WORKING PARTY FOR THE PREPARATION OF THE FOURTH MULTILATERAL CONSULTATION OF PARTIES TO THE EUROPEAN CONVENTION FOR THE PROTECTION OF VERTEBRATE ANIMALS USED FOR EXPERIMENTAL AND OTHER SCIENTIFIC PURPOSES (ETS 123)

Meeting of the Drafting Group
Paris, 22 – 23 May 2002

Species-specific provisions for Rodents and Rabbits

Background information
for the proposals presented by the Group of Experts on Rodents and Rabbits

PART B

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Future principles for housing and care of laboratory rodents and rabbits

Report for the revision of the Council of Europe Convention ETS 123 Appendix A for rodents and rabbits

PART B

Issued by the Council’s Group of Experts on Rodents and Rabbits

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Preamble

In 1997, the Council of Europe (CoE) established four expert groups with the aim of advising the CoE Working Party whether, how and to what extent Appendix A of the Convention ETS 123 (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 1986) needed revision.

The report of the Council’s group of experts on Rodents and Rabbits comes in two parts: Part A describes the actions taken by the group and the proposals for amendments to Appendix A being presented to the CoE Working Party (GT 123 (2000) 8; GT 123 (2000) 57, 1st revision); Part B provides background information for these proposals, which are based upon scientific evidence as well as current good practice (quoted proposals for amendments of Part A are set in italics, while recommendations for future research are marked with an arrow). The group hopes that the explanatory Part B will be made available for future users of the revised Appendix A in some way.

The group proposes that the CoE Resolution on Training of Persons Working with Laboratory Animals (adopted May 30, 1997) and the European Commission DG XI Guidelines on Euthanasia (Close et al, 1996, 1997) be added to ETS 123 as separate appendices.

The proposals and their rationale are the outcome of extensive discussions within the group and are to be regarded as expert recommendations. The group is convinced that the proposed amendments are reasonable and pragmatic and will increase the welfare of animals used for research.

Since the group began its work in February 1998, proposals and drafts were frequently discussed by e-mail. Furthermore, the group met five times (London, 21.11.98; Copenhagen, 18./19.3.99; Utrecht, 10./11.6.99; Bicester, 2./3.11.99, London 25.10.00). During the meeting in Bicester the group made practical assessments of different stocking densities of mice, rats and hamsters. Several group members participated in the CoE Working Party Meetings in Strasbourg (27.-29.1.99, 9.-12.5.00). Axel Kornerup Hansen participated in meetings of the co-ordinators of the four groups in Paris (30.6.98, 17.11.98, 17.12.99) and presented the groups work at the 2nd Working Party in January 1999, and Markus Stauffacher participated in two co-ordination meetings in Strasbourg (27.11.99, 8.5.00) and presented the group's proposals at the 3rd Working Party in May 2000. The group has furthermore presented part of its work at the FELASA/ICLAS joint meeting in Palma de Mallorca (26.5-28.5.99), at the 3rd World Congress on Alternatives in Bologna (3.8.-2.9.99) as well as at conferences of SGV (29.11.99) and LASA (1.-3.12.99).

The group agreed that, where possible, it should make its recommendations on the basis of scientific evidence, but that where it was lacking or insufficient, the group should also use current good practice. The group started from the premise that basic laboratory housing should meet the behavioural and physical needs of the animals. It therefore considered factors such as appropriate enrichment, the need for social housing together with other important issues such as husbandry and practicability in order to make its recommendations on cage sizes and stocking densities. The group particularly took into account the varying needs of animals at different ages; for example, young animals tend to be more active and exploratory than older ones. The group also accepted that as more animals are housed in the same cage there is greater potential for the sharing of space allowances (the so called “omnibus effect”).

The group proposes to delete all figures, and to provide tables in their stead, introduced by a general section relating to rodents’ needs. The graphs for minimum cage dimensions (figs. 1-7 Appendix A, 1986) and for maximum stocking densities (figs. 8-12, Appendix A, 1986) are
based on simple correlations between weight and space, which neglect the different needs of animals of the same species depending on strain, age, sex, reproductive status, etc. Such correlations are not justified by current knowledge. A range of factors affecting the welfare of experimental animals cannot be reduced to purely mandatory regulations and minimum requirements of space dimensions and stocking densities.

The expert group also agreed that the layout of the old appendix A needed to be changed, to make it more informative and to meet species specific needs. For example, at present the minimum space requirements for the mouse, rat, Syrian hamster, Guinea pig and rabbit are provided in one table. It was the group’s view that such an approach could not meet the needs resulting from the very different biological characteristics of these species.

Hence the group has suggested that the tables and figures relating to Appendix A should be revised to provide separate specifications for each species together with a species-specific introduction similar to those in the UK Code of Practice for Laboratory Animal Breeders (1995). The General Introduction to Appendix A (1986) lays out broad principles relating to animal care, and the rodent and rabbit expert group has made a number of recommendations for changes to this section.

It should be emphasised that the dimensions and stocking densities proposed in the revised tables should be considered minimum requirements, and are based on our current state of knowledge. Limits have always to be set arbitrarily, and although they may be justified by science-based arguments, their exact values cannot be scientifically proved. Under most circumstances such values can be thought of as good practice, but may not necessarily be the best practice. Knowledge gained by further research may necessitate changes in the future.

As previously stated, the expert group has based its recommendations for space allowances in the document on the behavioural needs of the animals. Therefore, even if space recommendations are for some reason not implemented immediately in all research establishments, the group strongly recommends that group housing and the provision of a complex and enriched environment should both be given a very high priority.
I Recommendations for amendments to the General Part of Appendix A (ETS 123, 1986)

I.1. Introduction

To produce a scientific background for the proposals for amendments to the General Part of Appendix A (Council of Europe, 1986), the group has concentrated on published research on rodents and rabbits. Therefore, for background information the reader is referred to the species-specific sections (II Recommendations species-specific sections: IIa Rodents, IIb Rabbits).

I.1.1 Breeding animals

The current Appendix A (Council of Europe, 1986) does not emphasise the different requirements of breeding animals or the different constraints of breeding versus user establishments. There are very obvious differences between the needs of animals in breeding colonies and those kept for experiments, which must be taken into account to ensure the welfare of both types of animals.

The group has, therefore, made proposals for amendments to Appendix A (Council of Europe, 1986) with the aim of meeting the needs of animals in both experimental and breeding facilities, and proposes that the general introduction to Appendix A should refer to the special needs of breeding animals as follows:

“The purpose of this revised version of Appendix A is to establish minimum standards for the breeding, care and housing of laboratory animals in regulated facilities in Europe.

As breeding animals may be maintained for longer periods than animals used for scientific procedures, and have behavioural needs relating to their reproductive behaviour particular attention is required to ensure that the environment provides for the animals' behavioural as well as physiological needs. Provision for such needs includes providing suitable nesting materials. Young animals require an adequately complex social and physical environment during development to become normally behaving adults.”

I.2. The environment in the animal enclosure and its control

I.2.1 Lighting

Excessive light or exposure to continuous high level light may cause retinal damage, particularly in albino rats (O'Steen et al, 1972; Semple-Rowland and Dawson, 1987a; Weihe, 1976). Exposure to bright light should therefore be avoided, and darker areas for withdrawal from light should be provided e.g. into shelters, nestboxes, nesting material. On the other hand, adequate light must be available for the caretakers to inspect the animals and to perform tasks. A regular photoperiod should be maintained with minimal interruptions, e.g. no flashes of light during the dark period. Photoreceptors need a period of dark to enable them to regenerate (Clough, 1982), but extended periods of low light should be avoided because when the animals are later moved to a bright room retinal damage has been found to occur (Semple-Rowland et al, 1987).
The group concludes that 'Lighting' (paragraph 2.4, Appendix A, Council of Europe, 1986) should read:

“In windowless rooms, it is necessary to provide controlled lighting both to satisfy the biological requirements of the animals and to provide a satisfactory working environment. Exposure to bright light should be avoided and darker areas for withdrawal should be available within the enclosure. There must be adequate illumination for the performance of husbandry procedures and inspection of the animals. Regular photoperiods suitable to the species should be maintained and interruptions to these should be avoided. When keeping albino animals, one should take into account their sensitivity to light.”

I.2.2 Noise and alarm systems

Different species have different hearing ranges and sensitivities, which may include ultrasound (Clough, 1982). Rodents in particular are very sensitive to ultrasound (Olivier et al, 1994). Noise can cause stress in animals (Armario et al, 1985; Geber et al, 1966; Nayfield and Besch, 1981) and loud noise may even cause hearing damage (Fletcher, 1976). Sound can also be an uncontrolled source of experimental variation. It is, therefore important to be aware of its sources and of how sound in the laboratory can vary (Milligan et al, 1993; Sales et al, 1999). Consideration should be given to potential sources of ultrasound e.g. from electronic devices, such as a computer screens, squeaky glass stoppers and running taps (Sales et al, 1988). Alarms should be designed to operate outside the sensitive hearing range of the animals being held (Clough and Fasham, 1975). A softly playing radio may help to mask startling or frightening noises, however there has been little research to show whether this is a benefit to the animals.

To conclude, sudden loud noises should be avoided, and ultrasound should be minimised. Alarm systems should sound outside the sensitive hearing range of the species kept in the facility, but be audible to man.

Therefore, the group recommends that 'Noise' (paragraph 2.5, Appendix A, 1986) should read:

“Noise including ultrasound can be an important disturbing factor in the animal quarters and may cause changes in behaviour, physiology and pathological effects. Noise in the hearing ranges of man and the species being held, especially that which is sudden or loud, should be minimised in procedure and holding rooms. Alarm systems should be designed to sound outside the sensitive hearing range of the animals.”

I.3. Health

The group proposes amendments to the paragraph relating to 'health' (paragraph 3.1, Appendix A, 1986). As there are no further specific recommendations for rodents and rabbits, detailed background information is given below.

Infections in laboratory rodents and rabbits can interfere with animal experiments and thereby reduce their validity. This may lead to the use of a higher number of animals or a reduction in their welfare. Microorganisms may interfere with the function of certain animal models; they may make it difficult to interpret the final results or may induce a dose-related abnormal response to a test factor leading to false conclusions in pharmacology or toxicology studies. They may also increase the variation within the group thereby leading to the use of a larger number of animals in that particular study (Van Zutphen et al, 1993).
There is a variation in pathogenicity within the range of microorganisms, which may be present in animals, which are not of ‘clean’ health status. Many experiments have been ruined by specific disease-causing infections, but even subclinical disease may disturb essential parameters, e.g.: body weight may be reduced (Turnbull, 1983), behaviour may be changed (Andersen and Hanson, 1975; Mohammed et al, 1992; Yirmiya et al, 1994), and the presence of some microorganisms may cause changes in the organs, resulting in difficulties in the interpretation of histological findings e.g. in toxicology studies (Hansen et al, 1992; 1994). Respiratory disease of any aetiology can be responsible for deaths during anaesthesia (Hansen, 1994). Microorganisms may suppress or stimulate the immune system, which is an essential part of many experiments and in the manifestation of clinical disease (Bixler and Booss, 1980, 1981; Garlinghouse and van Hoosier, 1978; Griffith et al, 1982, 1984; Guignard et al, 1989; Hamilton et al, 1979; Huld et al, 1973; Isakov et al, 1982; Korotzer et al, 1978; Laubach et al, 1978; Mahmoud et al, 1976; Mims, 1986; Nicklas et al, 1999; Pollack et al, 1979; Ruskin and Remington, 1968; Simberkoff et al, 1969; Specter et al, 1978; Swartzberg et al, 1975; Tattersall and Cotmore, 1986; Ventura, 1967). Some microorganisms have a specific effect on enzymatic, haematological, and other parameters, which might be monitored in the animal during an experiment (Brinton, 1982; Notkins, 1971). Such organic function disturbances may unknowingly alter experimental results (Osborn, 1986; Tiensiwakul and Husain, 1979; Vonderfecht et al, 1984) and may be irreversible for some test compounds, while reversible for others (Frisi and Ladefoged, 1979). Some infections cause high mortality in neonates thereby interfering with experiments, adversely affecting breeding programmes and reducing the welfare of these animals (Cassell et al, 1981; Cassell, 1982; Hill and Stalley, 1991; Juhr, 1990).

In studies of experimental infection, spontaneously infecting microorganisms may propagate instead of the experimental infection (Bia, 1980). Some infections reduce the severity of disease caused by other agents, thereby destroying models of infectious disease (Barthold and Brownstein, 1988). Infectious agents may induce tumours, enhance the effect of certain carcinogens, or reduce the incidence of cancer in laboratory animals (Ashley et al, 1976; Barthold, 1985; Barthold and Jonas, 1977; Fox et al, 1994a, 1994b, 1995; Kimbrough and Gaines, 1966; Nettesheim et al, 1974; Toolan, 1967, 1968; Toolan et al, 1982). Microorganisms present in the animal may contaminate samples and tissue specimens, such as cells, sera etc, and thereby interfere with in vitro experiments or impose a risk to the animals kept in facilities performing in vitro tests (Nicklas et al, 1993; 1999).

Hence, there is overwhelming evidence that specific infections interfere with research, and consequently lead to the use of increased numbers of animals or a reduction in their welfare. During experiments, animals can be protected against infection by standard hygienic procedures. Guidelines for health monitoring in breeding and experimental colonies of rodents have been published by the Federation of European Laboratory Animal Science Associations (Kraft et al, 1994; Rehbinder et al, 1996). The vast majority of institutions use rodents and rabbits from breeders who carry out health monitoring, documenting the absence of a number of those infections described above. However, a few smaller institutions still use in-house colonies of undefined health status. This is a risk to animal welfare and also to the research performed on the animals. The experimental use of such animals should be abolished in the future or, at least, restricted to those instances where it can be proven that there will not be effects on animal welfare, science and other animals or personnel in the facility.

Therefore, the group recommends that the paragraph relating to ‘health’ (paragraph 3.1, Appendix A, 1986) should read:

“3.1.1. Animals should only be introduced into an animal facility if they are not harbouring infections which may be hazardous to other animals in the facility or the staff, or which interfere with the procedure to be performed on the animals. Appropriate health monitoring at the site of
origin must be in place to ensure this. If such data are not available animals should be kept isolated from other animals at least until health monitoring data have been generated at the user facility.

3.1.2. The person in charge of the establishment should ensure regular inspection of the animals and supervision of the accommodation and care by a veterinarian or other competent person. If animals are housed in the establishment the person in charge should also ensure that regular health monitoring by sampling for laboratory procedures is performed. Animals should be inspected at least daily by a competent person.

3.1.3. According to the assessment of the potential hazard to the animals, appropriate attention should be paid to the health and hygiene of the staff.”

