

## COMMENTARY

# EU guidelines for the care and welfare of an “exceptional invertebrate class” in scientific research

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### Abstract

Cephalopods have been defined as “advanced invertebrates” due to the complexity of their nervous system and to their sophisticated behavioural repertoire. However, until recently, the protection and welfare of this class of invertebrates has been mostly disregarded by EU regulations on the use of laboratory animals. The inclusion of “live cephalopods” in the Directive 2010/63/EU has been prompted by new scientific knowledge on the “sentience” of animals used for experimental or other scientific purposes, a fundamental criterion to which animal species are included or not under the protective umbrella of the Directive. In this scenario, the imminent publication of the Guidelines for the care and welfare of cephalopods in research as an initiative by the CephRes-FELASA-Boyd Group is a sign of ethical progress in the consideration of animals in research, and is likely to have a significant impact on both scientific and practical aspects of research conducted with these animals.

### Key words

- cephalopods
- animal welfare
- sentience
- Directive 2010/63/EU
- guidelines

*“Animals have an intrinsic value which must be respected. There are also the ethical concerns of the general public as regards the use of animals in procedures. Therefore, animals should always be treated as sentient creatures ....” [1]*

### INTRODUCTION

Europe has a long history of legislation on the use of laboratory animals that – in some countries – dates back to the 19<sup>th</sup> century (e.g. Denmark in 1891; Germany in 1883; and UK, one of the first to regulate, in 1876). In 1986 the EU Directive 86/609/EEC (Council of the European Communities, 1986) and the European Convention 123 (Council of Europe, 1986) were published with the main aim of harmonising local regulations on animals used for experimental and other scientific purposes. However, both the aforementioned Directive and the Convention were meant to protect only live vertebrates.

In the following period of twenty-four years (1986-2010) the scientific community has achieved significant progress in experimental techniques, and these have been paralleled by changes in the ethical approaches to animal experimentation, leading to new insights into

animal welfare. Moreover, the acquisition of new scientific knowledge on the capacity of animals to experience and express pain, suffering, distress and lasting harm has resulted in the need to improve the welfare of animals used in scientific procedures by raising the minimum standards protecting those animals still needed to be used for scientific purposes. The result of these new contexts has been the publication in 2010 of the European Directive 2010/63 on the protection of animals utilised in scientific procedures. Among the most important changes brought about by the new Directive it should be mentioned the welfare and protection of foetal forms of mammals (as from the last third of their normal development) and independently feeding larval forms; such protection also extends to genetically modified animals by defining their creation and maintenance as a procedure (see Article 3).

Noteworthy, and differently from the EU Directive 86/609/EEC and Convention 123, the Directive 2010/63/EU brings under its scope the welfare and protection of a whole class of invertebrates such as “live cephalopods” (both adults and juveniles). This is a major change in the legislation on the use of animals

in scientific procedures [2] that takes into account advances not only in research techniques but also the improved understanding and assessment of animal welfare, particularly in relation to invertebrates [3-5]. In this scenario, the recent publication of the Guidelines for the care and welfare of cephalopods in research [6] is a sign of ethical progress in considering animals in research, and is likely to have a significant impact on both scientific and practical aspects of research with these animals.

As reviewed by Smith and co-workers [2], in 1993 the UK pioneered the concern for the use of invertebrates in research by including the welfare of *Octopus vulgaris* under the scope of the Animals (Scientific Procedures) Act - ASPA -1986. Likewise, scientific procedures involving cephalopods and decapod crustaceans are regulated in Switzerland while Norway regulates the scientific use of squids, octopuses, decapod crustaceans and honeybees. The Australian Government National Health and Medical Research Council's Code of Practice regulates the use of "cephalopods such as octopus and squid"; the New Zealand Animal Welfare Act includes "octopus, squid, crab, lobster and crayfish" under its scopes while the Canadian Council on Animal Care in science includes "cephalopods and some other higher invertebrates [that] have nervous systems as well developed as some vertebrates" insofar as they may experience from little to severe pain, stress, discomfort or other suffering (for further details see [2] and references therein).

### WHY CEPHALOPODS SHOULD BE PROTECTED AND HOW TO BASE SUCH A CHOICE: THE ISSUE OF SENTIENCE

Why to protect a class of invertebrates? The concern for animal welfare in the Directive 2010/63/EU is focused on the ability of animals to experience and express pain, suffering, distress and lasting harm. This concept calls into question the complexity of the anatomy and function of an animal's nervous system that are in turn related to the "psychophysical" wellbeing and eventually to the sentience of the individuals.

