Humane endpoints

What is a humane endpoint?

Studies to better understand diseases and hopefully develop better treatment, testing efficacy or safety of substances are common aim for many studies in animals. In such cases, there is always a risk of pain, suffering or distress in the animals as the study proceeds. The EU directive - that is harmonized into national regulations in Europe - states clearly that any unnecessary pain, suffering, distress and lasting harm must be avoided [1]. Projects needs to be authorized by the authorities. Potential or actual pain, suffering or distress that is regarded necessary to achieve a certain aim must be justified in a harm-benefit assessment [1-3]. That means that the negative experience of the animal must be justified because of greater benefit for the majority by doing the study.

If the actual negative impact on the animal is higher than predicted in the harm-benefit analysis that was the terms and conditions for authorization of the study [4, 5] or when the condition for the animal reaches the level of unbearable [6] Pain, suffering and distress above that is required to achieve scientific objectives are regarded unnecessary and inhumane [4] and should be replaced by earlier, more humane endpoints.

There are also scientific reasons for applying earlier endpoints as an animal in advanced stages of disease is so physiologically deranged or disrupted that it does not provide useful, reliable scientific information any longer [4].

It is therefore an obligation of the researcher to plan and perform experiments in such a way that animals do not have more negative experiences than strictly necessary to achieve the aim of the study.

Chronic diseases gradually progress in the level of pain, suffering and distress for the animal, it gradually become more severe (figure 1). The only way to avoid this is to take appropriate actions to mitigate pain, suffering or distress. Means to identify the critical points were to take actions must be defined already in the planning phase of the study.

Severe clinical symptoms reflect alterations in the normal physiology and reduced ability to adapt to maintain homeostasis followed by intense pain accompanied by negative situational factors such as loss of control, fear or anxiety, or lack of social support [6]. The sense of being self-threatened over time cause severe distress in the animal. The severe state of moribund or dying reflects an irreversible situation with death as the only outcome[7].

Replacement of death as an endpoint earlier endpoints is explicitly expressed in the EU directive that has been translated into national regulations in most EU countries [1] state

> *Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. [1]*

The EU directive also state that if death is unavoidable as an endpoint the study should be designed to result in the deaths of as few animals as possible. In such cases were death is unavoidable the aim of the study must be evaluated against potential benefits.

Also, other international guidelines like the guide for the Gare and Use of Laboratory Animals address the importance of humane endpoints which better explains the scientific importance of applying early endpoints.

> *Studies that may result in severe or chronic pain or significant alterations in the animals' ability to maintain normal physiology, or adequately respond to stressors, should include descriptions of appropriate humane endpoints*[8]

Clinical symptoms or other signs of suffering develop when the animal cannot any longer compensate for the progressing disease. This is especially critical for pray animals – like rodents and several species of fish. Prey animals will use resources to compensate for and try to hide early signs of disease [9]. Hiding weakness for a prey animal is important to avoid attention from potential predators as this might make their lives at risk. The implication is that the animal might have suffered from coping distress for a while before humans are able to recognize the problem. The observation-window from no clinical signs to obvious clinical signs can be narrow for many species.

> *Sunken flanks, neglected grooming, piloerection, hunched back, immobility are clear evidence of severely impaired often moribund health status in mice [10]*

Therefore, we should search to define early signs of pain, suffering and distress and use them to define early humane endpoints in animal studies.

The concept of humane endpoints is about setting earlier endpoints and follow up with action points to minimize, reduce or eliminate unnecessary suffering and this can also be defined as an improvement strategy in accordance with the principle of refinement [11].

Implementing Early more humane endpoints is an important strategy to refine animal studies.

