Defining the Moribund Condition as an Experimental Endpoint for Animal Research

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Introduction

The obligation to alleviate unnecessary pain and distress is an important correlate of the responsible use of animals in biomedical research. Many strategies have been used to meet this challenge, including administering analgesics and anesthetics when appropriate, reducing the numbers of animals tested, and avoiding the use of death as an experimental endpoint. However, many types of research protocols are associated with high mortality rates or require the production of progressive and severe disease states that clearly could cause the deaths of experimental animals. These types of protocols generally specify conditions under which preemptive euthanasia will be performed and may state that animals will be euthanized when they become "moribund." However, persons with different biomedical backgrounds may have varying concepts of the term's implications, rendering it poorly defined and arbitrarily interpreted. Dictionary definitions of moribund include words and phrases such as "dying," "at the point of death," "in the state of dying," or "approaching death." However, these definitions are severely limited for laboratory animal research because they do not describe the moribund state in behavioral or physiologic terms. Developing a sound approach to identifying the moribund state is crucial to its effective use as an experimental endpoint.

The moribund condition typically implies a severely debilitated state that precedes imminent death. The following discussion describes a general data-based approach for predicting imminent death and defining specific moribund conditions in objective terms that are relevant to specific experimental models (Toth 1997). This process depends on the investigator's ability to identify objective data-based criteria that forecast the death of an experimental animal. Several measurable conditions appear to have good predictive value in specific experimental models. An objective data-based approach to predicting death in the context of specific experiments could provide an unambiguous signal that the experimental endpoint has been reached, thereby facilitating the implementation of timely euthanasia and reducing unnecessary pain and distress.

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[1]Abbreviation used in this article: EEG, electroencephalogram.

Pain and Distress in the Moribund State

The moribund state is preferred to death as an experimental endpoint because of the assumption that euthanizing a moribund animal will avoid or reduce terminal distress. However, the image triggered by the term *moribund* is often one of a prostrate, unresponsive, and perhaps seemingly comatose animal. From that perspective, one might seriously question whether such severely debilitated animals continue to experience pain or distress. If moribund animals are physiologically debilitated beyond the capacity for cognitive awareness of aversive sensations, euthanasia to avoid "spontaneous" death would not significantly reduce terminal distress.

A review of the literature on humans can provide insights into the relationship between behavioral responsiveness and awareness or consciousness. Clearly, in humans, as in animals, the absence of behavioral responses to painful stimuli does not prove lack of consciousness (Ashwal et al. 1994b; McQuillen 1991). A state of unresponsiveness may be similar under some circumstances to the condition of neuromuscular blockade, in which persons cannot generate motor responses but nonetheless maintain cognitive and sensory awareness. In human medicine, this condition is referred to as a "locked-in" state (Ashwal et al. 1994a; Giacino 1997).

A surprisingly large proportion of behaviorally unresponsive persons experience some type of awareness, ranging from clear sensory perception to "out-of-body" or paranormal experiences (Lawrence 1995; Schnaper 1975; Tosch 1988). Coma and persistent vegetative states in human patients are generally associated with severe brain damage, brain hypometabolism to a level consistent with that of general anesthesia, and an apparent unconsciousness as assessed by neurologic examination (Ashwal et al. 1994a). These findings suggest that such patients do not experience pain or suffering, although their eyes may be open, they may appear to be awake, and they may occasionally demonstrate apparent semipurposeful behavior mediated by residual islands of cortical function (Ashwal et al. 1994a; Plum et al. 1998; Schiff 1999). However, states of consciousness obviously have gradations. Patients may experience states of minimal awareness or minimal unconsciousness, particularly if brain function is adversely influenced by metabolic derangements or infectious agents whose effects could fluctuate or resolve (Ashwal et al. 1994b; Bernat 1992). Inferences about awareness of pain are less reliable under such conditions. In fact, many patients in vegetative states eventually experience the return of some degree of awareness (Beresford 1997; Childs and

Mercer 1996). Accurate assessment of consciousness and awareness is a significant clinical issue in human medicine (Andrews et al. 1996; Giacino 1997).

The difficulty inherent in assessing the cognitive state of unresponsive individuals is apparent from the literature on humans. Similarly, in animal research, an unresponsive apparently moribund animal cannot necessarily be considered unaware, and one cannot necessarily assume that an unresponsive animal can no longer experience pain. The possibility that even unresponsive subjects might experience pain or distress reinforces the need for a clear definition of moribundity and for a method of predicting death so that the potential for suffering can be minimized by timely euthanasia.