Sentience can be roughly defined as the individual capacity to feel, perceive and interpret a situation or a certain context. In a more refined definition, sentient beings are characterised by the capacity to assess the actions of other organisms in relation to themselves and third parties, to remember some of their own actions and the related consequences, to evaluate risks, to possess some feelings and a certain degree of awareness [7].

As pointed out by Donald M. Broom "It is of scientific and practical interest to consider the levels of cognitive ability in animals, which animals are sentient, which animals have feelings such as pain and which animals should be protected. (...) These abilities can be taken into account when evaluating welfare. There is evidence from (...) cephalopods and decapod crustaceans of substantial perceptual ability, pain and adrenal systems, emotional responses, long- and short-term memory, complex cognition, individual differences, deception, tool use, and social learning. The case for pro-

tecting these animals would appear to be substantial" [7]. Keeping in mind these considerations, EU State Members have certainly made a major step forward by deciding to include the welfare of live cephalopods under the scope of the Directive 2010/63/EU. Finally, it is worth emphasising that the very concept of "sentience" is the fundamental criteria to which animal species are included or not under the protective umbrella of the Directive.

### THE NEED FOR GUIDELINES TO PROTECT THE WELFARE OF CEPHALOPODS USED IN RESEARCH

The use of cephalopods in scientific research, particularly in the field of neuroscience, has a centenarian history. The reason for this success has its origin in some main features characterising this "exceptional class of invertebrates" [8]. First of all a complex and centralised nervous system resulting in a phenotypic plasticity and in a sophisticated behavioural repertoire that has been suggested to be evolved from their unique "embodiment" [8-10]. Moreover, it should be mentioned that their tractability for studies dealing with cellular and molecular mechanisms of learning and memory (e.g. long-term potentiation), likewise anatomical features facilitating physiological studies (e.g. squid giant axon and synapse) make cephalopods very suitable for scientific purposes.

As mentioned before, the Directive 2010/63 now includes cephalopods under the umbrella of animal species protected in Europe under the new legal text. As for other species, the Directive involves the justification and authorisation of projects dealing with cephalopods from local competent authorities and the revision of such projects includes a harm-benefit assessment and adherence to the 3Rs Principle [11].

To support project evaluation and compliance with the new EU law, specific guidelines for the care and welfare of cephalopods in research have then been developed following an international consensus based on an initiative by CephRes, FELASA and the Boyd Group [6]. The aim of the guidelines is to provide information as practical and effective support for researchers, animal care committees, veterinarians and technicians as well as animal care staff involved in research with these species.

Topics covered by the guidelines include (but are not limited to): implications of the Directive for cephalopod research; the application of the 3Rs Principle; project authorisation process; harm-benefit assessment and severity classification. Detailed aspects of requirements, care and welfare for the main laboratory species currently used are provided on: supply, capture and transport; housing conditions (e.g. water quality control, lighting requirements, vibration/noise sensitivity); cephalopod care (including tank main features, feeding, environmental and social enrichment); assessment of health and welfare; prevention of disease and treatment; general anaesthesia and analgesia and methods of humane killing. In addition, specific sections dealing with risks for operators, education and training requirements for carers, researchers and veterinarians

are reported. These guidelines will have an undoubted positive impact on the scientific environment by “guiding” investigators in the process of project authorisation possibly by also providing new inspiration for research on cephalopods. Personnel directly in charge of monitoring cephalopods’ psychophysical health such as veterinarians, animal care staff and technicians will also greatly benefit from the guidelines, since they currently rely only upon few aquatic medicine courses covering invertebrates and specific review papers on this topic. Furthermore, as detailed in the review by Fiorito and co-workers, the guidelines have a number of implications for different groups including not only working categories directly involved in the animal experimentation but also funders, journal editors and reviewers

[12]. As an example, funders could evaluate grants involving the regulated use of cephalopods to make sure that the proposed studies comply with the Directive and any national Codes of Practice related to care and welfare. Likewise, editors and reviewers of scientific journals will need to ensure that papers submitted for publication, when necessary, make reference to compliance with the Directive (in the philosophy of the ARRIVE guidelines).

The Guidelines for the care and welfare of cephalopods in research is a multifaceted and comprehensive document that will set an example for future similar initiatives.

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