Death as an endpoint

According to the EU directive death as the end-point of a procedure shall be avoided and replaced by early and humane end-points. Where death as the end-point is unavoidable – or have been authorized because of scientific needs, the directive state [1] that procedures must be designed so as to:

- *result in the deaths of as few animals as possible; and*
- *reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.*

The Canadian guidelines [12] also include the state of dying – the moribund state as

> *moribund animal is one that is close to death and may be comatose or unresponsive to stimuli, exhibit dyspnea or other severe breathing problems, hypothermia, prostration, etc. However, before the animal gets to the point of being moribund, detailed observations of the animal can help to set an earlier endpoint and thereby reduce the actual cost to the animal, in terms of pain and/or distress.*

Taking consideration to animal into account also the moribund state should be avoided as an endpoint equal to the use of death as an endpoint [7, 13].

Identify criteria to set humane endpoints and define follow up action-points

Endpoints can be defined based on how they impair welfare indicators that can be classified as

- Morphological welfare indicators
- Behavioural welfare indicators
- Physiological welfare indicators

For all of them - it is the absence of good welfare needs attentions, correction or follow up to avoid unnecessary pain, suffering, distress, or lasting harm.

Morphological welfare indicators must be based on knowledge about the specific species and life stage. Malformations that cause disabilities and problems to maintain the animal's normal functions and responses are relevant endpoint indicator. They are especially relevant in developmental studies and characterization of new genetically lines, where malformations may be an (unexpected) outcome. Examples include skeletal deformities [14, 15], and hypoplasia of organs [16-18]. Malformation can cause critical secondary malfunction as food intake caused by jaw or teeth deformities that limit growth and development as because of changes in connective tissue [19] or lip or cleft palate deformities [20] that will create problems already in the suckling phase for a mammal. Protocols for suggesting systematic approach to catheterization of new phenotypes [21] and assessment of severity of genetic altered animals [22] have been developed. Malformation of column vertebra in a fish might case extra strain with swimming both for search food and escape from treats [15, 23] Putting the animal under coping stress. Other examples of morphological welfare indicators may include damage to skin or eyes, ulcers, emaciation, change in body condition, body shape or fitness factor [15, 23]. The FISWELL project was made to define morphological welfare indicators in Atlantic salmon [24]. A collaboration with an experienced comparative pathologists is strongly recommended in defining morphological [25].

Several morphological welfare indicators are simple to register, and the optimum condition is usually well described. Others will demand more laboratory testing like CT/MR for internal organs or necropsy of sentinel individuals in an experimental group.

A main weakness of using morphological welfare indicators is that they are retrospective. That means that the condition that caused morphological deviation have happen back in time and the animal might have struggled with disabilities long before symptoms are detected during regular observation. Absence of morphological deviations is not a guaranty for good welfare and presence of a morphological deviation is not necessarily representing a welfare issue.

Behavioural welfare indicators are detected by observation of the animal and a great advantage is that they often reflect physiological responses in real time. Changes in behaviour may be the first visible response to adverse conditions and early warning. One example is response to daily husbandry practices like feeding. Animals fed at regular intervals will be hungry and come to be fed the food when it is served. Observing the animals during daily husbandry practices give valuable information of the physical condition – and such observations should be utilized to define end-points. Animals that do not respond as usual when the operator occur by the cage, pen or tank or net should be followed up for underlying cause so appropriate actions can be applied.

A natural behaviour in mice, for example, is to build nests. Healthy mice are highly motivated to build nests when they have access to nesting material [9] , and lack of nest building in mice that have access to nesting material may be an indicator of pain, suffering or distress and should be checked for underlying causes. Also reduces burrowing behaviour in mice [10, 26] or the normal vertical activity (rearing) in rats [27] have been use to assess pain and distress in laboratory animals. Other behaviours like hanging in the cage-lid is a strongly motivated, though not essential, behaviour and such behaviours can be used to assess pain and discomfort as for example hanging is reduced when mice are in pain [28].

Not all behavioural welfare indicators reflect real-time problems. Stereotypic behaviour can be a coping response to environmental distress that might have

last for a while [29, 30]. Attention should also be made to other animals in the same environment, they might also be suffering distress even if they have not developed stereotypies yet. Stereotypic behaviour might also have underlying physiological causes [31] – and this might be of especially interest when defining endpoints for newly generated genetically altered animals.