Data-based Definition of the Moribund State

The ability to predict death with high probability and accuracy could have several advantages to the research process. Animals would clearly benefit because unnecessary terminal distress could be eliminated or significantly reduced. Moreover, the research effort itself might benefit directly because experimental goals could be met more consistently. If one could accurately predict the time of death, euthanasia could be scheduled to permit timely collection of samples that would be lost if the animal died unexpectedly. For example, a recent study of rats with leptomeningeal tumors used hindlimb paralysis, rather than death, as an endpoint, thereby allowing the collection of tissues necessary for histopathologic documentation of the extent of the tumor and the possible response to therapy (Janczewski et al. 1998). In addition, imminent death might modify important physiologic variables, rendering data collected under those conditions unusually variable or even uninterpretable within the context of the study. For example, microbially infected mice that are near death develop both marked hypothermia (Soothill et al. 1992; Toth et al. 1995; Wong et al. 1997) and abnormal EEG findings (Gourmelon et al. 1986, 1991; Toth et al. 1995) that could render them physiologically unsuitable for some studies.

Thus, preemptive euthanasia could have several advantages for research: Data collected after severe physiologic derangements develop may not be useful or may be misleading for some purposes, and tissues that might otherwise be lost can be collected for postmortem analysis. If the research team recognizes the advantages of timely euthanasia when developing study endpoints, compliance with established endpoint criteria will undoubtedly be easier to achieve. Finally, a clear definition of the moribund state could also improve the ability of veterinarians and animal care personnel to promote animal well-being and the efficient collection of high-quality data.

The moribund state can be defined by identifying the values of various variables that precede imminent death and can serve as signals for preemptive euthanasia. To be most useful for routine application, these values should be derived from and tailored to specific experimental models and should be among the values evaluated as part of the normal process of data acquisition. Research data are generally subjected to close scrutiny by the research team, which increases the likelihood that predictive changes in values of key variables may be recognized. Furthermore, this strategy facilitates the routine collection of information relevant to moribundity as part of the experiment, without requiring extra work. Monitoring many diverse clinical signs may be labor intensive, and researchers using a generalized evaluation system may be required to evaluate variables that might appear arbitrary or even unrelated to the severity of the animal's condition. Conscientious compliance with that approach may be difficult to achieve. However, researchers' compliance may be increased if the measurement of the variables is related to research goals. Furthermore, the consideration of numerous variables that may be irrelevant can obscure the impact of pertinent factors, as occurred in a system for evaluating pain (Beynen et al. 1988). To be most useful, specific variables should be identified and weighted in terms of their predictive value. It is important to know which factors matter most in particular situations.

Comparing data collected from animals that die with data from animals that survive may reveal experimental variables that change before imminent death and could be valid predictors of death or moribundity. Information relating the frequency at which critical observations or measurements should be made, the time when specific conditions change, and the time of death is also useful. This type of evaluation can often be conducted during initial or pilot experiments, but long-term familiarity with the model may be necessary before useful but subtle indicators of imminent death are recognized. The identification and statistical validation of variables that correlate with and predict imminent death establishes indices that can be applied in subsequent studies using the same model. The validation data should be sound enough to convince the research team either that appearance of the predictor indicates imminent death or, alternatively, that data collected after the appearance of the predictor would be invalid. Because subjective evaluations require constant vigilance to avoid bias, objective or quantifiable variables may be preferable for routine use in standardized protocols.

Objective definition of the moribund state requires the differentiation of dying from illness, pain, and distress. An assessment of clinical and behavioral signs can certainly indicate that euthanasia is warranted for humane reasons, but many clinical signs, despite their severity, may not predict imminent or even eventual death. For example, seizures may indicate illness that is severe enough to warrant euthanasia for humane reasons, but obviously in many situations animals can live for a long time after having a seizure, and indeed they may never have another seizure. Scoring systems based on the severity of multiple behavioral or physiologic abnormalities (Morton and Griffiths 1985; Olfert 1996) can provide useful benchmarks for animal evaluation, and they can be used to support veterinary determinations that euthanasia is warranted for humane reasons. However, even severe illness or distress may not forecast imminent death.

