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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)

8th Meeting of the Working Party
Strasbourg, 22-24 September 2004

Species specific provisions for Amphibians

Background information for the proposals presented by the Group of Experts on Amphibians and Reptiles

PART B

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Background information

On the species-specific proposals for amphibians

Presented by the Expert Group on Amphibians and Reptiles

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Contents

Preamble

This document contains species-specific proposals for amendments to the Appendix A of the Council of Europe's Convention ETS 123 dealing with the protection of animals used or intended for use in any experimental or other scientific procedure which may cause pain, suffering, distress or lasting harm.

In 1997, the Council of Europe established working groups with the aim of advising the Council whether, how and to what extent the Appendix A of the Convention ETS 123 needed revision. The expert group appointed to deal with species-specific aspects of amphibians and reptiles was set by representatives of the following international organizations:

European Science Foundation, *ESF*
European Federation of Pharmaceutical Industries and Associations, *EFPIA*
European Federation of Animal Technologists, *EFAT*
Eurogroup for Animal Welfare, *EUROGROUP*
Federation of European Laboratory Animal Science Associations, *FELASA*
Canadian Council of Animal Care, *CCAC*

Representatives were:

Prof. Dr. Jörg-Peter Ewert (Coordinator)
ESF

Prof. John E. Cooper, DTVM, FRCPath, FIBiol, FRCVS
FELASA

Dr. Tom Langton
EUROGROUP

Prof. Dr. Gilbert Matz
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Kathryn Reilly
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Dr. Helen Schwantje
CCAC

The Group was complete in September, 2000. Unlike expected, no representative from the *EFAT* participated in this Group. Yet Mr. Chambers of *EFAT* let the Council's Secretariat know that *EFAT* remain very interested in the work of the Group and will give comments and suggestions on the proposal to be made by the Group.

The general tasks of the Group were defined as follows:

a. listing, for the species concerned, the main questions to be answered with a view to revising Appendix A;
b. examining results already available and practical experience acquired which could possibly answer these questions;
c. identifying areas where further research would be needed;
d. preparing proposals for amendments to Appendix A, providing information in particular to the ethological and physiological needs of the animals. These proposals (Part A) should be supported by background information in an explanatory report (Part B), presenting scientific evidence and/or practical experience.

The Group was expected to send a first draft of the proposal for the revision of the species-specific parts of Appendix A by 15 January 2001.

By the middle of November, 2000, the General Coordinator Dr. Wim de Leeuw suggested that Prof. Dr. J.-P. Ewert be the Coordinator of the Expert Group on Amphibians and Reptiles. Since there were no objections, Ewert accepted to do this job, presented a preliminary draft of a proposal to the group members on December 12, 2000, and asked for suggestions for improvements. In this draft, the presentation of the consensus proposals made by the Group of Experts on Rodents and Rabbits (Strasbourg, 21 February 2000) and the Standard Format for Species Specific Sections was used formally as a basis. The Resolution on the Accommodation and Care of Laboratory Animals adopted by the Multilateral Consultation on 30 May, 1997, was taken into account where appropriate. Furthermore, the Guide to the Care and Use of Experimental Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press, Washington, D.C., 1996) and the Guide of the Swedish Board of Agriculture (Department for Animal Production and Health, Animal Welfare Division) were considered. Since there were no suggestions for improvements on the draft proposal by the group members, the Coordinator sent the consensus proposal to the European Council's Drafting Group in due time by 14 January, 2001.

The original draft proposal for the revision of Appendix A concerning amphibians and reptiles presented by the Expert Group was sent to the members of the Drafting Group for consultation [see paragraphs 236 and 237 of the Summary Proceedings GT123(2000)39]. In agreement with the General Coordinator, the Chairman of the Working Party and the members of the Drafting Group, and in order to bring the document in line with the presentation previously adopted by other groups of experts, the document was divided into two parts: Part A containing paragraphs with proposals for Appendix A [doc. GT123(2001)1], and Part B providing detailed scientific background information supporting these proposals [doc. GT123(2001)23]. Paragraphs referring to Part A are in bold face italics, framed by boxes.