Animal general activity is also a useful endpoint indicator. Activity can be reduced because the animals is in general weakened, exhausted or in pian when moving. Increased activity might be a response to irritation, stress or a treat that upset the animals. Lethargy (apathy, drowsiness) is characterized by reduced sensitivity to stimuli, often occurring in the final stage of illness – a situation called moribund. It is questioned if the moribund stage can be regarded as a "humane" endpoint – as it is very likely that the animals has been subjected to pain, suffering and distress before it reached this stage and I is questioned if these distress should replace the moribund state as an endpoint. Willingness to move only when stimulated will be an earlier endpoint than no willingness to move when stimulated – but still this will reflect a severe situation for the animal.

Self grooming is important for many species. Rodent use much of its time budget on grooming its fur. Ruffled fur is therefore an important early indicator of poor welfare [32, 33]. Rodent fur should be smooth and dense coat. By age it might become duller and less shiny. Other early indicators of pain or distress in rodents include reduced activity, reduced food intake and isolation from group mates [32, 33].

When resting rodents are curled up – and in groups they group together when the sleep to better maintain preferred body temperature and avoid losing heat. Finding a rodent alone – laying stretched out – is and indicator of impaired welfare. Healthy rodents show exploratory behaviour rearing onto hind legs and sniffing. (VIDEOLINK)

Observing swimming pattern in fish give valuable information of the physical condition of the fish, however there are big differences between species and life stages. Zebrafish typically swim in the middle of the tank and swimming at the tank bottom is interpreted as an indicator of pain [34]. Salmon Parr are territorial, live in the demersal zone and show a cross-current swimming pattern in the

rivers [35]. But during the Parr-Smolt Transformation they reduced cross-current swimming and show tendency to move downstream against sea water [35]. In the lab in the tank salmon smolt typically swim in the same direction in a coordinated manner (they are schooling). Loss of equilibrium, position in tank or water and gill movements (hyperventilation, hypoventilation) are also useful welfare indicators in fish. Flared gills occur when fish have trouble breathing caused by low O_2 level in water or infection on the gills and thus must immediately be followed up by identifying the cause and thane correction actions. In rats [36], mice [37] and rabbits [38], specific postoperative pain behaviour have been described [39].

Striking painful body-part against surface has been used as an indicator of pain in rats [36] and rabbits [38] and behaviour changes cause by noxious stimuli has been demonstrated in several aquatic species including cephalopods [40, 41], hermit crabs [42] and several species of fish including rainbow trout [43] and zebrafish [34, 44].

Pain assessment by facial grimace scoring has been validated and showed to be a reliable method for pain assessment in several species [45-49].

Observations by humans may – depending on species and the adaption to human presence or interactions may cause observation bias. Some animals modulate behaviour to mask symptoms. This is especially a problem in prey animals [9] as predation is a strong selective force in evolution. Several species maintain a set of natural defence mechanisms (instincts) even when they are kept in captivity protected from natural predators. In some cases, natural defence mechanisms may be modulated by use of training, like with positive reinforcement. Some animals avoid predators by occupying areas or habitats that are nor suitable for their predators or they are active in times where predators are not. For mice – a nest function as a hiding place and offering animals a shelter gives the animal a better opportunity to self-control environmental control of treats that reducing the stress in the animal. Panic response in fish reflects that the fish are stressed for some reason. It can be poor water quality, fear of a predator presence, or just the animal caretaker dressing up in other colours than they are used to.

For animals in other habitats like fish in water, observation and evaluation of individual animals can be difficult as animals are not easily to access. By use of technology and remote recording like telemetry [9, 50], GPS-registrations and similar automated technology for [28, 34, 47, 51-53] behaviour observation can be possible without direct interfering with the animal.

A main weakness with behavioural welfare indicators is that interpretation of deviant behaviour is not always clear. Basic knowledge of natural behaviour for the species is a fundamental condition for using behavioural welfare criteria as endpoints. As a minimum the researcher should be able to answer the following according to Jobling [54]

- How do the species naturally move?
- How do they naturally feed, what do they eat and how much?
- How do the species natural respond to disturbances, potential dangers, or interactions with conspecific as well as humans?