I.4. Housing and enrichment

I.4.1 Social housing and environmental complexity

The aim of environmental enrichment is to improve the quality of the captive environment so that the animal has a greater choice of activity and some control over its social and spatial environment (Newberry, 1995; Stauffacher, 1995, 1998). When animals are deprived of the possibility to perform species-specific behaviour they may show signs of suffering such as behavioural disorders, chronic stress or other pathological conditions (Jensen and Toates, 1993; Würbel et al, 1996). Housing conditions of laboratory animals should provide opportunities for the animals to perform their species-specific behavioural repertoire by providing enrichment in the social, nutritional, sensory, psychological and physical environment (Baumans, 1997).

The group concludes that there is abundant evidence to show the value of providing group housing for social species and physical enrichment to meet the animals’ species-specific needs. Moreover, in accordance to the CoE Resolution of May 30 1997 (Council of Europe, 1997), the need for environmental enrichment should be stated. Therefore, the group proposes that the ‘General Introduction’ of Appendix A (1986) should introduce social and spatial enrichment as follows:

“Gregarious species should be group housed in stable harmonious groups. When for experimental reasons or welfare implications, group housing is not possible, animals should be housed within sight, sound or smell of one another and enrichment of their physical environment should be provided to relieve boredom.

Removing or replacing adult group members threatens harmonious group life.

Environmental enrichment provides the animal with some control over the environment and meets the need for exploration.”

And that 'Caging' (paragraph 3.6, Appendix A, 1986) should be modified to state that:

“Environmental enrichment appropriate to the animal’s needs, e.g. for social interaction, activity related use of space and for provision of appropriate stimuli and materials, should be provided.”

I.4.2 Feeding

The group recommends making a number of minor proposals for amendments to paragraph 3.7 of Appendix A (1986), in order to bring the paragraph up-to-date, in accordance with current good practice.
“3.7.1 Diets should be palatable, non-contaminated and meet the nutritional and behavioural requirements of the animal. In the selection of raw materials, production, preparation and presentation of feed, precautions should be taken to minimise the chemical, physical and microbiological contamination to acceptable levels.

The feed should be packed in bags that provide clear information on the identity of the product and its date of production. An expiry date should be clearly defined by the manufacturer and adhered to.

Packing, transport and storage should also be such as to avoid contamination, deterioration and destruction. Store rooms should be cool, dark, dry and vermin and insect proof. Quickly perishable feed like greens, vegetables, fruit, meal, fish etc. should be stored in cold rooms, refrigerators or freezers.

All feed hoppers, troughs or other utensils used for feeding should be regularly cleaned and if necessary sterilised. If moist feed is used or if the feed is easily contaminated with water, urine, etc., daily cleaning is necessary.

3.7.2 Provision should be made for each animal to have access to the feed. In some circumstances, diet restriction may be appropriate to avoid obesity.

3.7.3 The opportunity for foraging should be given wherever possible. Hay and straw satisfy the need for roughage. “

I.4.3 Identification

It is often necessary to identify animals individually, either temporarily or permanently. It is advantageous for animals to be individually identified to ensure good experimental practice and monitoring of breeding performance, and to enable animals with eventual abnormalities to be excluded from breeding programmes.

Ideally, non-invasive methods should be used. If permanent identification is required, consideration must be given to the degree of discomfort to the animal during the marking procedure, to the training of staff and to the use of sedatives or local anaesthetics.

Therefore, the group proposes to add a new paragraph on identification to paragraph 3 (Appendix A, 1986):

“In some instances it is necessary for animals to be individually identified e.g. when being used for breeding purposes or scientific procedures, to enable accurate records to be kept. The method chosen must be reliable and cause the minimum discomfort to the animal both when applied and in the long-term. Staff should be trained in carrying out the technique and sedatives or local anaesthetics used if necessary. Non-invasive methods should be used if appropriate.”
II Recommendations species-specific sections: Rodents and Rabbits

II.1 Preamble

II.1.1. Process of determining recommendations by the expert group

Regulations such as Appendix A of the European Convention ETS 123 have to set limits. There may be good scientific arguments why the limits should be set in some places and not others. But the exact numeric values for minimum cage sizes and heights as well as for maximum stocking densities can never be scientifically evaluated and “proved”. Working out minimum requirements with respect to animal welfare and to supposed well-being of laboratory animals is a political question. Nevertheless, the decision-making process should be based first and foremost on sound arguments on the biology of species and strains in question. During discussion it should be carefully distinguished between biological facts, scientific evidence and practical experience on one side and ethical principles of animal protection and the assessment of economical and political reason on the other side.

In the species-specific figures and tables of Appendix A (1986), minimum space requirements and maximum stocking densities are plotted in a double logarithmic system in order to get an allometric function of recommended floor area to body weight. These models have been developed some 20 years ago (Merkenschlager and Wilk, 1979). They were laid down pragmatically and without scientific justification on the basis of existing standard cage dimensions developed in the early sixties (e.g. Macrolon cages: Spiegel and Gönnert, 1961; rectangular shape tested and confirmed by Weiss et al, 1982). As a lot of the expensive infrastructure of an animal facility directly depends on cage dimensions (e.g. racks, cleaning machines, experimental design), cage dimensions have not been changed greatly during the past 40 years.

The main problem with such calculation models is that they try to approach the problem of establishing minimum limits with scientific methods, although they are not based on experimental studies of the physiological and behavioural needs of the animals in question. The group has reasoned that the straight-line weight:space relationships as well as the minimum cage dimensions required in Appendix A (1986) do not reflect species-specific biological constraints; they seem to be the result of a compromise between standard cage sizes, practical experience and economic reasoning.

Hackbarth et al (1999) consider the allometric measure a good scale for the inter-specific comparison of recommended floor space, and for the discussion of species-specific needs for more or less space per animal. They neglect that the need for space depends on evolved behaviour traits which differs already within a species (Stauffacher, 1997b). Ikemoto and Panksepp (1992) have shown that play fighting behaviour in young rats rapidly increases after day 20, has its maximum from day 30 to day 50, and then decreases to a low intensity. During the time of intensive social play, the rats learn to settle competitive situations. Adult rats are comparatively inactive and aggressive encounters are rare. Thus, in relation to their body weight, young rats need much more space than adult ones; a principle which applies to all mammals.

Except for locomotory playing behaviour, most animals do not use space for its own sake; they use resources and structures within an area. Minimum recommendations for cage/pen sizes (floor area and height) depend on the minimum enrichment requirements which have to be incorporated into the cage/pen in a way that the animals can perform a wide range of different behaviours and can cope successfully with their spatial and social environment.
Therefore, making recommendations and proposals for amendments to Appendix A (1996), the following questions have to be answered:

(a) What are the minimum requirements for environmental stimuli and objects to safeguard health, growth and reproduction in a way that the animals’ capacity to adapt is not overtaxed (allowing for variation eg due to species/strain, sex/age/group composition, stock/experimental/breeding)?

(b) How can these stimuli and objects be incorporated into (restricted) space in a way that the animals can perform a wide range of different adaptive behaviours, and that all individuals of a group can cope successfully with their spatial and social environment?

(c) What is the space allowance required for a successful incorporation?

During the working process, the expert group has followed a stepwise approach:

(i) Science-based evidence where and why Appendix A (1986) should be amended in order to allow the animals to satisfy their physiological and ethological needs in a way that their capacity to adapt is not overtaxed.

(ii) Working out physiological and ethological needs based upon scientific papers and science-based experience, e.g. on choice experiments, social mechanisms and strategies, near-to-nature behaviour, genetic variability and individuality.

(iii) Working out spatial and social enrichment (stimuli and objects) which safeguards well-being based upon experimental results, e.g. scientific papers and good/best practice.

(iv) Working out minimum cage and/or pen sizes which allow proper spatial and social enrichment. To determine the minimum recommendations for cage sizes, the quantity and the quality of space has to be taken into consideration. The crucial point is the interaction between the space, the structure of the cage, the animals and the type and quantity of enrichment provided. These have been based upon experimental results, good/best practice and scientific papers as well as existing cage types (sizes) for rodents.

(v) Working out maximum stocking densities in relation to age, size and breeding which allow proper use of enrichment and successful social interactions for all individuals of a group based upon good practice and experimental results (scientific papers).

Although the behavioural repertoires of all rodent species (Brain, 1992) and of rabbits (Kraft, 1979; Stodart and Myers, 1964) have not basically changed during domestication and during the selection process of the many stocks and strains, there is a considerable inter-strain variation in both the frequency and the intensity of behaviour performances (e.g. Brain and Parmigiani, 1990, Kraft, 1979; Nevison et al, 1999). Moreover, there is also some variation within the same strain corresponding to sex, age and individual experience (Stauffacher, 1997a). On the other hand, the group is aware that minimum requirements should, for practical reasons, be made in a way that is valid for the entire spectrum of different strains, genotypes and conditions within a species. It is, however, impossible, especially with regard to recent development within the genetics of rodent breeding, to be able to predict which strains and genotypes would be in use in future. Thus, the proposed minimum requirements for a certain species may not be appropriate for every individual bred and housed in the future. For specific strains and genotypes some of the text paragraphs of the revised Appendix A may have to be interpreted in a way, that justifies a demand for more space or lower stocking densities than given in the tables section.
The current Appendix A (1986) does not emphasise the different requirements of breeding animals or the different constraints of breeding versus user establishments. There are very obvious differences between the needs of animals in breeding colonies and those kept for experiments, which must be taken into account to ensure the welfare of both types of animals. Pregnant and lactating animals have a need for particular cage structure and content, such as nesting material and an area for withdrawal. A cage designed for adults may not be suitable for young animals, for example young rabbits may not be able to cope with perforated or slatted floors suitable for adults (Coudert, 1982). In some rabbit breeding units, pre-weaning losses may be as high as more than 20% (Koehl, 1999), and this can be due to poor nest quality and the permanent exposure of the mother to the stimuli of the pups (e.g. Coureaud et al, 2000, Hamilton et al, 1997, Wullschleger, 1987). Some infections such as rotaviruses cause high mortality in neonates (Vonderfecht et al, 1984) but are less hazardous to adult animals. Furthermore, in breeding units weaned animals may be stocked in more harmonious groups if kept with their littersmates, which is not usually possible in experimental facilities.

II.1.2. Impact of spatial and social enrichment on experimental research

Environmental enrichment can influence the animal's behaviour, physiology and brain anatomy, and Hebb (1947) showed that rats from enriched environments were better able to solve problems in the 'Hebb-Williams maze'. Animals that have been kept in enriched captive environments have improved learning abilities, increased cortical thickness and weight, increased size, number and complexity of nerve synapses and a higher ratio of RNA to DNA (Renner and Hackett Renner, 1993; Shepherdson, 1998; Widman et al, 1992). Factors such as age, sex, genetics and individual variation influence exploration and animals' responses to novelty (Mench, 1998), as will housing conditions (CornwellJones et al, 1992; Jahkel et al, 2000; Prior and Sachser, 1995; Rilke et al, 1998). How differences in housing conditions will influence experimental results depends on the particular housing conditions and scope of the experiment.

Based on the definition of animal well-being as the ability of the animal to cope successfully with its environment (Broom, 1986), it can be proposed that animals from an enriched environment may be better able to cope with environmental variations and hence would be less reactive to stressful experimental situations. This would result in less variation between results and thereby reduce the numbers of animals used (Baumans, 1997; Stauffacher, 1997b). Furthermore, as animals from enriched housing conditions are expected to be physiologically and psychologically more stable, they may be considered as more refined animal models, ensuring better scientific results (Bayne, 1996; Benn, 1995; Dean, 1999; Rose, 1994; Spinelli and Markowitz, 1985; Van de Weerd, 1996). If housing conditions do not meet the demands of a particular species, one cannot expect reliable and reproducible results (Fortmeyer, 1982). Conversely, animals from an enriched environment may be thought to show more variability in their response to experimental procedures, leading to more variation in results and to an increase in the number of animals used (Eskola et al, 1999b; Gärtner, 1998).

Standardisation of environmental conditions (and other factors) serves to reduce individual differences within animal groups (intra-experiment variation) in order to facilitate detection of treatment effects, and to reduce differences between studies (inter-experiment variation) in order to maximise reproducibility of results across laboratories (Van Zutphen et al, 1993). Nevertheless, Crabbe et al (1999) have shown that despite conditions being rigorously equated among sites, seven inbred mouse strains and one null mutant tested simultaneously at three well recommended labs revealed large effects of site for nearly all variables examined. Increasing reproducibility of results through standardisation accentuates and obscures the problem of reporting artefacts that are idiosyncratic to particular circumstances (Würbel,
The effects of enrichment on variability depend on the parameters measured. The same studies revealed increased, decreased or unchanged variability for mice and rats housed in enriched cages *versus* standard cages (Eskola et al., 1999b; Gärtner, 1999; Mering et al., in press; Tsai and Hackbarth, 1999; Zimmermann, 1999). Van de Weerd et al. (1997a, 1997b) showed that nesting material alone did not influence the behaviour and physiology of mice to a great extent. However, mice provided with objects and nesting material habituated faster to open field tests and did not show effects on their circadian rhythm of behavioural patterns (Wainwright et al., 1994). In some pharmacological experiments mice and hamsters housed in enriched cages showed a more sensitive response to anxiolytic drugs (Baumans, 1997) and fever (Kuhnen, 1997). Group-housed rabbits did not show any immuno-suppression (Turner et al., 1997). In mice, strain differences have been found in their response to environmental enrichment (Van de Weerd, 1994). Thus, depending on the type of enrichment, type of experiment and genetic background, animals may respond to environmental enrichment differently. It should be noted that in some strains of inbred mice enrichment has led to increased aggression (Haemisch et al., 1994). Whereas the barren standard environment can prevent the ontogeny of normal competitive behaviour, enrichment objects might trigger aggressive behaviour typical for male mice (e.g. territoriality). An enriched environment has to allow the subordinates to perform adequate behavioural responses (submission or escape) in order to prevent chronic stress or injuries (Stauffacher, 1997b).

- Specific studies are needed to provide information on effects of specific enrichment programmes on the animal itself and on specific animal models and experimental results. Strain differences should also be taken into account (Haemisch and Gärtner, 1994; Nevison et al., 1999).

