

Retrospective evaluation of clinical signs, pathology and related discomfort in chronic studies

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Summary

Animals that are kept to old age develop age-dependent health problems. Chronic toxicity studies or carcinogenicity studies carried out according to international guidelines are performed to detect any effects on health and tumour incidence of a test substance dosed chronically at different levels. It is required that a substantial number of the animals survive until the pre-defined end of the study, at which time all the remaining animals are killed for pathological evaluation. Typically, the incidence of health problems increases towards the end of the study, as the animals become older. Animals may die, or they may be killed prematurely for humane reasons and also to prevent loss of pathological information due to autolysis. The retrospective study presented here was designed to evaluate animal health and welfare and was based on an existing recent chronic bioassay. The clinical signs, body weights and development of masses were monitored throughout this bioassay and all animals were subjected to postmortem pathological evaluation. Discomfort was not assessed during the study but afterwards, based on clinical and pathological observations. All 507 rats (257 males and 250 females) were considered as one population, irrespective of their experimental treatment. The majority of the animals survived the 2 years of the study, but 149 died or were killed prematurely, mostly during the second year. Discomfort was considered minor when no health problems or only minor ones were observed (240 animals); the discomfort of 113 animals was evaluated as moderate; and a total of 154 animals suffered serious discomfort prior to death, of which 128 were in this state for more than one day, and 85 of these were for more than one week. The latter categories are of special interest for defining humane endpoints. Thus, the data in these categories were re-evaluated to identify any specific clinical signs that might qualify as reliable criteria in deciding humane endpoints. Some categories of clinical signs are typically related to serious health problems, such as anaemia or impaired locomotion. However, in a substantial number of animals with serious discomfort, only non-specific clinical observations were observed, e.g. a poor general appearance. It is concluded that criteria for humane endpoints should include not only specific clinical signs, but non-specific signs as well.

The results of toxicological investigations performed in accordance with international test guidelines should be as relevant, complete and reliable as possible, to ensure their acceptability in risk evaluation procedures in as many countries as possible. The application of criteria for humane endpoints should

be similarly harmonized and based on a critical analysis of animal welfare in relation to the aims and design of the study so as to minimize the discomfort of the animals. This study aimed to contribute insights into animal welfare in chronic toxicity and carcinogenicity studies and to evaluate putative

criteria for humane endpoints. Animals that are kept to an old age develop age-dependent health problems which have been well documented for many strains of laboratory rodents. Carcinogenicity studies made according to international guidelines (OECD 1981) are performed to detect any effects on health and tumour incidence of a test substance dosed chronically at different levels that are not considered toxic (based on effects observed after repeated dosage in studies of shorter duration). It is required that a substantial number of animals survive until the predefined end of the study, at which time all the remaining animals are killed for pathological evaluation. The incidence of health problems increases towards the end of the study, as the animals become older. Animals may die, or be killed prematurely for humane reasons and also to prevent loss of information due to autolysis. The present retrospective study was designed to evaluate animal health and welfare and was based on an existing set of animal data from a recent chronic study. An effort was made to identify any typical clinical signs that might be instrumental in defining humane endpoints.

Animals, materials and methods

Albino Wistar rats (CrI:WI(WU)BR) were purpose bred by Charles River Wiga GmbH at Sulzfeld, Germany and allowed to acclimatize after transfer to the test facility in The Netherlands. They were assigned to test groups in a randomized way and housed in the same small single-sex social group throughout the study. The test compound was added to the ground cereal-based diet (Rutten & de Groot 1992). The clinical signs, the body weight and the development of masses were monitored throughout the study and all animals were subjected to post-mortem pathological evaluation. Clinical signs were observed and registered on a daily basis by the animal care staff, advised by a veterinarian. No attempt was made to interpret the clinical signs to reach a clinical diagnosis on the nature or cause of any abnormality. The pathological evaluation included gross pathology, and the histopathology of a number of pre-defined organs

and tissues and any others with gross lesions. Discomfort was not assessed during the study but afterwards, based on clinical and pathological observations. A critical anthropomorphic approach was used to assess the discomfort caused by pain, metabolic disturbances and other sources of distress. Literature on the assessment and grading of discomfort in laboratory animals was consulted (Morton & Griffiths 1985, Montgomery 1987, Kuijpers 1988, Workman *et al.* 1988, Sanford *et al.* 1989, Morton 1990, Wallace *et al.* 1990, Kuijpers & Walvoort 1990, 1991, Griffiths 1991, Walvoort 1991, Booy *et al.* 1993).