Physiological welfare indicators can include clinical observation, like breathing pattern, hearth frequency or results from laboratory test of blood, saliva, urine or other samples. They can be early identification or disease markers. Many of them are sensitive, have validated methods and comparison with normal condition is reliable when normal values are known, as for example levels of hormones, blood parameters or metabolites.

The main problem is the tissue of sampling as it often involves manual handling and restrain, which can be more of a burden for example for a fish that has to be taken out of water or an animal that is not adapted to human interaction or restrain. Training and habituation can be used for many species like dogs, NHP and pigs to reduce the stress related to handling and sampling [55]. Surface hypothermia using infrared non-contact thermometer has been used as a predictor for death in infectious studies in mice [56].

When including analysis tissues or body fluids as endpoint indicators – it must also be evaluated how frequent samples are necessary – and what is the volume

needed for the analysis – as these might affect the level of stress and physiology of the animal.

Blood glucose used as pre-lethal surrogates [4]

Reduced body temperature measured by telemetry [50] or by infrared measurement [56] used as an early endpoint for sepsis in studies of infectious disease.

Physiological endpoints are typically used as disease markers and surrogate endpoints, whit great potential to define earlier, more humane endpoints. However, the idea to use early clinical signs to predict later ones require validation (6)

All three categories described in this section have their strength and weaknesses with regard to evaluating welfare or emerging pain, suffering or distress. However, by combining more of them like body weight with behavioural change [57] , they can provide useful indicators for early humane endpoints.

Also animals with a significant loss of body weight might be lively and have a good quality of life (4).

Generic, specific or unexpected events

Endpoints can also be classified as generic endpoints, project specific endpoints, or endpoints for unexpected events [5].

Generic endpoint parameters reflect unspecific welfare issues (morphologic, behavioural or physiological) like loss of appetite, lack of self-grooming, activity level, change in body weight or body condition [58], signs of pain or distress etc. They give an indication that the animal is not doing well, but do not specifically relate to a specific condition. Lack of highly motivated species specific behaviour like burrowing [10, 26, 57], nest building or cage-lid hanging [28] in mice can be used to determine earlier endpoint in studies.

Using weight loss as an endpoint parameter

Weight loss, typically 10-20% is a commonly used generic endpoint parameter. There are many good reasons for using weight loss as an endpoint. It is not a risk

of subjective bias and it is also easy to measure in land living animals also without too much restrain, and some species can even be trained to walk voluntary on the weight [59]. Other species like aquatics, however, weighing is not easily performed without handling brining the animals out of its natural habitat - that is usually stressful for the aquatic animals.

Weight loss as an isolated parameter is not very sensitive for the animal´s actual condition. Maintaining body weight or weight gain can be caused by conditions like tumour growth or accumulation of fluid in the peritoneal cavity (ascites). Stable weight in an animal in a growth phase is a poor sign as they are expected to increase weight according to the weight-gain curve. Weight gain can also be a problem as obesity increase the risk of other disease, complicate self-grooming and increase the load on the limbs and cardiac function. Also, obese animals take up more space and there might be a need to split groups in more cages or pens unless this is accounted for on from the start of the study already. Overweight is especially a problem in long term studies.

Body weight should be evaluated together with body score evaluation [58, 60]. The body score in mice is usually defined in five categories from emaciated (score 1) to obese (score 5) and each category is will defined. However, there is a risk of subjective evaluation so body weight (objective) however, body condition scoring can also be used more objectively by micro CT-imaging [58]. Body Exclusive application of weight loss criterion for euthanasia as an endpoint might result in an unnecessary loss of animals [61]

Weight loss is reflecting a "problem in the past", i.e. the animal might have reduced appetite or been under a catabolic process already for a while before we are able to detect it. Also, in small animals like mice weighing 10-30 grams, accuracy of the scale is crucial to be able to detect an early change of 10% (1-3 g) change in body weight. A complementary endpoint parameter could therefore be to measure food intake as an indication of appetite whenever this is possible.

