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Summary — Humane endpoints are a core refinement concept in animal experimentation. This paper identifies an urgent requirement for individuals and institutions to refocus on humane endpoints as part of the transposition of Directive 2010/63/EU into the national laws of the Member States, and to go beyond their legal construction when setting new guidance or applying humane endpoints in practice. It will be argued that requirements for humane endpoints within the Directive appear not to promote recent advances in best practice, but seem reliant on a narrow and potentially outdated definition of the term. We describe progress that has been made in encouraging change in the construction and application of humane endpoints, and suggest that Directive 2010/63/EU does not sufficiently acknowledge the conceptual complexity of this refinement strategy. For example, a useful development representing recent consensual views of best practice has been proposed by an EU consortium (in 2012). A complex approach to humane endpoints may place additional demands on institutions and raise challenges that would, unfortunately, not need to be overcome in order to remain within the Directive’s current requirements regarding humane endpoints. We argue that there is now a need for a practical tool to help structure appropriate ethical reflection during research planning and experimentation, in order to facilitate best practice in the application of this important refinement concept.

Key words: Directive 2010/63/EU, ethical reflection, experimental animals, humane endpoint, refinement, Three Rs.

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Introduction

With the advent of new international legislation and calls for the harmonisation of experimental animal use, there is a much welcomed increased discussion regarding the implementation of the Three Rs (1). In the European Union (EU), this has been driven by the implementation of the new Directive 2010/63/EU (2). This discussion provides opportunities to improve the application of the principles, and to embed good practice at national and institutional levels. However, it is argued within this article that this important opportunity may be missed in terms of the application of the refinement concept of humane endpoints, due to the nature of the articulation of this concept within the Directive. This may, therefore, lead to a narrow translation of the concept in practice.

Humane endpoints have recently been described by Hendriksen et al. as “a refinement strategy designed to minimise pain, suffering or distress experienced by animals during an experiment” (3; p. 344). As such, this concept represents an important mechanism for the application of the Three Rs principle of refinement in the prevention of avoidable suffering. The literature published over the last 15 years illustrates how the effective use of humane endpoints has developed alongside other refinement procedures, in response to continued efforts to improve animal welfare in the laboratory.

In this article, the historical and current meaning and application of the term ‘humane endpoint’ will be examined, alongside the specific articulation contained within Directive 2010/63/EU (2), in order to explore whether this legislation is likely to reduce disparities among Member States, whilst promoting best practice focused on animal welfare improvements.

Humane endpoints first became prominent in the published literature following a conference held in 1998 in Zeist, The Netherlands, entitled Humane Endpoints in Animal Experiments for Biomedical Research (4). In the following years, a number of important publications addressed this concept. This included, in 1998, international guidelines on practical application from the Canadian
Council on Animal Care (5). The Organisation for Economic Co-operation and Development (OECD) published their guidance document, No. 19, in 2000 (6), having started work in this area in 1994. We will contrast the conceptual and practical approach to humane endpoints at that time with more-recent publications that challenge traditional approaches (7), and which have built on these important earlier works to develop humane endpoints into a powerful refinement strategy (3).

Member States were required to implement Directive 2010/63/EU on the protection of animals used for scientific purposes into national law by 1 January 2013. The previous Directive, Directive 86/609/EEC (8), was adopted in order to “eliminate disparities between laws, regulations and administrative provisions of the Member States”. However, Directive 2010/63/EU responds to the fact that, whilst certain Member States adopted national implementing measures that ensured a high level of protection of animals used for scientific purposes, others applied only the minimum requirements laid down in Directive 86/609/EEC. The new Directive states its aim to “provide for more detailed rules to reduce such disparities” (2; Recital 1).

We propose that references to humane endpoints within the Directive appear to be reliant on a narrow definition of the term, which relates humane endpoints to near-death states. This definition could now be considered outdated, and is not consistent with recently published opinions on best practice (3, 7). Therefore, this article is a call to individuals and institutions, particularly through animal welfare bodies and national ethics committees, to review how they operationalise humane endpoints, emphasising the importance of focusing on a more-evolved and ‘humane’ definition of an endpoint in the use of animals. We suggest that there is a need for an ‘ethical tool’ to help structure and formalise a comprehensive practical application of this refinement strategy.

