Ann Ist Super Sanità 2019 | Vol. 55, No. 4: 398-399
DOI: 10.4415/ANN_19_04_15

THE 3R PRINCIPLE: 60 YEARS TAKEN WELL

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Preface

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The Principle of 3Rs (Replacement, Reduction, Refinement) represents the methodological and ethical backbone of the Directive 2010/63/EU on the protection of animals used in scientific procedures (the Principle was described by an Official of the European Commission as "embedded in the Directive", pers. comm.).

Although 60 years have passed from the publication of the Principle, it has not lost its relevance to animal experimentation, instead, the ideas put forward by Russell and Burch are very contemporary, and still are a flexible and valuable tool to improve both the quality of life of the animals we use in our laboratories, as well as the quality of the data we collect.

It is important to look at the historical context in which the Principle was first envisaged of [1]. In the 50s' Charles Hume, the creator of the University Federation of Animal Welfare (UFAW), perceived the existence of a gap between the world of laboratory technicians and researchers, and a humanist view of the conditions of animals used for research. The term "humanist" referred in this context to a point of view derived from other disciplines rather than just laboratory science, such as, philosophy, anthropology, social science and so on. This view would see animals not just as objects to obtain measurable results, but living beings able to experience negative and/or positive mental states. Hume was dreaming of a new experimental science, in which scientific practice would be strictly linked with humanist values. In order to lay down the characteristics of this new enterprise, he enrolled the skills of William Russell, a brilliant PhD in zoology from Oxford, with interests reaching beyond the experimental sciences, interested in a multi-disciplinary approach to the use of animals for research. Russell called Rex Burch, as a sort of "field assistant", to help him in his mission. Russell and Burch embarked on a review of the characteristics of the methodologies used in laboratory animal science, with special focus on those techniques that could be further developed to decrease the level of inhumanity. It is very important to notice that Russell and Burch did not want to write a manual of animal ethics, because they were not interested in considering whether the use of animals in research was morally wrong or right. Their use of terms such as "inhumane" or "humane", in their words, "must not be taken to imply ethical criticism or even psychologic description of persons practicing any given procedure" (p. 14, [2]). Therefore, the volume by Russell and Burch [2] was not aimed at understanding whether the use of animals is acceptable or not, but gave suggestions and new methodological approaches to ameliorate the quality of lives of animals still used (to emphasise positive mental states, towards a more "humane" experimental science). This approach makes the Principle in line with the philosophy of the Directive 2010/63/EU, which regulates in Europe the use of animal models. As a matter of fact, the Directive is not a series of norms which favour or prohibit the use of animals in research, instead it protects the animals which are still used in research laboratories, until the day we will be able to do biomedical and toxicological research applying alternative methods which do not contemplate the use of animals. Therefore the Principle, which deals exactly with the situation in which animals are still used, is very much contemporary and consistent with the existing laws in Europe today regulating animal research.

There is a conceptual mistake, in our opinion, when it is said that the Principle is obsolete, because animals are still used, and we do not witness a significant decline in the numbers. Although it is true that the Replacement is one of the 3Rs, it is not the only R, and the Principle must be considered and applied in its whole. As so rightly put by the two authors: "Desirable as replacement is, it would be a mistake to put all our humanitarian eggs in this basket alone. The progress of replacement is gradual, not is it ever likely to absorb the whole of experimental biology" [2]. If you cannot replace, that does not mean that the Principle is not adequate, you can always try to reduce and refine.

And all this can be done today, as it was possible to do it before, even more now perhaps because new experimental techniques and sophisticated statistical methods are available. The Principle is a flexible tool, which can be adjusted to any new laboratory technique aimed at diminishing the level of "inhumanity", while preserving the quality of science. It is not a relic of the past because, first of all, it must be intended like a *forma mentis* to approach modern animal experimental science, and this *forma mentis* must be promoted to the new generation of scientists.

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The contributions presented in the dedicate section of the present issue are a proof of how new technologies and methods can be utilised, in the path suggested by Russell and Burch 60 years ago. These are modern ways to apply Replacement, Reduction and Refinement, and we are sure that more have to come.

The present monograph is divided into three contributions, aimed to present innovative aspects of each R.

For Replacement, the paper by Chantra Eskes deals with the importance to combine different methods and information sources to allow the full replacement of an animal experimentation and how the 3Rs Principle supports and drives these combinations. In recent years, several integrated strategy approaches using Non-Animal methods have been implemented for hazard identification and classification without compromising human or environmental safety [3]. They have benefited greatly from the latest advances in basic biomedical research strengthening the scientific background of the new replacement approaches to achieve more physiologically-relevant and human-based models. As a whole, the reported approaches may be also considered as relevant tools for investigation of the biological mechanisms that underlie diseases, chemical hazard effects as well as drug safety and efficacy.

As for Reduction, the contribution of Axel Kornerup Hansen focuses on the role of microbiota as a source of variability that has to be taken into account when dealing with laboratory animal studies. The microbiota of the human gut has recently gained wide attention in clinical studies because of its association with an increasing number of diseases (metabolic, autoimmune diseases, cancer and neurodevelopmental disorders) [4]: these associations can also be studied "pre-clinically" in animal models. But besides direct studies on the microbiota composition and effects, microbiota by itself can represent a source of variations in the immune and neuro behavioural responses in studies with laboratory rodents, and this also applies to studies not aimed at studying the microorganism colonization. Although solutions are not readily available, awareness of this source of variability is a first necessary step in designing experiments and an important issue when comparing results of standardized studies carried out in different laboratories on the same models.

For Refinement, the paper by Luca Bonini introduces new methodologies that could represent a very significant improvement of the quality of life of experimental subjects, as well as providing results of better quality. He focuses in particular on the use of non-human primates in neuroscientific research. The standard procedure for collecting neuroscientific data from non-human primates is the use of restraining chairs, in which a macaque sits immobilized (except for the arms) and perform visual and/or manual tasks. In the meantime, electrodes registers the activity of neurons in selected areas of the brain. In this situation, the major factor of distress for the animal is not the use of electrodes, but rather the physical restrain caused by the chair. For any animal restrain means to be hopeless in the case of the attack by a predator. The wireless system described by Bonini for recording the activity of neurons allows the animal to move freely in its environment, interact with objects and even conspecifics. The advantage of this new system is twofold: on one hand, the animal is not restrained anymore, and therefore there is a very significant refinement of its condition; on the other, the data collected have an increased "ecological" value, mimicking a more natural situation (rather than acting while sitting on a chair). At the end of his contribution Bonini suggests fascinating future scenarios. This is an excellent example of Refinement, where improved experimental conditions actually lead to the collection of better data.

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