Generic endpoints are relevant for all studies of gradually progressing diseases.

Some authors that endpoints can rarely be generalized and are against using to many or too many and too general endpoints as scoring them is labour intensive

and research might be required to evaluate variables that might appear arbitrary or unrelated to the animals condition [7]. Compliance may be increased if the measurement of the variables are related to research objectives [7]. Other authors underline that humane endpoint cannot rely on a single variable, suggesting to use a combination of variables [62] and recommend a combination of assessment parameters for a robust welfare assessment in laboratory animals [63].

Project specific endpoints are endpoints that reflect disease progression of the phenomenon of interest in the study. For example, in a cancer study, tumour size, number of tumours, metastases, tumour's tumour location and interaction with other functions (mobility, food intake) can be relevant project specific endpoints. In a studies involving surgery – wound complications like redness, swelling, infection, broken sutures are relevant and should be included as potential endpoints with defined action-point for follow up. Preclinical screening of ALS-mice showed that loss of motoric function was used as an early sensitive and rapid indicator for the initial phase of denervation of muscle fibres [64] and reduced activity in the home cage running wheel as an early diagnostic sign [65]. For diabetes studies, examples can include blood glucose, urination, cages changes need (because of increased urination) or water consumption. Also, these endpoint indicators should be followed up by appropriate action points to mitigate pain, suffering distress or lasting harm.

Table 1 Refinement of study specific endpoints and alternatives to death

Study	Endpoint	Reference
Infectious diseases	Acute phase responses	[66]
	Body Temperature	[66]
	Weight loss	[66]
Intracranial Glioma in rat	Body weight algorithm	[67]
Post laparotomy	Telemetric recording of heart rate	[9]
Rheumatoid arthritis in rodents	Swollen digits, knuckle, midfoot and ancle/wrist scoring, Ulceration, gait and posture analysis	[68]
Amyotrophic Lateral Sclerosis (ALS)	Decline in motoric Function	[64]
	Home cage running wheel	[65]
Severe irradiations studies in NHP	Rapid decrease of body temperature for 3 consecutive days combined with general behaviour score	[62]
Total-Body Irradiation in Mouse	Body weight, temperature by telemetry	[69]
Murine model for cholestasis	Burrowing activity	[57]
Anti-urolithiasis activity of test compounds by zinc disc implantation	Imaging (X-ray radiographs) of the bladder deposits	[70]
Experimental liver metastases	Laparoscopy	[71]

Endpoints for unexpected events relate to pain, suffering or distress caused by other unrelated illness, accidents or unexpected adverse effects of the study that necessitate human interaction [5] to avoid continued unnecessary and unjustified pain, suffering or distress. Other unwanted events might be fighting and fighting ulcers or that an animal is hurt under procedures or husbandry practices.

End and action points for unexpected events should also in include disaster planning i.e. how to follow up in case of a disaster. Examples could be power failure, outbreak of infectious disease, water flood, fire, ear crake, burglary, sick leave among personnel in case of a pandemic among humans etc.

The guide for the care and use of laboratory animals say [8]

> *Well-planned experiments with clearly delineated scientific and humane endpoints will help to ensure that a contingency plan is in place for problems that may arise during the study*

Depending on the level of impact, this event might disturb or not disturb the study, and it must be made a decision case by case or the experiment can continue or if it should be terminated. Continuation of the study may be on the cost of animal harm and reliability of the results. Termination and restarting the experiment will be on the cost of animal lives.

Control animals

When defining studies and endpoints special consideration must be made to control animals. Especially negative controls (not receiving the test substance of interest) will not have the advantage of a potential treatment of protection effect of the test-substance – and they will therefore likely be that first to reach the end-point state – assumed that the test substance is effective. This applies for both the generic and project specific endpoints.