Initially, we will illustrate how humane endpoints can be constructed as a complex ethical concept, and how recent best practice recommendations have emerged, before examining the need to move beyond the Directive.

Humane Endpoints — a Complex Ethical Concept

As is widely recognised, progressive public concern for the welfare of experimental animals, alongside drivers from within the animal care community, have contributed to an increasing ethical analysis of their use. In the context of humane endpoints, such analysis has led to a deeper and more nuanced understanding of what this refinement strategy aims to achieve.

An examination of the ethical boundary between humanity and inhumanity arose during the international conference on humane endpoints held in Zeist in 1998, where Balls suggested that the conference “was really about less inhumane endpoints” (9). He embedded this argument within the context of necessity in animal experimentation, and suggested that if necessity could not be satisfactorily established, then any such use of animals would be inhumane. Therefore, the term ‘humane’ has long been identified as describing a relative rather than an absolute state and, as such, its use could be expected to alter in line with progressive challenges to the necessity of animal experiments. Balls referred back to Russell and Burch, and described them as surprisingly and courageously discussing the ‘concept of inhumanity’ in 1959 in The Principles of Humane Experimental Technique (10) — this ethical boundary being fundamental to their development of the Three Rs.

At the same conference, Morton introduced the concept of “avoidable suffering” (11), suggesting that humane endpoints will reduce animal suffering only to that which is unavoidable — likewise framing humane endpoints as a relative rather than an absolute boundary. Importantly, this suggests that humane endpoints are conceptually relevant in all animal experiments where suffering could be reduced, not only in those expected to cause severe suffering and death. Through these conceptual arguments, we can start to identify humane endpoints as a broad “refinement strategy” with enormous potential to reduce suffering.

What adds complexity to the debate is that the use of humane endpoints can also introduce ethical problems. One of the central concerns in the literature is the potential for the early termination of studies to have a negative effect on the quality of research outcomes:

“With the move to earlier endpoints, there is valid scientific concern that significant differences in experimental treatments might be masked; earlier endpoints should not alter the outcome of the experiment. Endpoints should be scientifically valid as well as meet the obligation to minimize distress and pain to the animals” (12).

Although this is widely acknowledged, it is still valuable to note that, in addition to potential scientific redundancy, earlier endpoints raise the potential for unjustifiable wastage of animals through unsuccessful studies. In contrast to the ethical advantages that humane endpoints could offer, such a situation would result in the infliction of harm with little or no benefit, and would be unjustifiable on ethical grounds. Importantly, this illustrates that the definition of endpoints is a complex weighing process, informed by specialised scientific and animal care professional judgement.
The use of the term ‘humane endpoint’ introduces a further ethical risk for some. Depending on the criteria used to define the humane endpoint, the use of an approach labelled as humane could have very little positive effect on animal welfare, but it may appear to be ethical, and further still, it could be used to promote the work as being more ethically acceptable. This point is particularly relevant when we consider the text of the Directive, as noted by Franco et al. “Merely replacing spontaneous death with euthanasia of moribund animals may fail to have a significant impact on the degree of poor animal wellbeing” (7; p. 2). This does not suggest that there is no benefit to the euthanasia of moribund animals — Toth (13) argues that even unresponsive subjects might experience pain or distress. Rather, whilst there might be an ethical justification for the euthanasia of moribund animals, there must be a greater imperative in intervention before that point is reached.

This brief discussion of the literature is intended to introduce the current understanding of humane endpoints as a complex ethical concept that relates suffering to scientific outcome and ethical acceptability, not merely to death. We will next explore how this conceptual understanding is represented in a recent view of best practice, which describes humane endpoints as “a refinement strategy designed to minimise pain, suffering or distress experienced by animals during an experiment” (3; p. 344; emphasis by the authors of this article).

**Humane Endpoints — Advancing Best Practice**

In order to illustrate the progress that has been made in realising the full potential of humane endpoints, it is important to examine two different definitions of the term.