Discomfort was classified according to the grading system used for the central registration of animal experiments up to 1997 in The Netherlands, as minor, moderate or serious discomfort. For serious discomfort, the duration was also specified (one day, one week, one month, or over one month). In the instructions on the use of this grading system, some typical examples were provided for reference, e.g. the recovery from general anaesthesia was classified as moderate discomfort, whereas any procedures causing lasting pain were classified as serious discomfort. Clinical and pathological signs recorded during the study, and body weight were classified into categories to enable a statistical analysis. A maximum of three clinical and three pathological signs for each animal were registered in the database, in a hierarchical manner according to their putative significance for animal welfare. The database was analysed statistically using standard software (MS Access), by the application of queries and selections. Thus, frequency tables of combined observations were generated and then transformed into graphics.

Results

All 507 rats (257 males and 250 females) were considered as one population, since no compound-related pathology was detected. The majority of animals survived the 2 years of the study, but 149 died or were killed prematurely, mostly during the second year (Fig 1). Discomfort was considered to be minor when no, or only minor, health

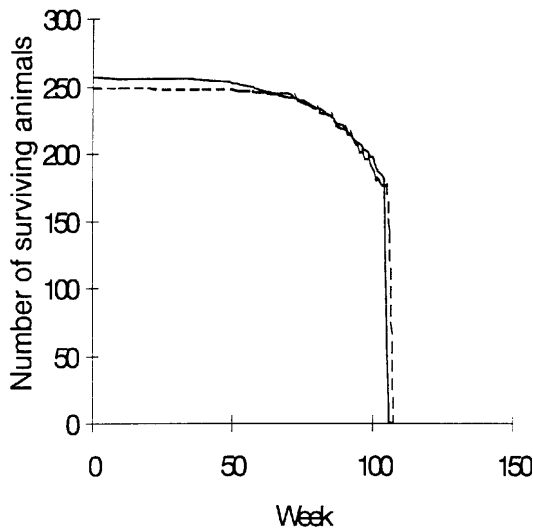


Fig 1 The number of surviving animals (males and females, respectively) during the course of the study

problems were observed clinically or pathologically (240 animals). The discomfort of 113 animals was evaluated as moderate, whereas a total of 154 animals suffered serious discomfort prior to death. The relationship between the discomfort and the endpoint (time and cause of death) is shown in Fig 2. The majority of animals with minor or moderate discomfort were killed at the scheduled end of the study. Only a minority

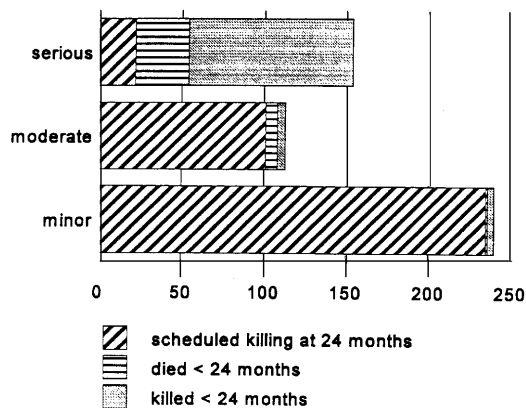


Fig 2 The relationship between discomfort and the endpoint for each animal. The discomfort is categorized for all animals in the study (minor, moderate or serious) and the absolute number of animals in each category is represented. The bars are subdivided to show the time and cause of death

of animals with serious discomfort survived until the end of the study; the others died or were killed humanely before that time.

The 154 animals with serious discomfort are of special interest for the task of defining humane endpoints. These were analysed in more detail to determine the time and cause of death (Fig 3). A total of 128 had experienced serious discomfort for more than one day and 85 of these for more than one week. To evaluate the decisions on humane killing (e.g. 'in time', 'too late'), three criteria were applied. As the quality of the pathological evaluation depends on the fresh state of the tissues, if this had been affected by autolysis, the time of killing was classified as 'too late'. Secondly, the retrospective assessment of the discomfort was applied, using the arbitrary criterion that more than one day of serious discomfort is to be avoided. Thirdly, the plausibility of a proper assessment of the condition of the animal based on the clinical signs was taken into account. In the absence of any distinct clinical signs, a proper assessment was considered to be quite impossible, and these animals were classified as killed 'in time'.

