves analyzing the results obtained during the study as effectively as possible. It is important to emphasize the fact that scientific analysis of the results obtained will not be possible if the animals have not been properly managed. In other words, ethics go hand in hand with scientific quality: "*Happy animals make good science*".