Part A. The species-specific proposals of Part A, concerning amphibians and reptiles, were revised based on suggestions and comments provided by the documents of the Drafting Group (*) and the representatives and observers at the meetings of the Working Party at Strasbourg from 2001 through 2003 [cf. also Summary Proceedings of the Working Party, GT123(2001)35, GT123(2002)41, GT123(2003)40, GT123(2003)41, GT123(2003)72, and of the Drafting Group GT123(2003)57rev].

Amphibians & Reptiles:
GT 123 (2001) 1)*
GT.123 (2001) 23)[A&B]*
GT 123 (2001) 30
GT 123 (2001) 31
GT 123 (2002) 5
GT 123 (2002) 10)*
GT 123 (2002) 25
GT 123 (2002) 40)*
GT 123 (2002) 56
GT 123 (2002) 59
GT 123 (2002) 62
GT 123 (2002) 64
GT 123 (2002) 69
GT 123 (2003) 15)*
GT 123 (2003) 27
GT 123 (2003) 33
GT 123 (2003) 35

Amphibians:
GT 123 (2001) 1E rev3)*
GT 123 (2003) 51)*
GT 123 (2003) 62
GT 123 (2003) 65
GT 123 (2001) 1
GT 123 (2004) 1E)*
[Rev. Appendix]

The revision of the proposals proceeded preferably via e-mail communication in the Expert Group on Amphibians and Reptiles. On September 10th and 11th 2001, a meeting of the Expert Group was organized by Kathryn Reilly at Harlow/Essex UK. This meeting was supported by the Merck Sharp & Dohme Company. Furthermore, the Coordinator participated in meetings at the *Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft* organized by the representative of Germany of the Working Party at February 19th and November 6th ,2003.

At the 6th meeting of the Working Party in March 2003 it was decided to separate the proposals of Part A in two documents: *Species-specific Provisions for Amphibians* and *Species-specific Provisions for Reptiles.* Both documents were adopted and finalized at the 7th and 8th meeting of the Working Party in December 2003 and September 2004.

Part B. The present background information provides, where possible, scientific evidence for the *Species-specific Provisions for Amphibians*. Where this is not available, they take account of established good laboratory practice, based both on the experience of the members of the Expert Group and also on consultations with other experts. Additional comments and suggestions from members of the Working Party are considered and incorporated in Part B where appropriate. The revised Part B including Part A [GT123(2003)69] was submitted to the Council of Europe in advance of the 7th meeting of the Working Party in December, 2003.

In reply to the general tasks a-d:

a. *Listing, for amphibians, the main questions to be answered:*

(1) The peculiarities of the body skin make amphibians significant bio-indicators of the environmental health. In view of the world-wide declines of populations of amphibians and [cf. Sections 1.1 and 4.8] a selection of suitable species for the use in scientific procedures should be recommended [Section 1.2]. "Suitable" means that captive breeding programs for this species exist, and/or the population of this species is not in danger. Captive breeding programs should be promoted [Section 3.3].
(2) Recommendations for housing amphibians (minimum cage sizes, heights) under consideration of the natural biotope and the species-specific needs are required [Section 4.3].
(3) Amphibians are ectothermic and thus strongly adapted to their different biotopes. This requires species-specific considerations regarding temperature and humidity preferences, homeostatic capabilities, and seasonal activity patterns [Sections 2.1 to 2.4].
(4) Knowledge on diseases of amphibians and their treatments should be incorporated [Sections 1.1 and 3.4].

b. *Examining results already available and practical experience acquired which could answer these questions:*