The pathological observations were classified according to the organ (system) involved and the tumours were sub-classified accord-

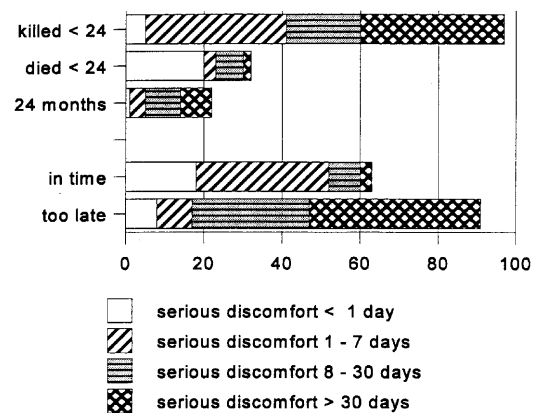


Fig 3 Animals with serious discomfort (absolute numbers). The endpoint (time of death, either at the end of the study, at 24 months, or prior to that time, and cause of death, either died or killed) is illustrated in the upper three bars. The evaluation of the time of humane killing (either in time or too late) is indicated in the lower two bars

ingly. Only pathological findings with an assumed impact on the health (physiology) or welfare of the animal were included, to a maximum of three per animal. For example, small benign tumours were not listed, as opposed to tumours that compressed other tissues or had malignant biological characteristics. The incidence of relevant pathological findings related to the classification of the discomfort is shown in Table 1. Some pathological findings are typically associated with serious discomfort (nephrosis, circulatory and respiratory disease) but most are found in animals of all discomfort categories. It is remarkable that a fair number of animals that were thought to have had moderate or even serious discomfort, based on the clinical findings, had no relevant pathological abnormalities.

The data on animals with serious discomfort were re-evaluated to identify any specific clinical signs that would qualify as a reliable criterion for defining a humane endpoint. The most objective clinical sign that is routinely measured in toxicological studies is the development of body weight. Body weight was assessed monthly during the later part of the study, as required by the OECD (Guideline 451). The monthly intervals were

analysed for each animal and the period with the most typical or extreme deviation of body weight was used for the classification. The classes were derived from the raw data to optimize the statistical evaluation. A normal age-dependent development of the body weight for the sex was taken as the standard reference point, and either excessive increase, lack of increase or decrease were considered deviations. The relationship between the discomfort grade and the development of body weight is shown in Fig 4. It can be seen that both the absolute and the relative numbers of animals with a substantial decrease in body weight were found in the group of animals categorized as showing serious discomfort. Moreover, an excessive increase in body weight was also associated with serious discomfort. Animals with a normal growth rate, a stable body weight or a slight decrease in body weight were found in the category of minor discomfort, and also in the other two categories.

The other clinical signs were related to the level of discomfort as shown in Table 2. Some categories of clinical signs are typically related to serious health problems, such as paleness (anaemia) or impaired locomotion. However, in a substantial number of animals

Table 1 The relationship between pathological findings and the discomfort as assessed by clinical and pathological

Pathological findings	Discomfort		
	Minor	Moderate	Serious
Nephrosis	8	17	40
Circulation		2	12
Respiratory		2	14
Mouth, teeth	1		1
Pituitary tumour	60	32	56
Thyroid tumour	40	21	15
Adrenal tumour	29	22	15
Ovarian/uterine tumours	33	16	20
Testicular tumour	5	6	6
Tumours of other endocrine organs		1	4
Mammary tumour	37	25	13
Skin tumour	7	8	14
Other tumours	18	17	23
Other pathology	1	5	19
No pathological signs	85	14	11

Only pathological findings that might have affected animal health and welfare in a significant way were included, at a maximum of three per animal