The group accepts that enrichment methods should be carefully chosen so that they are compatible with the type of study or use of the animals, and that standardisation of enrichment within a study can help minimise any variation or other interference with results. Care should also be taken to ensure that these would not cause any harm to the animals. Enrichment programmes should be focussed on high priority behaviour that is strongly motivated, such as foraging, nest building and social behaviour. Nevertheless, a potential impact of cage and pen enrichment on a specific type of experiment should not lead to negate the benefits and needs for enrichment at all. The European Convention focuses on laboratory animals in general, and on the entire life of an individual. For most laboratory animals, the time spent in the breeding facility and in stock exceeds the time spent in an experimental procedure by far. And, production and housing conditions are often more stressful than the experiment itself (Stauffacher, 1994, 1997a). Therefore, exceptions from housing standards for experimental reasons should be authorised by the national legislative system as for the whole the experimental protocol.
IIa  Recommendations species-specific sections
Rodents

IIa.1. Introduction

The group proposes introductions to the species mouse (*Mus musculus*), rat (*Rattus norvegicus*), gerbil (*Meriones sp.*), hamster (*Mesocricetus sp.*) and guinea pig (*Cavia porcellus*), covering the most important aspects of biology, behaviour and habitat use as well as of husbandry requirements. Background information is provided in section IIa.4, 'housing and enrichment'.

**Mouse**

The laboratory mouse is derived from the wild house mouse (*Mus musculus*) a largely nocturnal burrowing and climbing animal which builds nests for regulation of the microenvironment, shelter and reproduction. Mice are good climbers and make good use of grid cage roofs. Mice do not readily cross open spaces, preferring to remain close to walls or other structures. A wide range of social organizations have been observed depending on population density and intense territoriality may be seen in reproductively active males. Pregnant and lactating females may prove aggressive in nest defense. As mice, particularly albino strains, have poor eyesight they rely heavily on the sense of smell and create patterns of urine markings in their environment. Mice also have very acute hearing and are sensitive to ultrasound. There are considerable strain differences in the expression and intensity of behaviour. The cages and their enrichment should allow conspecifics to solve competitive situations adequately. Minimum enrichment should include nesting material.

**Rat**

Rats (*Rattus norvegicus*) should be housed in socially harmonious groups unless there are good veterinary or scientific reasons for not doing so. Disruption to social groups should be minimised. Rats are excellent climbers, avoid open spaces, and use urine to mark territory. Their senses of smell and hearing are highly developed, and rats are particularly sensitive to ultrasound. Daylight vision is poor, but dim-light vision is effective in some pigmented strains. Albino rats avoid areas with light levels > 25lux. Activity is higher during hours of darkness. Young animals are very exploratory and often engage in social play. The minimum enrichment should include refuges, such as nest boxes, pipes, nesting material.

**Gerbil**

The gerbil or Mongolian jird is largely nocturnal although in the laboratory they are active during daylight. In the wild, gerbils (*Meriones sp.*) build extensive tunnel systems, and in the laboratory often develop stereotypic digging behaviour unless provided with adequate facilities. For this reason gerbils need comparatively more space in order to allow them to build or use burrows of sufficient size. Gerbils should be housed in harmonious social groups. Although gerbils are relatively docile, mixing of adults can result in serious aggression. Gerbils require a thick layer of litter for digging and nesting and/or a burrow substitute, which may need to be up to 20 cm long. Nesting material (hay, straw, etc.) and wood sticks for chewing and gnawing may be considered for enrichment.

**Hamster**

The female hamster is larger and more aggressive than the male and can inflict serious injury on her mate. The wild ancestors (*Mesocricetus sp.*) were largely solitary. Group housing is possible but special care should be taken in forming socially harmonious groups and aggressive
animals should be separated. Hamsters often make a latrine area within the cage, mark areas with secretions from a flank gland, and females frequently selectively reduce the size of their own litter by cannibalism. Minimum enrichment should include nesting material, climbing rack, refuge area (e.g. tube, hut), roughage and gnawing objects. Careful control of environmental features and prevention of disruption during routine husbandry practices are of particular importance in this species.

Guinea Pig

Guinea pigs are cursorial rodents which do not burrow, but which in the wild may live in burrows made by other animals. Adult males may be aggressive to each other, but generally aggression is rare. Guinea pigs tend to freeze at unexpected sounds and may stampede as a group in response to sudden unexpected movements. Guinea pigs are extremely sensitive to being moved and may freeze as a result for 30 minutes or more. Guinea pigs should be housed in socially harmonious groups unless there are good scientific or veterinary reasons not to do so. Faulty mesh floors can lead to serious injuries so mesh must be closely inspected and maintained to ensure that there are no loose or sharp projections. When grid or perforated floors are used, a solid resting area must be provided. Hay is an important enrichment item, and copious provision can be used to provide a resting area on grid floors. Plastic or perforated floors are preferable to grid floors. Refuges such as tubes or shelters should be provided within the cage or pen to allow the animal to climb onto or hide under them. Hay or similar material should be provided as a substrate and for environmental enrichment unless there are good scientific or veterinary reasons for not doing so. Sterilized woodsticks for chewing and gnawing may be considered for enrichment.

IIa.2. The environment in the animal enclosure and its control

IIa.2.1 Ventilation

No specific recommendations for rodents; see General Part of Appendix A, GT 123 (2000) 54.

IIa.2.2 Temperature

The group proposes no changes to the information given in Table 1 of Appendix A (1986), but to add the following text to the rodent section of the revised Appendix A:

“Local temperatures among groups of rodents in solid floored cages will often be higher than room temperatures. Even with adequate ventilation, the cage temperatures may be 3-6 °C above room temperature. Nesting material and nestboxes give animals the opportunity to control their own microclimate. Special attention should be paid to the temperature in individually ventilated cages as well as to hairless animals.”

The text is meant to help the animal user to follow the recommendations of the appendix. It should also be pointed out that maintaining a stable room temperature with minimal fluctuation to which animals can acclimatise, is probably more important in terms of minimising stress to the animals.
IIa.2.3 Humidity

The group has consulted textbooks, existing recommendations and some of the few scientific papers in the field. The US Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) recommends a relative humidity of 30 to 70%. It is, however, the opinion of the group that this range is too wide to be applicable for all rodents.

The main problem experienced in relation to a low humidity is a disease called ringtail, i.e. a condition in which the animal develop necroses in its tail and occasionally also in its toes. This disease is not uncommon in rats, while it is rather rare in mice. General experience as well as recommendations of textbooks of laboratory animal science (Fox et al, 1984; Krinke, 2000; Laber-Laird et al, 1996; van Zutphen et al, 1993) state that this disease is unlikely to develop as far as the relative humidity is kept above 50%.

In Mongolian gerbils kept at too high a humidity the fur is most likely to become matted, which eventually may develop into dermatitis starting in the nasal region and at least induce an increased grooming behaviour (Fox et al, 1984; Hansen, 1990; Schwentker, 1968). This condition is unlikely to occur as far as the relative humidity is kept below 50% (Laber-Laird et al, 1996).

Too high a relative humidity favours the production of ammonia in rodent cages (Clough, 1982).

Both high humidity, i.e. around 70% and low humidity, i.e. around 40%, increases pre-weaning mortality in mice (Clough, 1988).

The group, therefore, proposes as follows:

*The relative humidity in rodent and rabbit facilities should be kept between 45% and 65%. Excepted from this principle are Mongolian gerbils, which should be kept at a relative humidity between 35% and 50%.*

IIa.2.4 Lighting

Excessive light or exposure to continuous high level light may cause retinal damage, particularly in albino rats (O'Steen et al, 1972; Semple-Rowland and Dawson, 1987a; Weihe, 1976). Rats seem to prefer a cage with a low light intensity to one with higher light intensity (Blom, 1993), and albino rats have been shown to prefer areas with a light intensity of less than 25 lux (Schlingmann, 1993b). Light intensity has an effect on the mouse oestrus cycle (Clough, 1982), and biorhythms such as circadian rhythm and reproductive cycles are affected and regulated by the light:dark cycle (Clough, 1982, Weihe, 1976). Furthermore, the behavioural activities of rodents (e.g. Harri et al, 1999) and rabbits (e.g. Jilge, 1991) follow a circadian rhythm with most activity at dawn and dusk. Light exposure during the dark period may disturb this regulation (Ellis and Follett, 1983). In many animal rooms light intensity is usually too high (Schlingmann, 1993a, 1993b). Moreover, there may be marked variation in the levels of light inside cages in different positions on a conventional rack (Schlingmann et al, 1993b). Exposure to bright light should therefore be avoided, and darker areas for withdrawal from light should be provided e.g. into shelters, nestboxes, nesting material. On the other hand, adequate light must be available for the caretakers to inspect the animals and to perform tasks. A regular photoperiod should be maintained with minimal interruptions, e.g. no flashes of light during the dark period. Photoreceptors need a period of dark to enable them to regenerate (Clough, 1982). Extended periods of low light should be avoided because when
the animals are later moved to a bright room retinal damage has been found to occur (Semple-

- It is necessary to study the effects of maintaining rodents under dim light conditions with
  periods of increased light intensity, e.g. while staff are working in the room.
- The effects of different cage materials (i.e. fully or partially ‘tinted’ polycarbonate walls),
  should be studied.

The group proposes that the rodents' general considerations section should contain the fol-
lowing paragraph:

"Light levels within the cage should be low. Animals should have the opportunity to withdraw to
shaded areas within the cage. All racks should have shaded tops to prevent retinal degenera-
tion, which is a particular risk for albino animals. Red light, which is undetectable by rodents,
can be a useful management technique."

IIa.2.5 Noise

Different species have different hearing ranges and sensitivities, which may include ultra-
sound (Clough, 1982). Rodents in particular are very sensitive to ultrasound (Olivier et al, 1994). Noise can cause stress in animals (Armario et al, 1985; Geber et al, 1966; Nayfield and
Besch, 1981) and loud noise may even cause hearing damage (Fletcher, 1976). Sudden, loud
noise can cause audiogenic seizures in rodents (Iturrian, 1973). Sound may have many ad-
verse effects on physiology (Clough, 1982) and ultrasound may affect prenatal development
in the mouse (Shoji et al, 1975). Sound can be an uncontrolled source of experimental
variation. It is, therefore important to be aware of its sources and of how sound in the
laboratory can vary (Milligan et al, 1993; Sales et al, 1999). Consideration should be given to
potential sources of ultrasound e.g. from electronic devices, such as a computer screens,
squeaky glass stoppers and running taps (Sales et al, 1988). Alarms should be designed to
operate outside the sensitive hearing range of the animals being held (Clough and Fasham,
1975). A softly playing radio may help to mask startling or frightening noises, however there
has been little research to show whether this is a benefit to the animals.

To conclude, sudden loud noises should be avoided, and ultrasound should be minimised.

- Further research is needed to study the effects of background music on animals and the
effects of vibration e.g. from engineering plants and from forced ventilation in individu-
ally ventilated cages.

The group proposes that the rodents' general considerations section should contain the fol-
lowing paragraph:

“As rodents are very sensitive to ultrasound, and use it for communication, it is important that
this extraneous noise is minimised. Ultrasonic noise can be produced by many common labo-
atory fittings, including dripping taps, trolley wheels and computer monitors and can cause
abnormal behaviour and breeding cycles. Steps should therefore be taken to monitor the acous-
tic environment over a broad range of frequencies and over extended time periods.”

IIa.2.6 Alarm systems

Alarm systems should sound outside the sensitive hearing range of the species kept in the fa-
cility, but be audible to man.

No specific recommendations for rodents; see General Part of Appendix A, GT 123 (2000)
54.
IIa.3. Health

See proposals of the group for amendments to the General Part of Appendix A (1986), Section I, Chapter 3.

IIa.4. Housing and enrichment

IIa.4.1 Social Housing

The group agrees with the CoE Resolution of May 30 1997 (Council of Europe, 1997) that it is preferable to group-house rodents. For gregarious species, such as mice, rats, gerbils and guinea pigs, housing together with conspecifics, either in groups or in pairs, should be the norm. The group composition should be stable and harmonious (Baer, 1998; Claassen, 1994; Hurst et al, 1997a; Sachser, 1994; Stauffacher, 1997a), and visual barriers or hiding places may be necessary to minimise aggression (Baer, 1998; Van de Weerd and Baumans, 1995; Van de Weerd et al, 1997a; 1997b).

Individual housing has frequently been shown to be stressful for mice. Detrimental effects of individual housing include both, behavioural and physiological abnormalities usually referred to as 'isolation stress' or 'isolation syndrome' (e.g. Baer, 1971; Brain, 1975; Haseman 1994). There is evidence that subordinate male mice prefer company to being housed individually, even if that companion is dominant (Van Loo and Baumans, 1998).

In general, rats are very tolerant to conspecifics. Whereas group-housing of male mice may be difficult, depending on strain, previous experience, and cage enrichment, housing of single sex groups of male and female rats does not pose problems. Detrimental effects of individual housing of rats have been reported, amongst others, by Ader and Friedman (1964), Gärtner (1968), Hatch et al (1965), Hokon et al (1991), Hurst et al., 1997b; Kaliste-Korhonen et al (1995), Perez et al (1997), Sharp and La Regina (1998), and Zimmermann (1999).

Hamsters are considered to be largely solitary in their natural habitat, but they do show a preference for social housing, although this may be linked with fighting and enlarged adrenals (Arnold and Gillaspy, 1994). Group-housed hamsters also have a higher growth rate, increased food consumption and increased fat deposition (Borer et al, 1988).

When for experimental or welfare reasons group housing is not possible, rodents should be housed within sight, sound or smell of each other and extra attention should be provided to enrich their environment to relieve boredom.

The group proposes that the rodents' general considerations section should contain the following paragraph:

"Gregarious species should be group housed as long as the groups are stable and harmonious. Such groups can be achieved, albeit with difficulty, when housing male mice. As hamsters are not a gregarious species, they may be housed individually if aggression is likely to occur in group or pair housed animals. Disruption of established groups should be minimised, as this can be very stressful. "
IIa.4.2 Environmental complexity

IIa.4.2.1 Activity-related use of space

Except for locomotory playing, animals do not use space *per se*; they use resources and structures within an area for specific behaviours. Most rodent species attempt to divide their living space into separate areas for feeding, resting and excretion. Structures within the cage may facilitate these divisions such as nestboxes, nesting material, tubes, empty bottles and platforms and allow the animals to control light levels. Boxes may serve as both hiding places and vantage points (Baumans, 1997, 1999; Blom, 1993; Manser, 1998; Schlingmann, 1993a, 1993b; Sherwin, 1997; Stauffacher, 1997b, Townsend, 1997; Ward, 1991).

IIa.4.2.2 Appropriate stimuli and materials for environmental enrichment

Stimulation of exploratory behaviour and attentiveness helps meet the need for information-gathering by the animal and may reduce boredom (Wemelsfelder, 1997). Animals become stressed when an environment is unpredictable and/or uncontrollable (Manser, 1992). Providing a shelter or refuge gives the rodents the opportunity to withdraw beneath it to avoid frightening stimuli or to climb on to use it as a look-out point (Baumans, 1997; Chmiel and Noonan, 1996; Orok-Edem and Key, 1994; Scharmann, 1991; Van de Weerd and Baumans, 1995).