Several other considerations influence the selection of an indicator for preemptive euthanasia. First, an indicator that is appropriate for one model may not predict imminent death in another. The variables discussed below as useful for predicting death are examples that undoubtedly are not applicable in all situations. Administering certain drugs, for example, could induce hypothermia and an unresponsive state from which the animal will eventually recover. In this situation, these conditions obviously do not indicate a moribund state because death is not the ultimate outcome. Second, the experimental endpoint can be modified as more is learned about the model. For example, a group studying mice inoculated with a myeloma cell line observed that the animals developed hind-leg paralysis, indicative of metastatic compression of the spinal cord, approximately 2 to 7 days before death (Huang et al. 1993). In a subsequent study, the experimental endpoint was redefined, and hind leg paralysis was used as an indicator for preemptive euthanasia (Huang et al. 1995). As experience with and data collected from a specific model accrue, more information will be available for developing endpoint refinements.

The choice of euthanasia indicators and the implementation of euthanasia must be compatible with the experimental design and maintain the scientific integrity of the experiment because the advancement of knowledge and the value of the research will depend on maintaining the animal long enough to collect crucial data. Furthermore, some studies may require prolonged clinical maintenance of animals before euthanasia, despite the occurrence of signs that forecast eventual death, or may have valid justification for using spontaneous death as the experimental endpoint. Finally, despite conscientious efforts by the research team, objective data-based criteria that predict imminent death and provide a signal for preemptive euthanasia yet allow the completion of experimental objectives may be difficult or impossible to identify in the context of some experimental paradigms. The establishment of requirements to identify criteria for and to implement preemptive euthanasia rests with institutional animal care and use committees, based on the assessment of specific protocols.

Human Outcome Research: Perspectives for Animal Experimentation

Subjective predictions of death may be prone to bias (Dawes et al. 1989; Forster and Lynn 1988; Heyse-Moore and Johnson-Bell 1987; Knaus et al. 1991; Parkes 1972; Seneff and Knaus 1990). For example, in one study, predictions of patients' life expectancy by experienced physicians and nurses were overwhelmingly optimistic rather than random (Parkes 1972). Such bias could be due to a variety of factors. Because patients are likely to receive supportive care and medication to relieve symptoms, those who are near death may appear less seriously ill than they actually are. Alternatively, they may remain in a relatively stable condition until death is imminent, or they may die unexpectedly. Another possible reason for the preponderance of optimistic predictions is the potential psychologic difficulty associated with stating definitively that a person will die very soon. Research personnel may be similarly influenced by a pragmatic view that if death cannot be predicted with relative certainty, an experiment may as well continue. Statistically based systems of outcome prediction are often more reliable and more accurate than is human subjective judgment (Dawes et al. 1989; Knaus et al. 1991; Seneff and Knaus 1990).

Objective approaches to predicting clinical outcomes in human medicine are frequently used to assess the effectiveness of medical or surgical treatments, evaluate the quality of hospital services, and develop changes in public health policy (Allard et al. 1995; Barriere and Lowry 1995; den Daas, 1995; Knaus et al. 1991; Le Gall et al. 1995; Lemeshow et al. 1987; Seneff and Knaus 1990). Studies of outcome prediction offer some insights into the potential problems and benefits associated with using similar approaches in animal research. In general, two basic strategies have been applied in human studies. The first involves the use of disease severity scores, which are typically based on the deviation from normal of various physiologic measurements, such as serum glucose concentrations and urine output. In some systems, these variables are weighted and integrated with patient factors such as age and other preexisting disease to arrive at a score that can then be correlated with outcome and that may be predictive or prognostic. Classification systems developed for the assessment of disease severity and the prediction of patients' clinical outcomes include the acute physiology and chronic health evaluation ("APACHE") (Knaus et al. 1985), simplified acute physiology score ("SAPS") (Le Gall et al. 1993), mortality probability model ("MPM") (Lemeshow et al. 1985), and sickness impact profile ("SIP") (Bergner et al. 1976).