Unregulated species and life stages

Regulations and guidelines define borders of which animal species and life stages that are included. Legally humane endpoints are relevant for studies in these species and life stages. However, the regulations are based on historical knowledge and new knowledge is continuously coming. The inclusion and protection of cephalopods in the 2010 EU directive [1] is an example of a new group of non-vertebrate animals that become protected based on scientific knowledge. As knowledge is constantly evolving – attention to pain, suffering and distress also to early life stages, like fish larva before they start feed or drosophila may be worth considering – even if they are not currently covered by regulations.

Actions to be taken when an endpoint is reached and consider possible options for refining methods to finish at an earlier endpoint

When an animal reaches a certain defined end-point it must be followed up by action points to reduce pain, suffering distress or lasting harm. "Action points" describe the actions we are going to take to mitigate the condition and avoid further pain, suffering, distress or lasting harm when or if an animal reaches certain stages – or endpoints - in a study.

In many cases, for many species this will be to kill the animal to avoid further suffering. However, with earlier endpoint other alternative action points should also be considered, as said in the directive [1] on care an accommodation

> *Article 33 (d) arrangements are made to ensure that any defect or avoidable pain, suffering, distress or lasting harm discovered is eliminated as quickly as possible*

That could include medical treatment to relieve pain, nausea, correct blood glucose, fluid therapy etcetera. An example of endpoints and action points for follow up a surgical wound after surgery (Figure 2).

Figure 2

	Clinical symptom/endpoint criteria	**Grade**	**Action-point**
Surgical Wound evaluation	Closed wounds, No erythema	0	New observation next day
	Slight erythema around, no edema/swelling	3	Frequent observation 2 times per day
	Moderate erythema, edema	6	Consider painkiller or antibiotics based on veterinary recommendation
	Severe erythema, swelling/edema, open wound	8	Provide painkiller, conservative wound care or reoperation of wound
	No effect of treatment	12	Humane killing of animal

Other action point than those mention above can include providing soft or alternative food, provide enrichment (for example for behavioural problems or fighting) or consultation by the veterinarian. The animal care staff, and the designated veterinarian should be involved in planning endpoint and action points as they can provide useful information. In all cases, the study director must consider whether these actions might bias the study in an unfortunate manner.

Only when the condition cannot be ameliorated, the animal must be killed as the end of a procedure as a final endpoint.

> *An animal shall be killed when it is likely to remain in moderate or severe pain, suffering, distress or lasting harm (article 17.2)*

The humane killing will only be applied if there is no effect of the treatment or mitigating actions and the animal is exposed to unnecessary pain, suffering or distress caused by the wound.

There is much to gain by implementing good endpoints and action points. Especially if severe studies are planned (FIGUR 3)

Define and apply appropriate humane end-points - criteria to identify when the humane endpoint has been reached

Score sheets – endpoints and action points

A score sheet is a protocol for systematically recording of key clinical observations and other test-results of the research animals [4, 72]. These observation and test results show to which degree the animals physiologically or mental state deviate from the normal and are used to determine when the animal reach predefined endpoints that trigger certain actions in the study. It helps to identify when the condition for an animal exceeds what has been defined and approved (by the competent authority) as the humane endpoint in the light of a harm-benefit analysis (the harm to the animals must be balanced against potential benefits)

> *The project evaluation shall be performed with a degree of detail appropriate for the type of project and shall verify that the project meets the following criteria…*
>
> *a harm-benefit analysis of the project, to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome (article 28) [1]*
>
> *"…the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns.[8]*

It can also be used to document when the condition for an animal exceeds what has been defined and approved (by the competent authority) as the humane endpoint

Scores as categories on a scale from no impact to severe

When defining scores for clinical signs they are typically categorized in distinct categories from 0 – or no impact to higher numbers depending on how sever the situation is for the animal and the highest score for the most severe condition – often that cannot be relieved or scientifically justified.

The Facial Grimace Score (FGS) for pain [45, 47] for example a score 0 is no signs of pain using the FGS, a score 1 is reflecting moderately present and 2 an obviously present sign of pain.