Appropriate structuring of the environment e.g. with climbing accessories, shelters, exercise devices or nesting material may be more beneficial than simply providing a larger floor area (Baumans, 1997). However a minimum floor area is needed to provide such a structured space (Stauffacher, 1997b).

Animals tend to be highly motivated to make use of enrichment based on food items. Food material can be scattered in the bedding giving the animal the opportunity to forage, as in nature a large part of the time-budget is spent on this activity. Animals will preferentially search for food even when it is readily available as this gives information about the location and quality of potential foraging sites (Mench, 1998). Additional food items such as hay or straw can satisfy the need for roughage and for chewing in guinea pigs (Baumans, 1997). Rats gnaw on aspen blocks (Eskola et al, 1999a), especially when housed without bedding (Kaliste-Korhonen et al, 1995). Hamsters (Niethammer, 1988) and gerbils (1999) routinely store food and should be provided with food pellets inside the cage.

Contact with humans, such as handling, training and socialising, will usually benefit both the animals and the outcome of experiments as it engages the animal on a cognitive level and allows positive interaction with animal caretakers, technicians and scientists (Baumans, 1997; Shepherdson, 1998; Van de Weerd and Baumans, 1995).

- Although a number of studies have investigated different methods of enrichment it is necessary to perform more research on the effects of environmental enrichment on different strains of animals, in particular its effect on aggression in different mouse strains. Future scientific work is likely to involve many genetically-modified strains of rodents, and it is highly likely that a single approach to enrichment will not be suitable for all.
IIa.4.2.3 Proposal

For all these reasons, the group proposes that the rodents’ general considerations section should contain the following paragraphs:

"Both bedding as well as nesting material and other refuges are important resources for rodents in stock or under procedure and should be provided unless there are overwhelming scientific or veterinary reasons against doing so. Nesting materials should allow the rodents to manipulate the material and construct a nest. Nest boxes should be provided if insufficient nesting material is provided for the animals to build a complete, covered nest. Bedding materials should absorb urine; they may be used by the rodents to lay down urine marks. Nesting material is important for rats, mice, hamsters and gerbils as it enables them to create appropriate microenvironments for resting and breeding. Nest boxes or other refuges are important for guinea pigs and rats. Hay is important for guinea pigs.

Many rodent species attempt to divide up their own cages into areas for feeding, resting, urination and food storage. These divisions may be based on odour marks rather than physical division but partial barriers may be beneficial to allow the animals to initiate or avoid contacts with other group members. To increase environmental complexity the addition of some form of cage enrichment is strongly recommended. Tubes, boxes, etc., are examples of devices, which have been used successfully for rodents, and these can have the added benefit of increasing utilisable floor area."

IIa.4.3 Enclosures - dimensions and flooring

IIa.4.3.1 State of knowledge

Literature searches (e.g. Medline, Biosis, Current Contents, Embase) have shown that little research has been performed on the influence of cage sizes on the behaviour and well-being of laboratory rodents, especially in recent years. On the other hand it is doubtful whether minimum space requirements should and can be worked out on a purely scientific basis; every limit is set empirically and minimum requirements are always the result of compromises between the different parties involved. Therefore, the group considers it essential that compromises be based upon biological reasoning as well as good practice.

IIa.4.3.2 Existing recommendations for minimum cage sizes and welfare consequences

Actual recommendations for minimum cage sizes for rodents in stock and during procedure are given in Table 1, and for breeding rodents in Table 2.

Table 1 Minimum space requirements for rodents in stock, and during procedure

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<tr>
<td></td>
<td>floor area</td>
<td>height</td>
</tr>
<tr>
<td>Mouse</td>
<td>180 cm²</td>
<td>12 cm</td>
</tr>
<tr>
<td>Rat</td>
<td>350 cm²</td>
<td>14 cm</td>
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<tr>
<td>Gerbil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hamster</td>
<td>180 cm²</td>
<td>12 cm</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>600 cm²</td>
<td>18 cm</td>
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Table 2 Minimum space requirements for breeding rodents (mother and litter)

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<tr>
<td>floor area</td>
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<td>Mouse</td>
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<td>Rat</td>
<td>800 cm²</td>
</tr>
<tr>
<td>Hamster</td>
<td>650 cm²</td>
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<tr>
<td>Guinea pig</td>
<td>1200 cm²</td>
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<tr>
<td>in harem</td>
<td>1000 cm²</td>
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The European Convention ETS 123 (Council of Europe, 1986) claims, that "any animal used or intended for use shall be provided with accommodation, an environment, at least a minimum degree of freedom of movement, food, water and care, appropriate to its health and well-being. Any restriction on the extent to which an animal can satisfy its physiological and ethological needs shall be limited as far as practicable..." (article 5). And the aim of the British Code of Practice (1989) "is to maintain animals in good health and physical condition; behaving in a manner normal for the species and strain with a reasonably full expression of their behaviour repertoire...". Both the European Convention and the British Code of Practice split their recommendations for accommodation of laboratory animals into qualitative recommendations on what the animals' environment should look like, and mandatory tables and graphs for minimum space allowance and maximum stocking densities. There is a considerable discrepancy between the qualitative recommendations of Appendix A (Council of Europe, 1986) and the minimum space allowances and maximum stocking densities which do not allow fulfillment article 5 of the European Convention ETS 123 (1986).

Behaviour is always the expression of a causal network between genetics, actual physiological status, ontogeny and factors of the actual spatial and social environment (Stauffacher, 1997a). If the space available does not allow proper provision of key stimuli and features, the performance of adaptive behaviour may be impaired. This may lead to the development of behavioural disorders and chronic stress (e.g. Brain et al, 1991; Würbel and Stauffacher, 1996). Although restricted space as provided in standard cages cannot be correlated with morphological damage in mice and rats (Gärtner et al, 1976), behavioural disorders, such as wire-gnawing, are widely accepted as signs of impaired welfare (Lawrence and Rushen, 1993).

IIa.4.3.3 Existing recommendations for stocking densities and consequences

Several attempts have been made to set up mathematical equations to calculate the individual space requirements and stocking densities for laboratory rodents (Bruhin et al, 1988; Gärtner et al, 1979; Hackbarth et al, 1999; Merkenschlager and Wilk, 1979; Sato, 1997; Weihe, 1978). The cm² of space required per gram body weight are species-specific. Application (stock and experiment versus breeding), as well as age (weanlings versus adults) have not been taken into account. Larger rodents are often the older animals, which do not necessarily need more
space than smaller subjects, while young animals tend to be more active as adults. After weaning until reaching sexual maturity, rodents are often engaged in extensive locomotory and social plays (Nagel and Stauffacher, 1994; Pfeuffer, 1996; Sachser, 1994; Scharmann, 1991; Schmitter, 1989). Even considering the multiplication of space available to individual animals in larger groups (the "omnibus effect"), it is questionable how 13 young rats of 100 grams, each, could manage to cope with a cage with a floor area of 810 cm\(^2\) (fig. 9, Appendix A, 1986). Thus, a step-wise progression might be more biologically accurate than a straight-line relationship. Following Weihe's suggestion (1978), the group considers it essential that cage sizes for rodent groups should relate to the individuals' final body weight, which gives the younger animal the benefit of more space.

IIa.4.3.4 Flooring

Rodents prefer solid floors with bedding to grid flooring if given the choice (Arnold and Estep, 1994; Blom et al, 1996), especially for resting (Manser et al, 1995). The degree of motivation to reach a solid floor is similar to that for exploring a novel environment (Manser et al, 1996). Wistar rats were more active and less emotional in the open field when housed in cages with solid floors and bedding (Eskola and Kaliste-Kohonen, 1998); but, a comparison of the behaviour and stress responses of groups of SIV male rats housed on grid floors versus siblings on solid floors did not reveal any significant difference with respect to animal welfare (Nagel and Stauffacher, 1994; Stauffacher, 1997b). It is already part of the CoE Resolution of May 30 1997 (Council of Europe, 1997) that rodents should be provided with solid floors with bedding instead of grid floors unless there are strong experimental or veterinary reasons for not doing so.

IIa.4.3.5 Proposals - General

To determine the minimum recommendations for floor area, the quantity and the quality of space have to be considered. Except for locomotory playing, animals do not use space per se, they use resources and structures within an area for specific behaviours. It has been shown that, when space additional to that of standard caging is provided, mice are highly motivated to enter it (Sherwin and Nicol, 1997). On the other hand, preference tests have shown that it is less the size than the degree of environmental variability, which is selected by mice (Baumans et al, 1987). Behavioural disorders in mice (Wübelt et al, 1996) and gerbils (Wiedenmayer, 1996) are more related to lacking or inadequate stimuli and objects than to space. Increasing amounts of empty space as well as inappropriate enrichment may stimulate territorial aggression among mouse males (Haemisch et al, 1994; Stauffacher, 1997b). The crucial point is the interaction between the space, the structure of the cage, the animals and the type and quantity of enrichments provided (Jennings et al, 1998). It may be quite difficult to provide proper spatial and social enrichment within very limited space. This problem is one of the main reasons for contradictory results of different studies.

Mice, hamsters and, to some extent, rats, make good use of the third dimension. Mice, for example, will climb on the cage lid, as well as on enrichment racks (Büttner, 1991). The ground-living guinea pigs mainly use the cage periphery and avoid open terrain (White et al, 1989). Mice and hamsters frequently use the cage lid for climbing and exploration (Scharmann, 1991), and rats regularly stand up-right for both exploration (Büttner, 1993) and social behaviour (e.g. boxing position, Nagel and Stauffacher, 1994).
A traditional approach to evaluate minimum cage dimensions is using body size. Lawlor (1990), for example, evaluated the minimum cage floor size for rats on the basis of the body length including tail, and of the body width. She claimed that a rat should be able to sit or lie without any torsion of the body or the tail. Accordingly, the minimum cage dimensions for an average sized rat (weight about 250 grams) would be at least 800 cm\(^2\), with a larger allowance for bigger rats. However, this approach neglects the need for social housing as well as for activity-related use of the space (Resolution of the 3\(^{rd}\) Multilateral Consultation to ETS 123, 1997). As stated earlier, the most important factor for devising minimum floor area recommendations for laboratory rodents is to consider the minimum enrichment requirements that have to be incorporated into the cage to allow the animals to perform a wide range of different behaviours and to cope successfully with their spatial and social environment. This applies to mice, hamsters and gerbils, and to a lesser extent to guinea pigs and rats. The optimal group size is determined by sex and age of the animals, cage size and experimental design. It is important to form harmonious groups and to keep group size and composition stable to avoid stress by altering the established hierarchy (Hurst et al, 1999; Jennings et al, 1998; Haemisch and Weisweiler, 1992; Peng et al, 1989; Stauffacher, 1997a).

The group's recommendations for minimum cage dimensions and stocking densities are based on scientific evidence and good practice. As stated earlier, figures for minima (cage sizes) and maxima (stocking densities) can never be scientifically "proved". To set limits (minima and maxima) is a political and not a scientific question. Any claim for proper experimental "proof" for such limits would be the consequence of a fallacy. Nevertheless, animal science can provide sound arguments why limits should be set in some instances and not others.

The group proposes that, prior to the species-specific tables for minimum cage dimensions and stocking densities, the following paragraphs should be inserted:

"The cages should be made of an easy to clean material and their design should allow proper inspection of the animals without disturbing them. Solid floors with bedding or perforated floors are normally preferable to grid or wire mesh floors. If grids or wire mesh are used for extended periods, a solid or bedded or slatted area should be provided for the animals to rest on unless specific experimental conditions prevent this.

Once young animals become active they require proportionally more space than adults do. In this and subsequent tables for all rodent recommendations “cage height” means the vertical distance between the cage floor and the upper horizontal part of the lid or cage, and this height should apply over greater than 50% of the cage floor area.

When designing procedures, consideration should be given to the potential growth of the animals to ensure adequate room according to this table in all phases of the procedures."

IIa.4.3.6 Proposal - Containment systems

Animals may be housed in isolation for various reasons. As genetically modified animals may be immuno-compromised, they may be more sensitive to infections. Also, newly purchased and potentially infected animals may be housed in quarantine prior to introduction into the animal facilities. Finally, as a precaution to protect the staff from allergen exposure, animals may be housed within a containment system to reduce the release of such allergens. There are, in principle, four levels of animal containment:

(i) Filter-topped cages.
(ii) Ventilated cabinets.
(iii) Individually ventilated cage systems (IVC).
(iv) Isolators.
In general, the group does not consider that stocking densities, minimum space or enrichment should be different for these systems. However, it may be necessary to change the approach when using isolators, for example, or to allow a specific technical performance such as in an IVC system. Some of the systems tend to be associated with specific problems such as humidity and trace gases (Corning and Lipman, 1991) for filter-topped cages. These problems may also be shown for IVC systems, where draughts and noise from the powerful ventilation will also need consideration. As IVC systems are becoming more common for more general use and not just for isolation purposes, the group considers it essential that producers should be urged to manufacture systems that do not reduce the welfare of the animals compared to traditional housing systems. Nonetheless, the group accepts that veterinary or scientific considerations may require certain divergences from Appendix A.

The group proposes that, prior to the species-specific tables for minimum cage dimensions and stocking densities, a paragraph on ‘Containment Systems’ should be inserted:

"The same principles regarding quality and quantity of space, environmental enrichment and other considerations in this document should apply to containment systems such as individually ventilated cages (IVC), although the design of the system may mean that this may have to be approached differently."

IIa.4.3.7 Proposals - Mice: minimum dimensions of enclosures and maximum stocking densities

For mice, the critical issues to be considered when discussing minimal cage sizes and stocking densities for mice, is the tendency for males to try to establish territories when there is something to defend (e.g. enrichment objects), and the need of a defeated subordinate male to escape successfully (Stauffacher, 1997b). Special attention should be given to group size and group composition (Chamove, 1989; van Loo and Baumans, 1998) and to distinct differences between and within outbred stocks and inbred strains (Bisazza, 1981; Brain and Parmigiani, 1990, Nevison et al, 1999).

Mice respond to increased group size with reduced levels of aggression, but show more evidence of stress, reflected in increased serum corticosterone levels and a higher gastritis incidence (Barnard et al, 1994; Manser, 1992). Removing or replacing adult group members threatens harmonious group life and may lead to serious aggressive encounters (Brain, 1990).

For mice, the group believes that, if the minimum floor area is raised enough to facilitate proper enrichment, there is no need to change stocking densities. Breeders may even house young animals up to 20 grams at a higher stocking density for the short period after weaning until issue, providing that larger cages are used and proper enrichment is guaranteed. Breeders are able to create socially harmonious groups based upon weaning of littermates. A larger minimum floor area will create an omnibus effect, which secures the welfare of these animals as well as if they were housed at lower stocking densities in smaller sized groups in a smaller floor area. Furthermore, stocking densities will be reduced as animals are issued from the cage, and as such the need for re-stocking may be reduced. Therefore, the higher stocking densities proposed for post-weaned mice may even improve the welfare of these animals.