As opposed to using broad-based scoring systems, numerous studies have attempted to relate patients' specific symptoms to survival or death. For example, several studies report that the incidence of dyspnea and other breathing problems increases before death (Coyle et al. 1990; Higginson and McCarthy 1989; Reuben and Mor 1986; Reuben et al. 1988; Ventafridda et al. 1990). A correlational study of nearly 1600 cancer patients showed that indicators of poor nutritive status (e.g., difficulty swallowing, recent weight loss, anorexia, and dry mouth) were also predictive of death, but pain (even when severe), gastrointestinal signs, and signs of central nervous system involvement were not (Reuben et al. 1988). Nutritional status as estimated by the change in mid-arm circumference was a predictive indicator of mortality in a series of hospitalized geriatric patients (Incalzi et al. 1998). That variable exemplifies several advantageous features, including ease of measurement, reproducibility, availability for all patients, marginal alteration due to hydrational status or edema, and incorporation of both muscle and fat mass (Incalzi et al. 1998).

Problems or signs associated with poor clinical outcomes are often assessed in patients with specific medical conditions. For example, for patients in ischemic coma, a poor prognosis (defined to include either survival in a vegetative

state or death) is associated with the bilateral absence of somatosensory evoked potentials during the first week of coma (Zandbergen et al. 1998) and with high serum levels of a glial calcium-binding protein (Martens et al. 1998). Documented seizures or loss of consciousness is associated with a poor prognosis for children with shigellosis (Khan et al. 1999), as is the need for ventilation or hemodynamic support for patients who have received bone marrow transplants (Jackson et al. 1998). Similarly, the development of hypothermia in septic patients is significantly correlated with death due to septic shock (Clemmer et al. 1992).

The prognostic significance of clinicopathologic variables has also been evaluated in humans. For example, in some groups of patients with malaria, factors significantly correlated with death include impaired consciousness and respiratory distress, creatinemia, bilirubinemia, hyperlactemia, hypoglycemia, and proportion of pigment-containing neutrophils (Krishna et al. 1994; Marsh et al. 1995; Phu et al. 1995; Waller et al. 1996). Levels of inflammatory cytokines are correlated with mortality in patients with burns (Marano et al. 1990), sepsis (Barriere and Lowry 1995; Debets et al. 1989; Girardin et al. 1988), adult respiratory distress syndrome (Meduri et al. 1995), and malaria (Krishna et al. 1994).

As strategies for predicting patient outcomes, broad-based evaluations and analysis of specific variables each have strengths and weaknesses. Broad-based scoring systems can be useful for statistical assessment of population tendencies, but they may be less accurate in predicting clinical outcomes for individual patients (Barriere and Lowry 1995; Dellinger 1988; Deyo and Inui 1984; Jackson et al. 1998; Jones 1998). For example, scores on the Karnovsky index, which evaluates overall performance capabilities of patients (Yates et al. 1980), generally correlate very well with the length of survival of populations of patients (Evans and McCarthy 1985; Mor et al. 1984; Reuben et al. 1988; Yates et al. 1980), but when the index is applied to individual patients, scores associated with a specific survival interval can span the entire scale (Evans and McCarthy 1985). Such broad-based scoring systems may accurately predict mortality rates if scores reflect very poor health, but overestimate or underestimate mortality rates for patients with more moderate disease (Jackson et al. 1998; Prytherch et al. 1998). Several factors probably contribute to the inaccuracy of broad-based scales in assessing the imminence or likelihood of death for individual humans or animals. First are issues of sensitivity. These scales may detect large changes in patients' conditions (e.g., the progression from inpatient to outpatient status), but some clinically significant changes in health status that are obvious to both clinician and patient may only modestly affect the overall score (e.g., symptom progression in ambulatory patients with chronic disease) (Barriere and Lowry 1995; Deyo and Inui 1984; Fitzpatrick et al. 1992). Related to the issue of sensitivity is the so-called "floor phenomenon," which refers to the inability of health status measures to detect clinically important changes in patients whose baseline health status is poor (Baker et al. 1997; Bindman et al. 1990). Another factor that can influence the predictive accuracy of broad-based scoring systems is the possibility that considering irrelevant variables can reduce the impact of pertinent factors.

In contrast to broad-based evaluation systems, analysis of specific factors may provide greater accuracy in specific situations. However, the weakness of the targeted approach is the difficulty in selecting the optimal variables from many possible options. Thus, the initial consideration of numerous variables that are relevant to the clinical condition is likely to be informative (Goldhill and Withington 1996), and in animal studies a broad-based system may be useful during the initial development of a model. When used to define moribundity, this type of analysis can help to identify those variables with the strongest prognostic implications, so that the assessment can ultimately be tailored to specific condition or situation. In general, accurate prediction of outcomes requires at the very least a standardized approach to data collection (Goldhill and Withington 1996).