The OECD published very detailed guidance on the use of humane endpoints in 2000 (6). Their definition states that a humane endpoint is “The earliest indicator in an animal experiment of severe pain, severe distress, suffering or impending death” (p. 10). It is important to note that prior to this, death was a commonly-used endpoint in many animal experiments — and so, choosing to euthanise animals shortly before this point was deemed a relatively humane act. As illustrated above, humanity is seen as a relative concept in this context. We will later suggest that there are clear links between this definition and the language used in the Directive. However, the pertinent question is, does this approach fulfil current Three Rs expectations and does it still represent best practice?

Recent work funded by the EU through a COST Action (COST B24: Laboratory Animal Science and Welfare; http://biomedicum.ut.ee/costb24/about.html), resulted in an informative manual of laboratory animal care and use (3) that provides a view of best practices for individuals and institutions caring for, and working with, laboratory animals. Within this work, Hendriksen et al. (3) identify a variety of differing published definitions for humane endpoints, including the one proposed by the OECD. They suggest a new definition, which encompasses the most important elements of each:

“The earliest indicator in an animal experiment of (potential) pain and/or distress that, within the context of moral justification and scientific endpoints to be met, can be used to avoid or limit pain and/or distress by taking actions such as humane killing or terminating or alleviating the pain and distress” (3; p. 344; emphasis by the authors of this article).

There are many important practical elements to this more recent definition of humane endpoints, which are underlined in the quotation above. These aspects, which are further discussed in Table 1, support our argument that this recent definition of humane endpoints reflects the conceptual complexity discussed earlier. We have illustrated how the conceptual complexity of humane endpoints is recognised in recent best practice guidance, in that this strategy is not merely presented as an alternative to death, but as something with the potential to completely elimi-

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nate suffering, whilst also prioritising the production of accurate scientific data. The benefits for the animals, in terms of the reduced suffering that can be achieved by pursuing such an approach, should not be underestimated. To provide a specific example, Krug et al. (14) proposed the use of serological test methods as a replacement for infection trials in piglets to test the potency of Escherichia coli vaccines. Such information illustrates how the more-recent approach to humane endpoints could define the in vitro testing of a blood sample, that satisfies scientific requirements, with the infliction of very little suffering, as the humane endpoint of the experiment. This contrasts with the “severe suffering or impending death” of a clinically infected animal, which would previously have been necessary and defined as a ‘humane endpoint’ by the OECD.

A very important consideration regarding this best practice approach to defining humane endpoints is that it demands much more from the researcher and animal care professionals at all stages of a study than would near-death endpoints. It requires simultaneous consideration of often-competing factors, such as scientific objectives, severity limits and cost–benefit analysis, alongside the identification of individual staff responsibilities and detailed knowledge of animal welfare criteria; in some instances, it is likely that these demands may represent a barrier. We will next argue that such barriers would, unfortunately, not need to be overcome in order to remain within the Directive’s current requirements regarding humane endpoints.

**Directive 2010/63/EU Guidance on Humane Endpoints**

The use of the phrase ‘humane endpoints’ within Directive 2010/63/EU represents the first legal recognition of the term, yet beyond recognising the concept of humane endpoints by the inclusion of a phrase, it could be argued that it is important for the legislation to clearly define and promote best practice use of this refinement strategy. Directive 2010/63/EU initially addresses humane endpoints, as follows (2; emphasis by the authors of this article):

“*The methods selected should avoid, as far as possible, death as an end-point due to the severe suffering experienced during the period before death. Where possible, it should be substituted by more humane end-points using clinical signs that determine the impending death, thereby allowing the animal to be killed without any further suffering*” (Preamble No. 14).

This phrase clearly sets out that humane endpoints are being proposed as an alternative to death as an endpoint. Specific guidance provided in the Articles of Directive 2010/63/EU does provide a legal requirement for ‘early and humane endpoints’. However, beyond this provision, it again limits the discussion of humane endpoints to a contrast with death. In fact, the article provides more detail on how death as an endpoint should be managed, than on guidance about the use of humane endpoints:

“*Death as the end-point of a procedure shall be avoided as far as possible and be replaced by early and humane end-points. Where death as the end-point is unavoidable, the procedure shall be designed so as to:*

a) *Result in the death of as few animals as possible*
b) *Reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death*” (2; Article 13[3]; emphasis by the authors of this article).