It should be noted that the group only considers these assumptions valid as long as proper enrichment is guaranteed in the breeding facilities, and cages contain sufficient enrichment furniture to allow shelter and a degree of separation. After having observed animals in different size cages at different stocking densities and having scrutinised photographs of the animals distribution within the cage in different set-ups over a 24-hour period, the group considers that 950 cm$^2$ represents the lowest area fulfilling such enrichment needs for mice when held at increased stocking densities.
At present, the group does not find any justification for changing cage height requirements as given for mice in the present Appendix A (1986).

For mice in stock, during procedure and breeding, the group proposes the following minimum cage dimensions and floor areas per animal:

"Guidelines for caging mice in stock, during procedure and breeding."
[GT 123 (2000) 57, Rodents, Table 1]

<table>
<thead>
<tr>
<th>Body weight gms</th>
<th>Minimum floor area cm²</th>
<th>Minimum cage height cm</th>
<th>Floor area per animal cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>In stock and during procedure</td>
<td>&lt;20</td>
<td>330</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>21-25</td>
<td>330</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>26-30</td>
<td>330</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>&gt; 30</td>
<td>330</td>
<td>12</td>
</tr>
<tr>
<td>Breeding</td>
<td>330</td>
<td>For a monogamous pair (out-bred/inbred) or trio (inbred). For each additional female plus litter 180 cm² should be added.</td>
<td>12</td>
</tr>
<tr>
<td>Stock at breeders*</td>
<td>&lt; 20</td>
<td>950</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>&lt; 20</td>
<td>1500</td>
<td>12</td>
</tr>
</tbody>
</table>

* Post-weaned mice may be kept at these higher stocking densities, for the short period after weaning until issue, provided that the animals are housed in larger enclosures with adequate enrichment. It must be demonstrated to the regulatory authority that the housing conditions do not cause any welfare deficit such as: increased levels of aggression, morbidity or mortality, stereotypies and other behavioural deficits, weight loss, or other physiological or behavioural stress responses.

IIa.4.3.8 Proposals - Rats: minimum dimensions of enclosures and maximum stocking densities

To determine minimum cage sizes for rats, the minimum spatial enrichment objects as well as the rats’ need for rearing up should be taken into account (Büttner, 1993; Ernst, 1994; Lawlor, 1990; Weiss and Taylor, 1985). Nesting material (e.g. loose straw, paper towels) and some objects providing shelter (e.g. huts, tubes, barriers, in addition to the food trough) are highly recommended (Anzaldo et al, 1994; Bradshaw and Poling, 1991; Zimmermann, 1997).

A series of studies have shown that rats prefer cage heights of 18-20 cm (Büttner, 1993; Lawlor, 1983; 1990; Weiss and Taylor, 1985), but it has also been shown that rats spend most of their time in burrows if given the choice (Boice, 1977). Thus, the lid on a rat cage should allow enough height for both grooming, i.e. performing face washing while sitting upright on the hind legs, as well as withdrawal into lowered parts, for example underneath the food trough (Blom et al, 1995). As rats do not perform stereotypic digging, the provision of thick substrate layers or artificial burrows is not necessary (Nagel and Stauffacher, 1994). The group considers that there is a scientifically valid basis for raising the minimum demands for cage heights for rats to 18 cm.

When evaluating the stocking density for rats, the group did not consider that the weight range of 50 - 350 grams given in the present Appendix A was appropriate. If adult rats are to be housed in a cage with a minimum floor area of 800 cm², any rat of a weight less than 200 grams should be allowed the same space because of the higher activity level of younger animals. The group is aware that breeders have practical experience to show that post-weaned rats can easily be harmoniously housed at a higher stocking density. However, the group does not consider that an 800 cm² cage contains enough space for adequate enrichment at higher
stocking densities. Therefore, increased stocking density should only be allowed if the minimum floor space is increased to 1500 cm². Furthermore, the present Appendix A (Council of Europe, 1986) seems to ignore the fact that certain outbred stocks of rats can easily grow to a weight larger than 600 grams. Therefore, it is necessary to define decreased stocking densities for rats larger than 350 grams so that the rats can still perform behaviours that require extra space, e.g. grooming, rearing and locomotion.

For rats in stock, during procedure and breeding, the group proposes the following minimum cage dimensions and floor areas per animal:

"Guidelines for caging rats in stock, during procedure and breeding. [GT 123 (2000) 57, Rodents, Table 2]

<table>
<thead>
<tr>
<th>Body weight gms</th>
<th>Minimum floor area cm²</th>
<th>Minimum cage height cm</th>
<th>Floor area per animal cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>In stock and during procedure</td>
<td>&lt; 200</td>
<td>800</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>201-300</td>
<td>800</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>301-400</td>
<td>800</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>401-600</td>
<td>800</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>&gt; 600</td>
<td>1500</td>
<td>18</td>
</tr>
<tr>
<td>Breeding</td>
<td>800</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother and litter. For each additional adult animal permanently added to the cage add 400 cm²</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Stock at breeders*</td>
<td>&lt; 50</td>
<td>1500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>51-100</td>
<td>1500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>101-150</td>
<td>1500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>151-200</td>
<td>1500</td>
<td>18</td>
</tr>
<tr>
<td>Stock at breeders*</td>
<td>&lt; 100</td>
<td>2500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>101-150</td>
<td>2500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>151-200</td>
<td>2500</td>
<td>18</td>
</tr>
</tbody>
</table>

Post-weaned rats may be kept at these higher stocking densities, for the short period after weaning until issue, provided that the animals are housed in larger enclosures with adequate enrichment. It must be demonstrated to the regulatory authority that the housing conditions do not cause any welfare deficit such as: increased levels of aggression, morbidity or mortality, stereotypies and other behavioural deficits, weight loss, or other physiological or behavioural stress responses."

IIa.4.3.9 Proposals - Gerbils: minimum dimensions of enclosures and maximum stocking densities

The term 'laboratory gerbil' is currently almost exclusively applied to the Mongolian gerbil (Meriones unguiculatus), the most common of five species of the subfamily Gerbillinae (Havenaar et al, 1993).

Gerbils develop extensive stereotypic digging if they are not given the chance to dig burrows, or if they are not provided with an artificial burrow (Wiedenmayer, 1997). When a gerbil structures the cage for this purpose, several centimetres of the height is taken up by the bottom layer, and, therefore, the group proposes that gerbils are provided with more cage height than, for example, the hamster. Gerbils also frequently adopt a rearing posture and so should have adequate headroom to accommodate this.
Gerbils have a particular need for structuring their cage into rest, toilet area and food store while still being able to dig burrows (Brain, 1999). This cannot be realistically performed on less than 1200 cm$^2$.

In the wild, gerbils form large colonies or family groups (Agren, 1978). In the laboratory, it is suggested, that the best social grouping is a male-female pair (Agren, 1984; Havenaar, 1993). If not for breeding, they should be housed in stable and harmonious unisex groups; gerbils are generally intolerant of intruders (Brain, 1999). For gerbils the weight-bands proposed by the group have been based upon the weight at which these species reach maturity and have to be restocked.

For gerbils in stock, during procedure and breeding, the group proposes the following minimum cage dimensions and floor areas per animal:

"Guidelines for caging gerbils in stock, during procedure and breeding.
[GT 123 (2000) 57, Rodents, Table 3]

<table>
<thead>
<tr>
<th></th>
<th>Body weight gms</th>
<th>Minimum Floor area cm$^2$</th>
<th>Minimum cage height cm</th>
<th>Floor area per animal cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In stock and during procedure</td>
<td>&lt; 40</td>
<td>1200</td>
<td>18</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>&gt; 40</td>
<td>1200</td>
<td>18</td>
<td>250</td>
</tr>
<tr>
<td>Breeding</td>
<td>1200 Monogamous pair or trio with offspring</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IIa.4.3.10 Proposals - Hamsters: minimum dimensions of enclosures and maximum stocking densities

Hamsters housed in small cages seem to be more stressed than hamsters housed in larger cages; in smaller cages they have increased body temperatures (Kuhnen, 1998; 1999a) independent of their age (Kuhnen, 1999b). The effect of the small cages is confirmed by crossover studies (Kuhnen, 1999b). Furthermore, there is evidence that hamsters housed in non-enriched cages are more stressed than hamsters housed in enriched cages (Kuhnen, 1997). Therefore, and as it is difficult to provide sufficient enrichment in a smaller cage, the group recommends for hamsters a minimum cage floor area of 800 cm$^2$. As hamsters do not seem to have the same needs as gerbils for structuring the cage to the same extent, a legal demand for a minimum floor area more than 800 cm$^2$ is hard to justify.

Caging should allow hamsters to adopt their grooming posture, bury food and build a nest to completely cover them when sleeping. The group considers that an increase in cage height for hamsters from the present 12 cm to 14 cm can be justified, considering the size of hamsters and the thickness of the substrate.

As for gerbils, the weight-bands proposed by the group for hamsters have been based upon the weight at which these species reach maturity and have to be restocked.

Although golden hamsters (Mesocricetus auratus) are solitary animals in nature, there is an indication that group housing actually is preferable from a reduced stress and optimal welfare point of view, as group housed hamsters have a higher growth rate, increased food consumption and increased fat deposition (Borer et al, 1988). Male golden hamsters spend more time in social proximity than out of proximity, especially if they have had prior group-housing experience (Arnold and Estep, 1990). The group recommends that hamsters should be housed.
in harmonious social groups but accepts that strain-dependent or unusual aggression in individual animals might be used as a valid reason for single housing. As is the case for other rodents, younger hamsters are more active than the older ones. However, the group feels that there might be some validity in allowing larger hamsters more space as hamsters tend to become more aggressive with age and aggression seems to be reduced by more space. On the other hand, post-weaned hamsters at breeders may be housed at a higher stocking density if a larger cage is provided according to the same logic, which has been described for rats and mice.

For Chinese hamsters (*Cricetus griseus*), no studies in relation to these features seem to be available. Experience suggests that they tend to be more aggressive than golden hamsters; thus, group housing may be more problematic for Chinese hamsters than for golden hamsters. However, practical experience also shows that it is possible to house Chinese hamsters under the same conditions as for golden hamsters, and as such, there is no reason not to base housing of Chinese hamsters on principles for housing golden hamsters. However, it should be underlined that they should be housed individually if aggression is likely to occur in groups or pairs.

The larger sized European hamster (*Cricetus cricetus*) and the dwarf, mouse-like Djungarian hamster (*Phodopus sungorus*) are used to a lesser extent in the laboratory (Whittaker, 1999).

For hamsters in stock, during procedure and breeding, the group proposes the following minimum cage dimensions and floor areas per animal:

"Guidelines for caging hamsters in stock, during procedure and breeding."

[GT 123 (2000) 57, Rodents, Table 4]

<table>
<thead>
<tr>
<th></th>
<th>Body weight gms</th>
<th>Minimum floor area cm²</th>
<th>Minimum cage height cm</th>
<th>Floor area per animal cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In stock and during procedure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>800</td>
<td>14</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>60-100</td>
<td>800</td>
<td>14</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>&gt; 100</td>
<td>800</td>
<td>14</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td><strong>Breeding</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother or monogamous pair with litter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stock at breeders</strong></td>
<td>&lt; 60</td>
<td>1500</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>

*Post-weaned hamsters may be kept at these higher stocking densities, for the short period after weaning until issue, provided that the animals are housed in larger enclosures with adequate enrichment. It must be demonstrated to the regulatory authority that the housing conditions do not cause any welfare deficit such as: increased levels of aggression, morbidity or mortality, stereotypies and other behavioural deficits, weight loss, or other physiological or behavioural stress responses.*

IIa.4.3.11 Proposals - Guinea pigs: minimum dimensions of enclosures and maximum stocking densities

Guinea pigs are the only rodents for which pen housing is strongly recommended and also practical (North, 1999). Other rodent species are sometimes housed in floor pens for experimental reasons (e.g. behavioural studies), but there are no validated concepts known for housing and breeding laboratory mice, rats, gerbils and hamsters in floor pens at large scale.
In the wild, the cavy (*Cavia apera*) lives in small groups of five to ten individuals, but in the laboratory, the domestic guinea pig (*Cavia apera porcellus* or *Cavia porcellus*, depending on the authors) can be housed in large mixed-sex breeding colonies (3-10 males and 15-30 females), gradually developed from small compatible breeding nucleus, or in all female groups (Sachser, 1986a, 1990). Males can be kept in groups up to 4 month of age; then housing in duos is recommended (Beer and Sachser, 1993). Whereas males form dominance hierarchies (Coulon, 1975b), females are less competitive and may display a weak and flexible social hierarchy (King, 1956). The scientific evidence that guinea pigs should be housed and bred in pens (in large mixed-sex groups) rather than individually or in pairs in cages is reviewed by Sachser (1994).

Housing conditions and social status significantly affect the guinea pigs' hormonal activities and their social development (Sachser, 1986a, 1986b, 1990; Sachser and Kaiser, 1996; Sachser et al, 1994; Stanzel and Sachser, 1993). According to group size, guinea pigs change their social organization, a mechanism for adjusting to increased population density, avoiding the negative effects of increased dominance-aggression (Beer and Sachser, 1993; Sachser, 1986a, 1998; Sachser and Beer, 1995).

The cage sizes recommended in the present Appendix A (Council of Europe, 1986) are far too small to be properly enriched. Standard rodent cages, such as Macrolon type IV (1800 cm$^2$), are only adequate for young and small-sized guinea pigs, while larger guinea pigs should be allowed more space (Beer et al, 1995). The group considers that 2500 cm$^2$, at least, is necessary to accommodate the animals’ shape and movement and to allow the addition of a suitable sized shelter. Guinea pigs need to have adequate protection when in floor pens and they need careful management to prevent panic. They are easily disturbed and startled so they also need adequate provision of cover and subdivision of space.

It is the opinion of the group that the stocking densities for guinea pigs given in the present Appendix A are above what should be considered good practice. In particular, younger and smaller animals should be allowed more space, as they are more active (Beer et al, 1995). For guinea pigs in stock, during procedure and breeding, the group proposes the following minimum cage dimensions and floor areas per animal:

"Guidelines for housing guinea pigs in stock, during procedures and breeding in cages or floor pens."