I theory any of morphological, behavioural or physiological welfare indicators can be used to define endpoints and action-points in a score sheet. Some might be sensitive to subjective evaluation unless very strict categories are defined. It is advisable to consider they relevance for the particular study, how feasible it is to collect data during regular observation and to what degree can we define them in an objective manner so that we avoid observation bias when scoring the animals. Ambiguous score sheets including many observation points may look impressive but there is also a risk they are completely impractical in use.

Binary endpoints

An alternative to score signs in several severity categories - is to use binary scoring. Observation-points are not scored and categorised into several degrees

of severity – but they are scores as either present OR not present. For example, you observe:

- Indication of pain (+/-) OR no indication of pain (1/0)
- Normal behaviour (+/-) OR Abnormal behaviour (too low, too high) (1/0)
- Normal food intake (+/-) OR change in food intake (1/0)

The convention is that – or 0 indicate the normal situation while +or 1 indicate that the animals is outside the normal range.

Binary scoring represents a simplification that may reduce risk of misinterpretation or subjective evaluation (which can be the case when too many poorly defined score categories are used). It is therefore less sensitive to observer bias. The principal investigator must, define how many 1/+ that defines actions to follow up.

Score-sheets should not be a static "copy-and-paste" document from study to study – but should be constantly developed and updated with further experience [4]

How humane is your endpoint?

Humane endpoint is a professional concept that is defined both in regulations as well as in literature and guidelines for animal research [1, 5, 8, 72]. The most widely use, but also most narrow definition as by the directive [1] and OECD guidelines [73] promote humane endpoints as an alternative to death as endpoint [5]. However humane end points was redefined by Hendriksen [72] also been defined as a refinement strategy designed to minimise pain, suffering and distress in animal studies.

The term "humane" reflects an altruistic position marked with kindness, care, compassion or sympathy for others (74, 68, 69) [74][74][68][69] – especially humans but also animals. For animals we also use the term humane in connection with humane killing – which in veterinary practice means mercy killing performed by specially trained personnel generally to prevent suffering from incurable or painful conditions [75].

Humane endpoints can similarly be recognized with studies designed with end- and action points assuring kindness, care, compassion or sympathy for the animals avoid unnecessary pain, suffering, distress or lasting harm. The word "unnecessary" is a word causing cognitive dissonance when we talk about humane end-points – especially among lay persons. Pain, suffering or distress might be approved necessary to reach certain scientific aims – when they are justified by potential benefits.

Accepting pain, suffering or distress - even when justified by potential benefits - is contradictory to beliefs, ideas or values of common peoples understanding of «humane» actions - that should be characterize with compassion, sympathy, or consideration to animals' wellbeing.

If the question is of brining in the humanity cause more confusion and contribute to blurring the message of what actually happens in animal research, it might be suggested to only talk about endpoints – and leaving out the "humanity".

The directives definition applies for avoiding death as an endpoint and replace it by earlier and humane endpoints [1]. It does not explicitly apply for the moribund state. However, the

> *moribund animal is one that is close to death and may be comatose or unresponsive to stimuli, exhibit dyspnea or other severe breathing problems, hypothermia, prostration, etc. However, before the animal gets to the point of being moribund, detailed observations of the animal can help to set an earlier endpoint and thereby reduce the actual cost to the animal, in terms of pain and/or distress.* CCAC[12]

It is therefore questioned if endpoints – like moribund that involve a severe status for the animal can be regarded as humane.

The Guide for the care and use of laboratory animals link the experimental and humane endpoints.

> *The experimental endpoint of a study occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or*

relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental end- points that result in unrelieved or severe animal pain and distress, including death. For many invasive experiments, the experimental and humane endpoints are closely linked.

Key check question

- Will our endpoint be perceived as humane by the common public?
- Will the aim of the study be perceived as a justification for the endpoints we have defined?
- Is there room for further regiment to reduce harm to animals in this study and till reaching our scientific goals?

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