Other factors also complicate the evaluation of the moribund state in human populations. The precise time of disease onset may be unknown, and various clinical therapies are likely to have been applied. Even the diagnosis or the cause of the medical condition may be uncertain. Another complicating factor is "lead-time bias," or the inaccuracy introduced into risk prediction if treatment is initiated before physiologic measurements relevant to scoring have been completed (Tunnell et al. 1998). This situation arises, for example, if patients receive supportive medical treatment (e.g., the administration of intravenous fluids) in an ambulance, but physiologic data used for outcome assessment (e.g., hematology values) are not collected until the patient arrives in the emergency room or intensive care unit. Moreover, in humans, preexisting or secondary medical conditions are common, and their importance may vary depending on the patient population (Jackson et al. 1998; Jones 1998; Tunnell et al. 1998). Demographic factors such as age, history, life style, and genetic background also vary widely in patient populations. Finally, terminally ill patients generally receive intensive supportive care that may delay, modify, or blunt the clinical signs predictive of dying. Such issues complicate assessment of the moribund state in human patients.

Predicting Death in Experimental Animals

The evaluation of the moribund state may be somewhat easier in animal populations than in humans. In experimental models, the precise nature, time, and magnitude of challenges and subsequent interventions are known and are generally standardized. Furthermore, animal populations may be very homogeneous in terms of age, history, environment, and genetic background. Thus, accurate prediction of imminent death may be more feasible in experimental animal models than in human populations.

Hypothermia is perhaps the most commonly reported predictor of experimental animals' imminent death (Gordon et al. 1990; Kort et al. 1997; Soothill et al. 1992; Stiles et al.

1999; Wong et al. 1997). Its use as an endpoint requires determination of a specific index temperature that is invariably associated with imminent death. Preemptive euthanasia is then performed if an animal's temperature drops below the predetermined value. In one study, for example, mice with acute experimental bacterial infections developed rectal temperatures of less than 34°C before the onset of clinically overt illness that eventually warranted euthanasia (Soothill et al. 1992). In studies of influenza-infected mice, rectal temperatures of less than 32°C were inevitably associated with death in one study (Wong et al. 1997), whereas another found that mice recovered after even more profound hypothermia and used a core temperature of 28°C as the indication for euthanasia (Toth et al. 1995). A toxicity study found a linear relationship between the 50% lethal dose and the dose of metallic salts that reduced body temperature to 35°C (Gordon et al. 1990). Premorbid variability in temperature, uninterrupted wheel-running for 3 hr, and hypothermia below 30°C were sequential temporal markers of moribundity and death in rats studied in an activity-stress model (Morrow et al. 1997).

Several considerations are relevant to the use of temperature as a sign of imminent death: (1) Body temperature can be significantly influenced by ambient temperature and by other aspects of the environment, such as the type of bedding or the presence of cage mates. This consideration is particularly important for mice. Thus, index values developed under one experimental situation may not be applicable in all situations. (2) Different strains or species of animals may react differently to the same challenge. For example, BALB/c mice are more sensitive than C57BL/6 mice to influenza challenge, demonstrating higher mortality rates (Toth et al. 1995) and perhaps a different critical index temperature. In contrast, staphylococcal enterotoxin A elicits more severe hypothermia and greater rates of mortality in C57BL/6 mice than in BALB/c mice (Stiles et al. 1999). (3) The method of temperature measurement may influence the interpretation of specific values. Intraperitoneal transmitters that provide a continuous record of core temperatures can be used to evaluate the duration of hypothermia (Toth et al. 1995), but data collection with this type of system requires expensive equipment and surgical manipulation of the animal. Implanted microchips (Kort et al. 1997; Stiles et al. 1999) and rectal measurements using hand-held thermometers or probes (Gordon et al. 1990; Soothill et al. 1992; Wong et al. 1997) are less expensive and less invasive, but they provide only snapshot evaluations of temperature. In some cases, prolonged hypothermia may reflect imminent death, whereas transient or short-term hypothermia may resolve and be associated with eventual recovery. Furthermore, rectal measurement of temperature requires significant animal manipulation and may be associated with stress.