<table>
<thead>
<tr>
<th>Body weight gms</th>
<th>Minimum floor area cm$^2$</th>
<th>Minimum Cage height cm</th>
<th>Floor area per animal cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In stock and during procedure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>1800</td>
<td>23</td>
<td>200</td>
</tr>
<tr>
<td>200-300</td>
<td>1800</td>
<td>23</td>
<td>350</td>
</tr>
<tr>
<td>301-450</td>
<td>1800</td>
<td>23</td>
<td>500</td>
</tr>
<tr>
<td>451-700</td>
<td>2500</td>
<td>23</td>
<td>700</td>
</tr>
<tr>
<td>&gt; 700</td>
<td>2500</td>
<td>23</td>
<td>900</td>
</tr>
<tr>
<td>Breeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair with litter. For each additional breeding female add 1000 cm$^2$</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IIa.4.4 Feeding

No specific recommendations for rodents; see Section I, Chapter 4.2 of this report, and General Part of Appendix A, GT 123 (2000) 54.

IIa.4.5 Watering

No specific recommendations for rodents; see General Part of Appendix A, GT 123 (2000) 54.

IIa.4.6 Substrate, litter, bedding and nesting material

Apart from serving obvious hygienic purposes, bedding allows a certain degree of burrowing (mice, rats) or dwelling (hamsters, guinea pigs, rabbits) and makes it easier for them to deposit odour patterns in the environment. As such, bedding also serves as environmental enrichment. The CoE Resolution of May 30 1997 (Council of Europe, 1997) states that pens as well as cages should include bedding material. Dirty bedding leads to the accumulation of volatile compounds in the cage, which has a negative impact on the well-being of the animals, and may affect the outcome of the research (Vesell et al, 1973). Hygroscopic material should not be used for neonates because of the risk of dehydration (Baumans, 1999). There is also clear evidence that rodents housed on different types of bedding give different experimental results, especially if the study involves hepatic metabolism (Cunliffe-Beamer et al, 1981; Vesell et al, 1976) and care should be taken to standardise this between studies.

The group proposes that the rodents' general considerations section should contain the following paragraph:

“Various materials are commonly placed into the animal enclosure to serve the following functions: to absorb urine and faeces and thus facilitate cleaning; to allow the animal to perform certain species-specific behaviours such as foraging, digging or burrowing; to provide a comfortable yielding surface or secure area for sleeping; to allow the animal to build a nest for breeding purposes. Certain materials may not serve all of these needs and it is therefore important to provide sufficient and appropriate materials. Any such material should be dry, nontoxic and free from infectious agents or vermin or any similar form of contamination. Materials derived from wood that has been treated chemically should be avoided. Certain industrial by-products or waste such as shredded paper may be used. Nesting materials should be provided for nest making species.”

IIa.4.7 Cleaning

There is no doubt that good hygiene prevents a range of disease conditions in laboratory animals and improves the health of the animals (Vesell et al, 1973). However, the procedures involved in maintaining a good level of hygiene may have a negative impact on the animal, leading to stress and increased aggression (mice: Hurst et al, 1993; Jones, 1991; McGregor et al, 1991; van Loo et al, 2000).
It is advisable to maintain odour patterns left by the animals, especially from the nest area. Male mice show less aggression when a small amount of material from the nesting area is introduced in a clean cage (Van Loo et al, 2000), but more aggression if the remaining soiled material was the cage itself, sawdust or a marking block (Gray and Hurst, 1995). Given the rather limited evidence, the group considers that it is preferable that Appendix A gives advice in this field rather than giving mandatory instructions.

The group proposes that the rodents' general considerations section should contain the following paragraphs:

"High hygiene standards should be maintained, however it may be advisable to maintain odour patterns left by animals. Over-cleaning cages used by pregnant animals and females with litters should be avoided. Such disturbances can result in mis-mothering or cannibalism. Decisions on frequency of cleaning should therefore be based on cage system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality."

IIa.4.8 Handling

No specific recommendations for rodents; see General Part of Appendix A, GT 123 (2000) 54.

IIa.4.9 Humane killing

The group proposes that the CoE Resolution on Training of Persons Working with Laboratory Animals (adopted May 30, 1997) and the European Commission DG XI Guidelines on Euthanasia (Close et al, 1996, 1997) be added to ETS 123 as separate appendices.

IIa.4.10 Records

No specific recommendations for rodents; see General Part of Appendix A, GT 123 (2000) 54.

IIa.4.11 Identification

It is often necessary to identify rodents individually, either temporarily or permanently. It is advantageous for animals to be individually identified to ensure good monitoring of breeding performance and to enable animals with eventual abnormalities to be excluded from breeding programmes. It may also be necessary to provide accurate details of individual parentage, such as, for studies involving reproduction. In animal experimentation, individual data sampling makes accurate identification necessary, especially for group-housed animals. In Table 3, some proven methods of identification are listed for rodents and rabbits (Jennings et al, 1998; Keely et al, 1988; Morton et al, 1993). The different methods have both advantages and disadvantages; which one is selected depends on the specific purpose for which identification is needed. Ideally, non-invasive methods should be used. Toe clipping should not be done. If permanent identification is required, consideration must be given to the degree of discomfort to the animal during the marking action, to the training of staff and to the use of sedatives or local anaesthetics.
### Table 3  
Marking methods for rodents

<table>
<thead>
<tr>
<th>Non Invasive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Labels:</strong></td>
<td>Cages or pen labels for individually housed animals.</td>
</tr>
<tr>
<td><strong>Marker pens:</strong></td>
<td>Tail or coat, short term, depending on grooming and on housing conditions.</td>
</tr>
<tr>
<td><strong>Hair clipping:</strong></td>
<td>Lasts 2 to 6 weeks.</td>
</tr>
<tr>
<td><strong>Dyes:</strong></td>
<td>Sheep markers may be used, last longer but require renewal after moulting.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Permanent Methods</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microchips:</strong></td>
<td>Subcutaneous implant, insertion by competent staff. Requires a decoder (no external indication of animal’s identity). Microchips can migrate if not positioned properly; some types can be reused after cleaning and sterilisation.</td>
</tr>
<tr>
<td><strong>Tail tattooing:</strong></td>
<td>Rats, mice, (ears – guinea pigs) – local anaesthetics should be used to minimise discomfort. Requires trained competent staff to ensure legibility.</td>
</tr>
<tr>
<td><strong>Ear notching/punching:</strong></td>
<td>Only sharp punches should be used to avoid tearing the tissue and damaging veins.</td>
</tr>
<tr>
<td><strong>Ear tags:</strong></td>
<td>Difficult to read, can fall out or get caught in caging.</td>
</tr>
<tr>
<td><strong>Freeze marking:</strong></td>
<td>With spots of liquid nitrogen, useful for pigmented strains</td>
</tr>
</tbody>
</table>
IIb  Recommendations species-specific sections - Rabbits

IIb.1. Introduction

The group proposes an introduction to the species rabbit (*Oryctolagus cuniculi*), covering the most important aspects of biology, behaviour and habitat use as well as of husbandry requirements. Background information is provided in section IIb.4, 'housing and enrichment'.

"The rabbit (*Oryctolagus cuniculi*) is a naturally gregarious species. Young and female rabbits should be housed in harmonious social groups unless there are good veterinary or scientific reasons for not doing so. Adult males may perform territorial behaviour and should not be housed together with other entire males. Rabbits should be allowed adequate space and an enriched environment. There is increasing evidence to show that rabbits denied such freedom can lose normal locomotor activity, and suffer skeletal abnormalities. Wire floors should not be used without the provision of a resting area large enough to hold all the rabbits at any one time, unless there are good veterinary or scientific reasons for not doing so. Enriched floor pens have been used with success to house young rabbits and adult female rabbits although groups may need to be carefully managed to avoid aggression. Ideally rabbits for group housing should be littermates that have been kept together since weaning. Where individuals cannot be group housed, consideration should be given to housing them in close visual contact.

**Enrichment**

Suitable enrichment for rabbits includes roughage, hay blocks or chew sticks as well as an area for withdrawal. For breeding does, nesting material and a nestbox or another refuge should be provided. In floor pens for group housing visual barriers should be provided. Structures to provide refuges and look out behaviour should also be included.

**Cages**

It is preferable for cages to be rectangular. A raised area must be provided within the cage. This shelf should allow the animal to lie and sit on and easily move underneath, it should not cover more than 40% of the floor space. While the cage height should be sufficient for the rabbit to sit upright without its ears touching the roof of the cage, this degree of clearance is not considered necessary for the raised area. If there are good scientific or veterinary reasons for not using a shelf then cage size must be 33% larger for a single rabbit and 60% larger for 2 rabbits. When rabbits are kept in cages regular access to an exercise area is recommended."

IIb.2. The environment in the animal enclosures and its control

IIb.2.1 Ventilation

Rabbits should be able to avoid draughts caused by ventilation systems. Special attention should be paid to ammonia concentrations especially in floor pens with solid walls. Values of 10 ppm should not be exceeded (Batchelor, 1999).

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.
IIb.2.2 Lighting

Rabbits are intrinsically nocturnal with activity peaks at dawn and dusk (Jilge, 1991), but external noise and feeding schedules during the light period can make them predominantly diurnal (Batchelor, 1995, 1999). Although rabbits display activity peaks at dawn and dusk under near-to-nature conditions (Lehmann and Wieser, 1985), there is not sufficient evidence to mandate a substitute of dawn and dusk by gradual light change.

The group proposes that the section on general considerations for rabbits should include the following paragraph; see also background information in the Rodent Section IIa, chapter 2.4.

“Light levels within the cage should be low. Rabbits should have the opportunity to withdraw to shaded areas within the cage. All racks should have shaded tops to prevent retinal degeneration, which is a particular risk for albino animals. Red light, which is undetectable by rabbits, can be a useful management technique.”

IIb.2.3 Noise

The rabbit's sensitivity to high sound frequencies (Milligan et al, 1993) should be taken into account when considering sound levels in animal rooms. Background music may mask sudden loud sounds and reduce the rabbits' excitability. A softly playing radio may help to mask startling or frightening noises. There has, however, been little research to show whether this is a benefit to the animals.

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.2.4 Alarm systems

Alarm systems should sound outside the sensitive hearing range of the rabbit, but be audible to man.

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.3. Health

See proposals of the group for amendments to the General Part of Appendix A (1986).

IIb.4. Housing and enrichment

IIb.4.1 Social Housing

It is already part of the CoE Resolution of May 30 1997 (Council of Europe, 1997) that young and female rabbits should be housed in socially harmonious groups, unless the experimental procedure or veterinary requirements make this impossible. Adult male rabbits may become territorial (Lehmann, 1992), and their fighting strategy may lead to lethal injuries (Bigler and Oester, 1996). Contact with humans, such as handling, training and socialising, will usually benefit both the animals and the outcome of experiments as it engages the animal on a cognitive level and allows positive interaction with animal caretakers, technicians and scientists (Denenberg et al, 1973; Wyly et al, 1975).
The group composition should be stable and harmonious (Love, 1994; Morton et al, 1993; Stauffacher, 1997a; Turner et al, 1997), and visual barriers or hiding places may be necessary to minimise aggression (Stauffacher, 1993, 2000). Even in harmonious rabbit groups, it is necessary to allow individuals to initiate contact by approach, or avoid contact by withdrawal out of sight (Stauffacher, 1986a, 1986b). For social living animals such as the rabbits, a social partner is the most challenging enrichment factor: Whereas enrichment objects are static and of interest for specific activities only, a social partner always creates new and unpredictable situations to which the animal must react. A social partner leads to an increase of alertness and exploratory behaviour and it provides diversion, occupation and probably also some feelings of "security" (Stauffacher, 1995).

Adult females of most laboratory rabbit strains (medium and large size, e.g. New Zealand Whites, Chinchilla, Russian, Belgian Hare, Sandy Lop) are well suited to group housing, but differences in the expression and frequency of aggressive behaviours (Kraft 1979) may raise problems with some small strains. Advantages of group-housing females and young rabbits are: improvement of physical health and psychological well-being, availability of social partners, larger pen or cage size allowing functional subdivision of the available space, more docile animals; furthermore, there are some economic advantages (e.g. basic investment, maintenance and energy costs), and greater job satisfaction (Held, 1995; Love, 1994; Morton et al, 1993; Podberscek et al, 1991; Stauffacher 1993; Whary et al, 1993). This form of rabbit husbandry encourages animal technicians and researchers to see animals as living creatures rather than just as tools for research.

If individuals of common laboratory rabbit stocks are grouped at an early stage of life few problems of compatibility seem to be reported. In established female groups fights being more or less harmful are rare (Albonetti et al, 1990, Stauffacher 1986a, b), but aggression within groups of adult does correlates with sexual activity (Held, 1995) and stages of pregnancy (Stauffacher, 1986a), and the degree of compatibility of grouped rabbits will depend on factors such as strain, individuality, age and weight, sex, size and structuring of pens, methods of husbandry, and, last but not least, the motivation of the animal. Worries that subordinate females will be more stressed and immuno-suppressed have not been realised (Held, 1995; Morton et al, 1993; Turner et al, 1997; Whary et al, 1993).

The introduction of unfamiliar animals into established groups is difficult except for pre-mature rabbits (less than 3-4 month of age, depending on strain and feeding regime). Adult rabbits form individual-specific relationships (Stauffacher, 1986a), and an exchange of partners should be avoided as the newly introduced female may be seriously attacked, especially in cages for pair housing (Stauffacher, 1994). In floor pen groups, the use of sedatives (Fentanyl/Doperidol 0.08 ml/kg) prior to mixing unfamiliar rabbits has been successful (Love and Hammond, 1991).

For breeding, the scientifically developed husbandry system of breeding rabbits in permanent groups (Maier, 1992; Stauffacher, 1986a, 1986b, 1992) is most appropriate in animal welfare terms, but is very difficult to handle. Its practical introduction into laboratory animal breeding facilities needs further study.

Despite all advantages of group housing, individual housing is the only practicable system for entire adult males and for incompatible females (due to the unacceptable injuries which can result from repeated fighting), as well as for certain experimental purposes (Whary et al., 1993). Sexually mature male rabbits may be extremely violent against other males (Heath, 1972; Lehmann, 1992).
When group housing is not possible for biological, experimental or welfare reasons, rabbits should be housed within sight, sound or smell of each other and extra attention should be provided to enrich their environment to relieve boredom.

The group proposes that the rabbits' general considerations section should contain the following paragraph:

"Young and female rabbits should be housed in harmonious social groups unless there are good veterinary or scientific reasons for not doing so. Adult males may perform territorial behaviour and should not be housed together with other entire males. Enriched floor pens have been used with success to house young rabbits and adult female rabbits although groups may need to be carefully managed to avoid aggression. Ideally rabbits for group housing should be littermates that have been kept together since weaning. Where individuals can not be group housed, consideration should be given to housing them in close visual contact."