Although this can be viewed as a positive change, it is suggested here that the use of this phrase is very limited in terms of what it aims to achieve, and we will argue that it is unnecessarily conservative. With reference to the Directive’s stated aim, it appears that ‘detailed rules’ in the context of humane endpoints appear sadly lacking. Whilst the significant consultation and dialogue amongst Member States that led to the text of the Directive is recognised, it is argued that there are further opportunities across Member States, through the development of National Ethics Committees and within each institution’s Animal Welfare Body, to formalise the embedding of the Directive’s requirements within practice. As such, it is important that these further opportunities to operationalise a more-progressive understanding of humane endpoints are not missed.

Through our examination of the broader conceptualisation of humane endpoints and recent best practice guidance, we have illustrated how this phrase can ultimately define something much more complex than the Directive suggests. This would have a greater potential to alleviate suffering, thereby capturing the essential imperative of the refinement principle.

**Discussion**

When considering the adequacy of Directive 2010/63/EU with regard to humane endpoints, this paper presents an argument that the Directive may be based on a narrow and outdated perspective of humane endpoints which fits the definition proposed in 2000 by the OECD, but not a more recent and more appropriate definition. The published literature clearly illustrates that good practice has moved beyond merely challenging death as
an endpoint, to embracing humane endpoints as a complex conceptual idea, and, practically, as a powerful refinement tool.

In a practical sense, countries, institutions, and individuals will already be at different points along the scale of progression in terms of their understanding and application of this concept. Furthermore, during the transposition of Directive 2010/63/EU, it is likely that, due to the observed lack of detailed guidance and congruency between the Directive wording and published best practice, there has been, and could be, further variation in national recommendations.

It is both concerning and encouraging to note that there exist, within the Directive, all of the necessary components to allow a full use of this refinement strategy, as presented by Hendriksen et al. (3). This includes the provisions made for cost-benefit analysis, retrospective review, severity banding, and international validation through the European Centre for the Validation of Alternative Methods (ECVAM). To illustrate just one of these examples, there is much potential for improved international validation of humane endpoints via ECVAM (2; Recital 47). This mechanism should allow researchers to use humane endpoints that have been validated by others working within a particular field. However, this benefit is likely to be mainly limited to those experimental processes that are highly specific and repetitive, such as toxicity testing or vaccine batch testing. This is due to the fact that humane endpoints are specific to a particular animal species, strain and experimental procedure. For researchers working outside these specific areas, the harmonisation of best practice will now depend on institutions and individuals taking ownership of this refinement strategy, and choosing to apply the most recent guidance — i.e. taking humane endpoints beyond the requirements of the Directive. For this to be universally undertaken, there is a need for formal recognition that humane endpoints are a more complex refinement strategy than is set out by the Directive.

Lindl et al. (14) recently proposed, in response to the Directive, a formal procedure that aims to ensure the ethical defensibility of applications for animal experiments by determining indispensability and balancing potential benefits against harm to the experimental animals. Lindl’s work (15) provides a useful example of the need for structured processes that assist in the practical application of ethical concepts — in this case, the sequential consideration of several predetermined criteria is used to create an ethical appraisal. The authors note that “even if the details of national regulations vary, the essential points of ethical assessment remain the same” (15; p. 219). Another example of a structured tool, the Ethical Matrix (16, 17), which applies prima facie ethical principles to interest groups, in order to characterise ethical implications, was originally developed for use in the analysis of biotechnology development, and also aimed to facilitate ethical analysis. This tool has subsequently been developed and applied in a number of different ways (18).

Building on the potential value of these approaches, we propose that there is a need for the development of a practical tool, such as those discussed above, to facilitate the full use of this important refinement strategy by guiding researchers through the processes involved. In particular, it must be made clearer that ‘humane’ endpoint is a concept that goes far beyond the euthanasia of animals in near-death states. It is a complex ethical concept relating suffering to scientific outcome and ethical acceptability that can be very effectively applied in practice to eliminate any avoidable suffering.

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