Another simple but specific endpoint marker that is visually obvious, objective, and easy to assess is the inability to rise or ambulate. This condition was a good predictor of imminent death in guinea pigs with *Pseudomonas*-induced sepsis (Louie et al. 1997) and in mice with endotoxemia (Krarup et al. 1999). Moreover, mice with severe or palpable hypothermia are also likely to be recumbent and unresponsive to handling or other stimuli (Krarup et al. 1999). Although a moribund state is commonly interpreted to mean a prostrate, unresponsive condition, substitution of the specific physical definition of "unable to walk" illustrates how the use of precise terminology can limit ambiguity in the decision-making process for euthanasia.

A study of the survival of rats with central nervous system tumors provides another example of defining morbidity predictors according to the experimental model (Redgate et al. 1991). When evaluating animals after tumor inoculation, these researchers noticed that all animals underwent three phases of weight change: an initial period of weight loss associated with irradiation and tumor implantation, a period of weight gain when the animals appeared to be in a clinically stable condition, and a terminal period of weight loss that preceded death. Total survival time varied primarily because of the length of the first two phases, but statistical analysis showed that imminent death could be accurately predicted for animals that lost weight for 7 to 8 consecutive days during the terminal period. This criterion could be used in future studies to permit timely euthanasia of animals without biasing experimental outcomes. Interestingly, related variables (e.g., percentage of weight loss or reduction in food intake) were not predictive of imminent death in this model, indicating that careful analysis of the data may be needed to find the best variable for accurate prediction of moribundity.

Other experimental variables that may be useful for predicting moribundity can be developed based on specific experimental models. For example, experiments that monitor EEG patterns have shown a terminal flattening of EEG tracings in rabbits with infectious diseases (Toth et al. 1993), rats experiencing chronic sleep deprivation (Rechtschaffen et al. 1983), and mice that die after infectious challenge (Gourmelon et al. 1986, 1991) or spontaneously from age-related conditions (Welsh et al. 1986). Studies of rats and mice have shown that hind-limb paralysis, rather than death, can sometimes be used as the endpoint in tumor models (Huang et al. 1993, 1995; Janczewski et al. 1998).

Biochemical variables can also provide useful prognostic markers in animals. For example, plasma lactate concentrations were a good predictor of clinical outcome in dogs with gastric dilatation-volvulus (de Papp et al. 1999). However, in some research situations, timely biochemical measurements may be difficult to obtain because serum or other samples collected periodically over the course of a study may not be analyzed until a minimum number of samples have been accrued, or until the experiment has ended. Nonetheless, experience with the experimental model can sometimes provide clues about an animal's condition even before precise biochemical or physiological analyses are completed. For example, marked hypertriglyceridemia and distinct sleep patterns develop in rabbits that succumb to bacterial infections compared with those that survive (Toth et al. 1993). Although plasma triglyceride assays and quantitative evaluation of sleep may not be performed until days or weeks after

the end of the experiment, the severe hypertriglyceridemia causes a marked milky opacity of the plasma that is visually obvious after centrifugation. Similarly, qualitative changes in the EEG are visible on polygraph tracings even before the EEG amplitudes are accurately assessed. Thus, experience with the physiologic responses of terminally ill animals can teach the investigative team to recognize signs of imminent death so that timely euthanasia can be performed.

Criteria that are used experimentally to define moribundity and that have been validated as predictive markers for imminent death should be reported (Clarke 1997). Unfortunately, these types of observations are often published only in methods sections of research articles, and therefore many researchers and veterinarians may not encounter or be aware of such refinements. Furthermore, many researchers may not have a practical appreciation of humane, as opposed to scientific, refinement of an experimental model. Providing simple but practical examples and encouraging dissemination of information about refinements are important facets of training of research personnel to use experimental animals humanely.

Summary

Our obligation to alleviate the unnecessary pain and distress of experimental animals mandates the implementation of timely euthanasia. Subjective clinical judgments are essential for the evaluation of the animal's well-being and support the veterinary prerogative of euthanasia for humane reasons. However, subjective evaluations may be biased when used to predict imminent death. Objective data-based approaches to predicting imminent death developed for specific experimental models could facilitate the implementation of timely euthanasia before the onset of clinically overt signs of moribundity and could thereby reduce pain and distress experienced by experimental animals.

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