IIb.4.2 Environmental complexity

IIb.4.2.1 Activity-related use of space

Except for locomotory playing in young rabbits (Lehmann, 1987), rabbits do not use space per se; they use resources and structures within an area for specific behaviours (Lehmann, 1989; Stauffacher, 1986b, 2000). If given the chance, domestic rabbits attempt to divide their living space into separate areas for feeding, resting, nesting and excretion (Lehmann, 1992; Wieser, 1986). Structures within the floor pen or the cage may facilitate these divisions even within limited space (e.g. blinds, platforms for use of the third dimension). Shelters and platforms may serve as both, hiding places and vantage points (Batchelor, 1991, 1999; Hansen and Berthelsen, 2000; Heath and Stott, 1990; Held et al, 1995; Lehmann, 1989; Love, 1994; Morton et al, 1993; Stauffacher, 1992, 1993, 2000).

IIb.4.2.2 Appropriate stimuli and materials for environmental enrichment

Stimulation of exploratory behaviour and alertness helps to meet the need for information gathering by the animal and may reduce the development of behavioural disorders (Stauffacher, 1998) and boredom (Wemelsfelder, 1997). Animals become stressed when an environment is unpredictable and/or uncontrollable (Manser, 1992). Providing a shelter or refuge gives the rabbits the opportunity to withdraw beneath it to avoid frightening stimuli, or to jump on and to use it as a look-out point (Stauffacher, 1993).

Appropriate structuring of the environment may be more beneficial than simply providing a larger floor area; however, a minimum floor area is needed to provide such a structured space (Stauffacher, 2000).

Rabbits tend to be highly motivated to make use of enrichment based on food items. Additional food items such as hay or straw (Berthelsen and Hansen, 1999; Lehmann, 1990, Lidfors, 1997), as well as of gnawing-objects (e.g. soft wood: Lidfors, 1997; Stauffacher, 1993, 2000) can satisfy the need for roughage and for chewing.

In commercial rabbit breeding, there can be a relatively high average mortality of 20% between birth and weaning (Delaveau, 1982; Koehl, 1999) due to poor nest quality, injuries, failed thermoregulation and cannibalism: The following aspects are necessary to increase breeding success and for the prevention of behavioural disorders in the female rabbit: location of the nest (nestbox, separate compartment), nest quality (Canali et al, 1991; Delaveau, 1982), access to the nest (permanent, or limited: Coureaud et al, 1998; Wullschleger, 1987), and
nursing frequency (naturally this means 1-2 times a day, or abnormally this may be increased due to open nestbox and disorders in maternal care: Hudson and Distel, 1989; Hudson et al, 1996; Jilge, 1993; Seitz et al, 1998; Stauffacher, 1988; Zarrow et al, 1965). Welfare may be improved greatly by adding a nestbox outside the nest so the female rabbit cannot use its roof for resting, the provision of nesting material (e.g. long straw, hay) which allows the doe to build a nest by own activity, the possibility for withdrawal from the olfactory stimuli of the pups (e.g. by a door, restricting access to the nestbox), and for successful escape from pups who have left the nestbox about two weeks after birth (by provision of a shelf), may improve welfare greatly (Baumann and Stauffacher, in prep.; Canali et al, 1991; Coureraud et al, 1998, 2000b; Wullschleger, 1987).

Based on the current science-based knowledge of the rabbit's physiological and behavioural needs in both conventional and enriched cages, as well as in floor pens and in near-to-nature enclosures (e.g. Brooks et al, 1993; Lehmann, 1992; Lidfors, 1997; Stauffacher, 1992; 1993; Wieser, 1996), the group considers the following recommendations as a minimum:

(i) The rabbit should have a choice of resting places (e.g. floor and a shelf), and the possibility for withdrawal (e.g. underneath a shelf). The shelf should be large enough to allow the animal to lie down and to sit, and to easily move underneath it.

(ii) For the prevention of oral deficits, gnawing objects (e.g. wood-blocks) and roughage (e.g. loose hay/straw or pressed hay-rolls) should be provided ad libitum.

(iii) For group housing, the environment should be subdivided by partitions in such a way that each animal is able to initiate or to avoid social contact.

(iv) During breeding, the mother should be allowed to build a nest, and to be apart from the litter and other stimuli, either by her own activity or by management measures.

Therefore, the group proposes that the rabbits' general considerations section should contain the following paragraph:

"Suitable enrichment for rabbits includes roughage, hay blocks or chew sticks as well as an area for withdrawal. For breeding does, nesting material and a nestbox or an other refuge should be provided. In floor pens for group housing visual barriers should be provided. Structures to provide refuges and look out behaviour should also be included."

IIb.4.3 Enclosures  -  dimensions and flooring

IIb.4.3.1 State of knowledge

In contrast to rodents, there is a considerable amount of literature on the influence of cage sizes on the behaviour and well-being of laboratory rabbits (see below). On the other hand it is doubtful whether minimum space requirements should and can be worked out on a purely scientific basis as every limit is set empirically and minimum requirements are always the result of compromises between the different parties involved. Therefore, the group considers it essential that compromises be based also upon biological reasoning as well as good practice.

IIb.4.3.2 Existing recommendations for minimum cage sizes and welfare consequences

Actual recommendations for minimum cage sizes for rabbits in stock and during procedure are given in Table 4, and for breeding does with litter in Table 5.
Table 4  Minimum space requirements for rabbits in stock, and during procedure

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<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>when housed singly</td>
<td>when housed singly</td>
</tr>
<tr>
<td></td>
<td>floor area</td>
<td>floor area</td>
</tr>
<tr>
<td></td>
<td>height</td>
<td>height</td>
</tr>
<tr>
<td></td>
<td>when housed in groups</td>
<td></td>
</tr>
<tr>
<td></td>
<td>floor area</td>
<td>height</td>
</tr>
<tr>
<td></td>
<td>height</td>
<td></td>
</tr>
<tr>
<td>up to 2000 g</td>
<td>1400 cm²</td>
<td>2000 cm²</td>
</tr>
<tr>
<td>up to 3000 g</td>
<td>2000 cm²</td>
<td>2000 cm²</td>
</tr>
<tr>
<td>above 5000 g</td>
<td>3600 cm²</td>
<td>4500 cm²</td>
</tr>
<tr>
<td>up to 5000 g</td>
<td>3000 cm²</td>
<td>2600 cm²</td>
</tr>
<tr>
<td>above 6000 g</td>
<td>4000 cm²</td>
<td>5000 cm²</td>
</tr>
<tr>
<td>up to 4000 g</td>
<td>2500 cm²</td>
<td>2600 cm²</td>
</tr>
<tr>
<td>up to 6000 g</td>
<td>5400 cm²</td>
<td>5000 cm²</td>
</tr>
<tr>
<td>above 6000 g</td>
<td>6000 cm²</td>
<td>5000 cm²</td>
</tr>
</tbody>
</table>

Table 5  Minimum space requirements for breeding rabbits (mother and litter)

<table>
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<tr>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>floor area</td>
<td>nestbox inclusive</td>
</tr>
<tr>
<td></td>
<td>height</td>
<td>floor area</td>
</tr>
<tr>
<td></td>
<td></td>
<td>height</td>
</tr>
<tr>
<td></td>
<td></td>
<td>remarks</td>
</tr>
<tr>
<td>up to 2000 g</td>
<td>3000 cm²</td>
<td>1000 cm²</td>
</tr>
<tr>
<td>up to 3000 g</td>
<td>3500 cm²</td>
<td>1000 cm²</td>
</tr>
<tr>
<td>above 3000 g</td>
<td>4000 cm²</td>
<td>1200 cm²</td>
</tr>
<tr>
<td>up to 5000 g</td>
<td>4500 cm²</td>
<td>1200 cm²</td>
</tr>
<tr>
<td>above 5000 g</td>
<td>5000 cm²</td>
<td>1400 cm²</td>
</tr>
</tbody>
</table>

With respect to housing standards for laboratory rabbits, a series of studies have shown that in cages that comply with the minimum dimensions required in Appendix A of the European Convention (Council of Europe, 1986), the rabbits’ welfare is impaired: The consequences of limited freedom of movement are changes in locomotory patterns and sequences (e.g. inability to hop), resulting in skeletal damage, e.g. in the femur proximalis and in the vertebral column (Bigler, 1995, 1998; Drescher, 1993b; Drescher and Loeffler, 1991, 1996; Lehmann, 1987, 1989; Martrenchar, in press; Rothfritz, 1992).

The barren cage environment with a severe lack of stimulation leads to behavioural disorders such as wire-gnawing and excessive wall-pawing, as well as to panic reactions and to signs of “boredom” (e.g. Gunn, 1994; Krohn et al, 1999; Lehmann and Wieser, 1985; Metz, 1987; Oester and Lehmann, 1993; Stauffacher, 2000; Wieser, 1986).

During breeding, an open nest-box inside the cage and poor quality and quantity of nesting material do not permit the doe to perform natural behaviour (e.g. closing up the nest entrance triggered by odour cues of the litter: Canali et al, 1991; Wullschleger, 1987). In addition, these conditions do not allow the doe any chance to withdraw from the litter, which can result in behavioural disorders in the mother and in high rearing losses (Bigler, 1985; Coureraud et al, 2000a, 2000b; Hamilton et al, 1997).
IIb.4.3.3 Existing recommendations for stocking densities and consequences

In Appendix A of the European Convention (Council of Europe, 1986), the graph on stocking densities for rabbits (fig. 12) reflects weight:space correlations with the slopes set arbitrarily; like the recommendations for rodents, it is mainly based on a model proposed by Merkenschlager and Wilk (1979). The slopes represent weight-bands and allow to read the space requirements for a given number of rabbits. The heavier the rabbits, the less cm² are required per weight unit. The calculation model only refers to body weight; it does not make any distinction between strains, sex and age. Young rabbits of one strain (e.g. New Zealand White) may have the adult size of another strain (e.g. Belgian Hare).

In the laboratory, medium-sized strains are mostly used; they are weaned at 500–800 g and reach adulthood not before 2.5–3 kg. But the weight-bands for stocking densities are set at 250–500 g, 500–750 g, 750–1000 g, 1000–2000 g and >2000 g (fig. 12, Appendix A, 1986). In growing rabbits, the intensity of locomotory patterns is much higher than in adults (Lehmann, 1987, Wieser 1986). Thus, the model does not adequately reflect the fact that young growing animals need much more space in relation to their body weight than adults.

IIb.4.3.4 Flooring

The CoE Resolution of May 30 1997 (Council of Europe, 1997) states that wire floors should not be used without the provision of a solid resting area. The materials, design and construction of slatted or perforated floors should provide surfaces which do not produce welfare problems. The disadvantages of wire flooring are many; e.g. they are uncomfortable for resting and locomotion and they may lead to paw lesions which can be painful and become infected (Drescher, 1993a; Drescher and Schlender-Bobbis, 1996; Marcanto and Rosmini, 1996, Rommers and Mejerhof, 1996). The insertion of a solid floor area for resting large enough to hold all the rabbits at any one time is an easily made and cheap improvement.

Besides wire flooring, the group does not find it applicable to provide specific recommendations for flooring, e.g. slatted versus perforated versus solid floors. The best floor design can be disadvantageous for health and behaviour if processing and hygiene are of poor quality. With breeding, special attention has to be paid to the young, which often cannot cope with floors suitable for adults. Bedding (preferably straw) allows exploration activity and some dwelling, but bedding of poor quality is worse for the rabbits health and normal behaviour than adequately perforated plastic or metal floors (Fleischner, 1998). Morisse et al (1999) have shown that fattening rabbits kept under intensive conditions in floor pens preferred a wire floor to a straw deep litter. Floors should be slip-proof, securely fixed within the cage and easy to clean and to remove. Furthermore, metal slatted floors should be constructed of flat bars with smooth, un-broken edges, perforated floors should be slip-proof and should carry the animals weight without vibration. Holes (diameter) and bars (width and distance) of metal and plastic floors should be adapted to the size of the strain to be housed to avoid damage to the paws and hygienic problems. Floor pens should have an easy to clean, slip-proof and well-insulated floor, e.g. securely fixed rubber matting, straw-bedded or slatted plastic floors (Morton et al, 1993; Stauffacher, 1993).

Further research is needed to study the impact of different floor types on the welfare of rabbits.
The group proposes that the rabbits' general considerations section should contain the following paragraph:

"Wire floors should not be used without the provision of a resting area large enough to hold all the rabbits at any one time, unless there are veterinary or scientific reasons for not doing so."

IIb.4.3.5 Proposals - General

The group's recommendations for minimum cage and pen dimensions and stocking densities are based on scientific evidence and good practice. As stated earlier, figures for minima (cage sizes) and maxima (stocking densities) can never be scientifically "proved". To set limits (minima and maxima) is a political and not a scientific question. Any claim for proper experimental proof for such limits would be the consequence of a fallacy. Nevertheless, animal science can provide sound arguments why limits should be set in some places.

The number of groups housing rabbits in floor pens is increasing continuously for animal welfare and economic reasons. So far, Appendix A of the CoE Convention ETS 123 (Council of Europe, 1986) provides no specific figures for housing rabbits in pens. Detailed information recommended by various authorities on minimum floor areas for groups of rabbits housed in pens is given in Morton et. al. (1993). The group, however, does not discriminate between minimum space requirements for cages and pens, as this may lead to difficulties in defining systems as either pens or cages. Moreover, overly stringent demands for floor pen housing might encourage cage housing. For both, cages and pens, it is strongly emphasised that structuring the space according to the principles described in this report is of the utmost importance.

Based on the current science-based knowledge of the rabbit's physiological and behavioural needs gained in conventional and in enriched cages, as well as in floor pens and in near-to-nature enclosures (references, see above), the group considers the following recommendations as a minimum:

(i) Young and sub-adult rabbits should be allowed the same space as adults, since they are more active and perform more rapid locomotion. For rabbits, minimum space allowances and stocking densities should always refer to the final weight that rabbits will reach (of a certain strain, sex, feeding regime) while housed in a particular compartment.

(ii) In cage-housing, each rabbit should be allowed to stretch full length along one side of the cage (not just diagonally). The height should allow the rabbits to sit up straight.

(iii) To facilitate the physiological development of their locomotory abilities, rabbits should be allowed to perform sequences of hopping steps.

(iv) Even in very limited space, e.g. in a cage, each rabbit should have a choice of resting places (e.g. floor and a shelf), and the possibility for withdrawal (e.g. underneath a shelf). The shelf should be large enough to allow the animal to lie and sit on it and to move easily beneath it. If there are good scientific or welfare reasons for not using a shelf, then the cage size should be enlarged by about a third. To make best use of the space available, the cage should be rectangular (e.g. 1.5:1) with a larger width than depth.
Most of the time, animals do not use space for its own sake; they use resources and structures within an area. Thus, minimum space requirements depend on the minimum spatial enrichment objects, which have to be incorporated into the cage or pen in a way the rabbits can cope with successfully. Furthermore, younger rabbits need more space for exercise than older and larger subjects, e.g. to perform intensive locomotory plays (Lehmann, 1989). Therefore, the group bases space requirements on the final adult weight that rabbits (of a certain strain, sex, feeding regime) will reach while housed in the particular facility.