CITES lists of protected amphibian species are cited in Section 1.1. Examples of amphibian species from the four main habitats (aquatic, semi-aquatic, semi-terrestrial and arboreal) frequently used in experimental and other scientific procedures are listed in Section 1.2. Furthermore, a selection of amphibian species is recommended and an example of a breeding program is elaborated. A reference list is provided. Information on caging amphibians (cage dimensions, temperature/humidity preferences) is provided in Section 4.3 and in an Appendix.

c. *Investigating what research is being carried out within the field and identifying areas where further research would be needed*:

An Internet MEDLINE search on research activities in amphibians among different science disciplines is provided in Section 1.2. See also reference list.

d. *Providing information in particular to the ethological and physiological needs of amphibians and reptiles:*

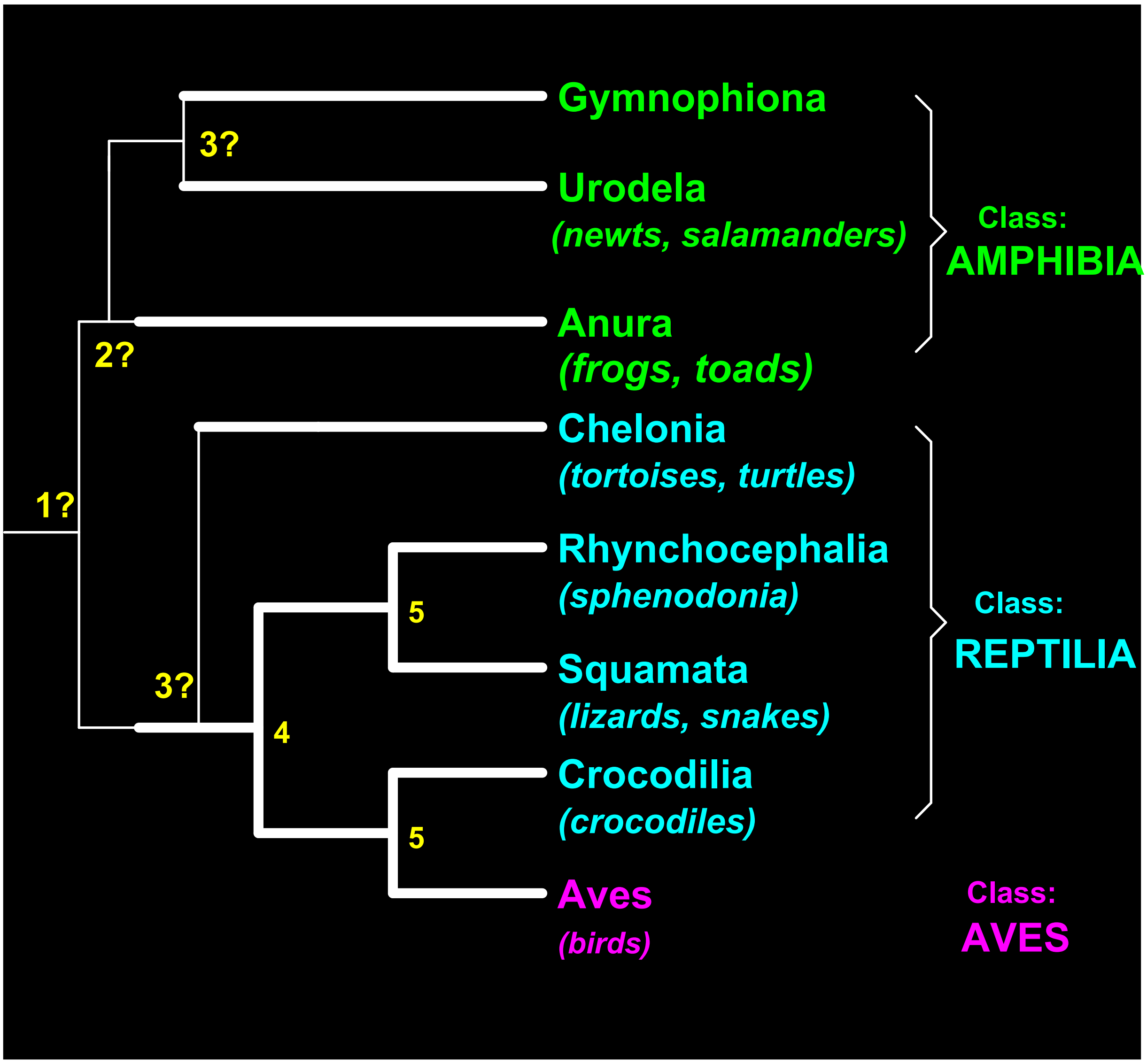
The standard format of the species-specific Sections 2-4 is provided with explanatory reports for the proposals and recommendations and these are supported by scientific evidence and practical experience.