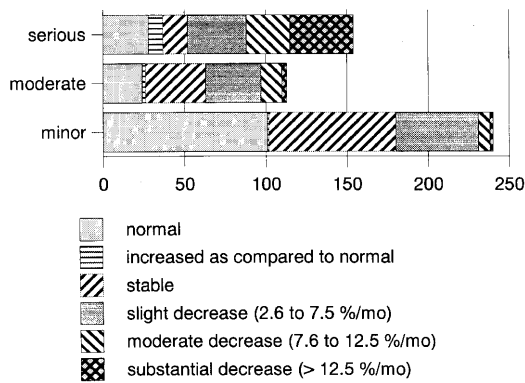


Fig 4 The relationship between changes in body weight and discomfort. The discomfort is categorized for all animals in the study (minor, moderate or serious) and the absolute number of animals in each category is represented. The bars are subdivided to show the changes in body weight

Table 2 The relationship between clinical signs (a maximum of three per animal) and the discomfort as assessed by clinical pathological evaluation

Clinical signs	Discomfort		
	Minor	Moderate	Serious
General		2	72
Coat	25	16	34
Pale		1	18
Mouth	42	20	25
Eyes	57	43	86
Masses	55	35	39
Locomotion		1	19
Rest	2	6	38
None	150	1	9

with serious discomfort, only non-specific clinical observations were made, such as a poor general appearance.

Discussion

The results of toxicological investigations performed in accordance with international test guidelines should be as relevant, complete and reliable as possible, to ensure their acceptability for risk evaluation procedures internationally. The application of criteria for humane endpoints, to minimize the discomfort of the animals, should also be harmonized, based on a critical analysis of animal welfare in relation to the aims and

design of the study. The present study aimed to contribute insights into animal welfare in long-term toxicity studies with rodents and to evaluate putative criteria for humane endpoints. In this study, the survival rate at the end of the study met the requirement of 50% with a margin to spare, but this is not always going to be clear in an ongoing study.

Essential to the retrospective approach was the availability of pathological information on each animal. We considered whether some of the pathological findings could be directly related to specific clinical signs, but no fixed relationship between a typical clinical sign and a specific internal pathological disorder could be found. If this had been possible then it might have contributed to a reliable prognosis for the course of an animal's disease. A diagnostic as opposed to a descriptive approach was not considered essential to make an informed decision on a humane endpoint for an animal. Non-specific clinical signs were often associated with significant pathological disorders and so could play a major role in the assessment of discomfort and any subsequent decision-making. We conclude that criteria for humane endpoints should rely not only on specific clinical signs, but should include non-specific (general) signs as well.

It is remarkable that many animals with significant pathological disorders did not show explicit clinical signs. This could be related to the natural defence mechanisms that are functional in social groups so as to deceive predators. In addition, the animals were rather old and may not have shown clear behavioural changes because their basic behavioural pattern was rather passive. Alterations of behaviour in aging rats have been documented (DiLoreto *et al.* 1998, Miyagawa *et al.* 1998). We conclude, however, that any clinical observation is significant in the evaluation of health and welfare.

The single most objective but non-specific clinical sign is body weight. It was found that both an abnormal increase and a substantial decrease in body weight were associated with serious discomfort. This association was perhaps not as strong as some would have expected. However, changes in body weight

are very helpful in identifying animals that have significant health disturbances and can also be used to see how an animal's condition progresses. A reduction in body weight reflects a catabolic state, whereas an abnormal increase is associated with the development of masses or with fluid retention. To use the measure of body weight to its potential, it should be assessed weekly rather than monthly and the weight should be compared to the previous weight and also to the highest weight known for that animal (Kuijpers *et al.* 1991).

The application of humane endpoints requires tailored study management. Most of the health problems occur towards the end of the study and are first observed by the animal care technicians. In case there is need for an informed decision to be made about an animal, the investigator responsible for the study, and the veterinarian responsible for the laboratory animals, should be involved. There should be a common awareness and agreement on the sort of clinical observations that are important for decision-making.

The degree of discomfort acceptable for these types of studies is partly an ethical matter and partly a practical one. In this case, serious discomfort for one day was adopted as an arbitrary limit for the humane endpoint. Clearly, if this limit had been set lower, the objective of the study would not have been reached, but serious discomfort for more than one week may have been ethically unacceptable. The choice of any period between one day and one week could be debated, and this choice should also allow for a well informed and balanced decision to be made by the team responsible for the execution of the study and the welfare of the animals.

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