Rabbits may be conditioned for successful use of an exercise area (Lehmann, 1987; 1989). This allows the rabbits to perform rapid locomotion and to exercise their locomotory apparatus. Furthermore, the use of a run may result in calmer animals during the rest of the day. Given access to an arena for half an hour at the same time every day, young fattening rabbits housed in conventional cages performed 65% of their rapid locomotory playing in the run, and the rest during the two hours before daily exercise. The control animals scattered about the same amount of rapid locomotory activity over many hours, and rapid locomotory activity was reduced to rotations around the body axis due to lack of space.

The optimal group size is determined by sex and age of the animals, cage size and experimental design. It is important to form harmonious groups and to keep group size and composition stable to avoid stress by altering the established hierarchy. A lot of research has been done on optimal group sizes for fattening rabbits with some contradictory results (e.g., Bigler and Oester, 1996; Morisse and Maurice, 1997; Rommers and Meijerhof, 1998). In the laboratory, it is good practice to house adult rabbits in groups of 5-20; this enables proper inspection of the animals and makes the pens easy to manage. However, in the production of fattening rabbits group size may reach 100 or more (Bigler and Oester, 1996), but in such large groups individual handling and monitoring is impossible. Depending on strain and feeding regime, young males need to be separated from each other from day 70-80 onwards to prevent serious injuries caused by repeated dominance fights (Bigler and Oester, 1996; Lehmann, 1989; Rommers and Meijerhof, 1998), but also from females to prevent intensive pre-mature sexual driving (Heath, 1972).

The group proposes that, prior to the species-specific tables for minimum cage and pen dimensions and stocking densities, the following paragraphs should be inserted:

"It is preferable for cages to be rectangular. A raised area must be provided within the cage. This shelf should allow the animal to lie and sit on and easily move underneath, it should not cover more than 40% of the floor space. While the cage height should be sufficient for the rabbit to sit upright without its ears touching the roof of the cage, this degree of clearance is not considered necessary for the raised area. If there are good scientific or veterinary reasons for not using a shelf then cage size must be 33% larger for a single rabbit and 60% larger for 2 rabbits. When rabbits are kept in cages regular access to an exercise area is recommended."

IIb.4.3.6 Proposals - Cages and pens for rabbits > 10 weeks of age

To determine the recommendations for minimum sizes of cages and pens, the quantity and the quality of space has to be taken into consideration. The crucial point is the interaction between the space, the structure, the animals and the type and quantity of enrichment provided. Beside provision ad libitum of gnawing objects (e.g. wood-blocks) and roughage (e.g. loose hay or straw, or pressed hay-rolls) to prevent oral deficits due to restricted possibilities for occupation (Lehmann, 1990; Lidfors, 1997), it is important that rabbits move in a way that proper development and maintenance of the locomotory apparatus is guaranteed even within restricted space. This can be realised either with daily access to a run (see above), or with the
insertion of a solid shelf. The shelf is very attractive, especially in pair housing; it helps to prevent skeletal problems even under restricted spatial conditions in cages (Bigler, 1998; Oester and Lehmann, 1993; Stauffacher, 2000). Furthermore, the shelf allows a choice for resting and for withdrawal (e.g. underneath the raised area), as well as a possibility for looking out from an elevated position.

Another advantage of the structured rabbit cage is that it allows the housing of two compatible female rabbits together (Bigler and Oester, 1994; Huls et al 1991; SOAP, 1991; Stauffacher, 1992, 1993, 2000). The benefits of having one social partner, at least, have been discussed earlier. With respect to locomotory development, the need for social interaction and control stimulates the rabbits to move around.

Group housing in pens increases the need for spatial subdivisions (i.e. by partitions or the use of the third dimension) to allow each animal to initiate or avoid social contact (Morton et al, 1993; Podberscek et al, 1991, Stauffacher, 1992, 2000).

Therefore, for cages and pens for rabbits > 10 weeks of age, the group proposes the following minimum floor areas and minimum heights:

"Cages and pens for rabbits > 10 weeks of age.
[GT 123 (2000) 57, Rabbits, Table 1]

<table>
<thead>
<tr>
<th>The final body weight kg that any rabbit will reach in this housing</th>
<th>Minimum floor area for one or two socially harmonious animals cm²</th>
<th>Minimum height cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3</td>
<td>3500</td>
<td>45</td>
</tr>
<tr>
<td>3-5</td>
<td>4200</td>
<td>45</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>5400</td>
<td>60</td>
</tr>
</tbody>
</table>

The table is to be used for both cages and pens. In cages a raised area must be provided (see Table 4). Pens should contain structures that subdivide the space to allow animals to initiate or avoid social contacts. The additional floor area is 3000 cm² per rabbit for the third, the fourth, the fifth and the sixth rabbit, while 2500 cm² must be added for each additional rabbit above a number of six."

The proposed minima are a substantial increase in comparison with the existing cage standards. However, since two rabbits can be successfully housed in one structured cage, the welfare gain and the economic costs can be balanced. The weight-bands reflect the sizes of the three categories of rabbit strains: small, medium and large. Most rabbits used for experimental purposes are of medium size (e.g. New Zealand White, Chinchilla, Sandy Lop, Russian, Belgian Hare).

IIb.4.37 Proposals - Cages for a doe plus litter

The group recommends that nest boxes for breeding does are placed outside the cage. Up to the age of about 8-10 days, young rabbits huddle in the hair nest for thermoregulatory reasons. They have only very brief contact with the mother (with a nursing time of only three minutes, once a day: Hudson et al, 1996; Stauffacher, 1988), and they react on vibrations by intensive movements preparing themselves for being nursed (Hudson and Distel, 1982). If the nestbox is within the cage, the doe uses its roof as a resting place. Jumping on the nestbox results in vibrations, and thus in repeated disturbance of the litter.
Under near-to-nature conditions (Wieser, 1986) and in breeding groups (Stauffacher, 1988) the doe stays away from the nest except for daily nursing. As soon as they have left the nest at about two weeks of age, the pups try to suck whenever they get the chance (Stauffacher, 1988). As the mother does not change the nursing frequency, she tries to escape these attempts (Bigler, 1986). Successful escape is only possible to places, which cannot be reached by the pups. Therefore, the group strongly recommends that in cages or small pens for breeding rabbits a shelf is made mandatory, unless specific experimental conditions or veterinary reasons prevent this.

For cages for a doe plus litter, the group proposes the following minimum floor areas, minimum heights and additional floor areas for the nestbox:

"Cages for a doe plus litter."
[GT 123 (2000) 57, Rabbits, Table 2]

<table>
<thead>
<tr>
<th>Doe weight kg</th>
<th>Minimum floor area cm²</th>
<th>Addition for nestboxes cm²</th>
<th>Minimum height cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3</td>
<td>3500</td>
<td>1000</td>
<td>45</td>
</tr>
<tr>
<td>3-5</td>
<td>4200</td>
<td>1200</td>
<td>45</td>
</tr>
<tr>
<td>&gt;5</td>
<td>5400</td>
<td>1400</td>
<td>60</td>
</tr>
</tbody>
</table>

At least 3-4 days before giving birth does should be provided with an extra compartment or a nestbox in which they can build a nest. The nestbox should preferably be outside the cage. Straw or other nesting material should be provided. The cage should be designed such that the doe can move to another compartment or raised area away from her pups after they have left the nest. After weaning the littermates should stay together in their breeding cage as long as possible. Up to 8 littermates may be kept in the breeding cage from weaning until 7 weeks old, and five littermates may be kept on the minimum floor area from 8 to 10 weeks of age.

IIb.4.3.8 Proposals - Cages and pens for rabbits < 10 weeks of age

For young rabbits, it is beneficial to stay together with littermates, as long as possible. To lower weaning stress, induced by the gradual change in the feeding regime and in the forced separation from the mother, it is advisable to take away the doe and to keep littermates in the breeding cage for some time. With the start of dominance-related and sexual behaviour (week 7 onwards), males have to be separated from females, and the stocking density has to be adapted to the increased need for space.

For cages and pens for rabbits < 10 weeks of age, the group proposes the following minimum floor areas, and minimum heights:

"Cages and pens for rabbits < 10 weeks of age."
[GT 123 (2000) 57, Rabbits, Table 3]

<table>
<thead>
<tr>
<th>Age</th>
<th>Minimum floor area cm²</th>
<th>Maximum number of animals on minimum floor area</th>
<th>For each additional animal add cm²</th>
<th>Minimum height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning - 7 weeks old 8-10 weeks</td>
<td>4000</td>
<td>5</td>
<td>800</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>3</td>
<td>1200</td>
<td>40</td>
</tr>
</tbody>
</table>

The table is to be used for both cages and pens. Pens should contain structures that subdivide the space to allow animals to initiate or avoid social contacts. After weaning the littermates should stay together in their breeding cage as long as possible."
IIb.4.3.9 Proposals - Shelf dimensions for rabbit cages

The subdivision of the cage with a shelf is only beneficial if the rabbits can make proper use of it. This is necessary to prevent accidents and is also a precondition for a harmonious relationship between two cage-mates. It is important that the size of the shelf allows a rabbit to rest in a stretched position. The distance of the shelf from the top of the cage should allow to sit on the shelf for grooming, and the shelf’s height above floor level should allow the rabbit to move rapidly underneath. There should be enough floor space with full cage height for the performance of hopping sequences and for jumping onto the shelf. The ratio of the size of the shelf to the total floor area should be about 2:5. Thus, the minimum size of a rabbit cage is given by the adequate subdivision of the cage: for one or two medium-sized rabbits with an adult weight of 3-5 kg, the minimum cage dimensions are 4200 cm$^2$ floor area, 1650 cm$^2$ (55 x 30 cm) shelf area, and an overall height of 45 cm, at minimum. To allow proper sitting and grooming on the shelf, 50-55 cm would be needed.

If there are good reasons for not using a shelf (e.g., veterinary or scientific), then the cage size should be enlarged by about a third for one rabbit, and 60% for two rabbits, respectively, to facilitate the physiological development of the rabbit’s locomotory abilities and to give a second rabbit some chance to escape (also realised in SOAP, 1991).

Therefore, the group proposes the following dimensions for the shelf in rabbit cages:

"Recommended shelf dimensions for rabbit cages.
[GT 123 (2000) 57, Rabbits, Table 4]

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Final body weight (kg)</th>
<th>Approximate size (cm x cm)</th>
<th>Approximate height above cage floor (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>-</td>
<td>55 x 25</td>
<td>-</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>&lt;3</td>
<td>55 x 25</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>3-5</td>
<td>55 x 30</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>60 x 35</td>
<td>30</td>
</tr>
</tbody>
</table>

To allow proper use of the shelf and of the cage as a whole the approximate shelf size is an optimum with minima and maxima very close (+/- 5-10%). If there are scientific or veterinary reasons for not using a shelf then cage size should be 33% larger for a single rabbit and 60% larger for 2 rabbits."

IIb.4.4 Feeding

No specific recommendations for rabbits; see Section I, Chapter 4.2 of this report, and General Part of Appendix A, GT 123 (2000) 54.

IIb.4.5 Watering

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.4.6 Substrate, litter, bedding and nesting material

Bedding allows exploration, oral activity and a certain degree of dwelling. As such, bedding also serves as environmental enrichment. Turner et al (1997) have shown that dust-free straw and shredded paper are preferred to sawdust and wood shavings in pens. But bedding of poor quality can have a worse effect on the rabbits' health and behaviour than good quality perforated plastic or metal floors. If roughage is offered in trays, bedding is not specifically recommended in animal rooms with controlled climate.
Rabbits do not construct nests for resting, such as mice, hamsters and gerbils. In breeding, the importance of the provision of good quality nesting material has been discussed earlier.

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.4.7 Cleaning

There is no doubt that good hygiene prevents a range of disease conditions in laboratory animals and improves the health of the animals (Vesell et al, 1973). However, for behavioural guidance, it is advisable to deposit some soiled bedding on places where the rabbits should preferably eliminate.

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.4.8 Handling

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.4.9 Humane killing

The group proposes that the European Commission DG XI Guidelines on Euthanasia (Close et al, 1996, 1997) be added to ETS 123 as a separate appendix.

IIb.4.10 Records

No specific recommendations for rabbits; see General Part of Appendix A, GT 123 (2000) 54.

IIb.4.11 Identification

In group-housing, it is often necessary to identify rabbits individually, either temporarily or permanently. It is advantageous for animals to be individually identified to ensure good monitoring of health and behaviour. In animal experimentation (e.g. production of polyclonal antibodies, pyrogenicity testing), individual data sampling makes accurate identification necessary, especially for group-housed rabbits. In Table 6, some proven methods of identification are listed for rodents and rabbits (Keely et al, 1988; Morton et al, 1993). The different methods have both advantages and disadvantages; which one is selected depends on the specific purpose for which identification is needed. Ideally, non-invasive methods should be used. If permanent identification is required, consideration must be given to the degree of discomfort to the animal during the marking action, to the training of staff and to the use of sedatives or local anaesthetics.
### Table 6  Marking methods for rabbits

<table>
<thead>
<tr>
<th>Non Invasive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coat colour:</strong></td>
<td>Only usable with white or mixed coloured strains.</td>
</tr>
<tr>
<td><strong>Felt marker / marker dye:</strong></td>
<td>On ear, needs regular renewal, can cause skin irritation. Coats can also be marked but require regular renewal.</td>
</tr>
<tr>
<td><strong>Leg rings:</strong></td>
<td>Numbered, aluminium or plastic. Usually fitted at weaning. Can cause irritation, need regular checking to ensure they do not become too tight.</td>
</tr>
<tr>
<td><strong>Fur clipping:</strong></td>
<td>Grows out quite quickly, needs regular renewal.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Permanent Methods</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microchips:</strong></td>
<td>Subcutaneous implant, usually inserted at weaning. Need competent trained staff and decoding equipment (no external indication of animal’s identity). Can migrate if not positioned properly. Some types are reusable after cleaning and sterilisation.</td>
</tr>
<tr>
<td><strong>Ear tattooing:</strong></td>
<td>Forceps or tattoo gun, less disturbance with forceps, usually done at weaning so tattoo expands with the growth of the animal. Local anaesthetics should be used to minimise trauma. May be difficult to read on pigmented animals. Does not require any special equipment for interpretation.</td>
</tr>
<tr>
<td><strong>Ear tags:</strong></td>
<td>Can fall out, get caught in caging or become infected. Can be painful to insert.</td>
</tr>
</tbody>
</table>
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