Amphibians

I. Species-specific provisions for amphibians

1. Introduction

Cladogram



modified after Hennig and other authors

1: early, 2: middle, 3: late Carbon

4: middle Perm

5: late Trias

1- 5

Fig. 1. Cladogram of amphibians and sauropsides (birds and reptiles).

According to systematics, amphibians involve three main orders: Urodela (Caudata), Gymnophiona (Apoda), and Anura (Ecaudata). The Anura belong to the super-order Salientia. For the present provisions, Urodela (salamanders, newts) and Anura (frogs, toads) are of interest. They differ greatly in their patterns of geographic distribution and in the diversity of living types, such as aquatic (for example, Xenopus laevis), semi-aquatic (for example, Rana temporaria), semi-terrestrial (for example, Bufo marinus) and arboreal (for example, Hyla cinerea). Amphibians occupy a wide range of habitat types from arid deserts to deep freshwater lakes. Some may spend most of their life underground or high in cloud forest canopy. Some are found north of the Arctic Circle and can tolerate freezing conditions, while others have evolved a range of adaptations to avoid desiccation in hot areas of the world.

In the cladogram shown in Fig. 1, amphibians and reptiles are traced back to a common ancestor living in early Carbon. Lizards and snakes, on the one hand, and crocodiles and birds, on the other hand, can be traced back to a common ancestor living in the middle of Perm. The common ancestor of crocodiles and birds probably lived in late Trias. Among the amphibians, the urodela (newts and salamanders) and the anura (frogs and toads) are of importance for the present proposals.

Amphibians and reptiles are different in many other aspects of which some will be mentioned. An ontogenetic developmental aspect points to the fact that reptiles – like birds and mammals – belong to the Amniota due to the existence of embryonic sheaths. Amphibians are Anamniota. The eggs of reptiles are mostly laid on land and develop into animals already adapted for land life. Amphibian eggs are mostly laid in the water and develop into tadpoles which after metamorphosis mature to the adults. Whereas almost all salamanders and gymnophiona (caecilians) are internal fertilizers, most anurans are external fertilizers.

From an evolutionary point of view, basic research both in amphibians and reptiles is of fundamental interest in order to evaluate comparable functional principles in mammals and, thus, also in humans (e.g., see Ewert, 1998). Phylogenetically, reptiles and mammals can be traced back to a common ancestor from that amphibians are derived. Current research suggests that certain morphological and physiological basic principles, present in amphibians and reptiles, were conserved during evolution up to the primates, in an appropriate differentiation and specification.

Amphibians are very much adapted to the substrate on/in which they live. In this context, the body skin plays an important role in the transfer of water, soluble substances, including toxic substances and oxygen. Therefore it plays key roles in the survival of amphibians, their interaction with their environment, and their ability to exploit a wide range of habitats and ecological conditions. An amphibian's health depends on certain properties and peculiarities of its body skin, thus making amphibians significant bio-indicators of environmental health.

A variety of functions in amphibians is performed by the skin as a complex organ:

- Sensing of mechanical, thermal, and chemical stimuli [cf. Sections 1.1, 2.2 and 2.3].
- Exchange of respiratory gases [cf. Section 2.1] which requires a moist body surface [Section 4.5].
- Hormone-controlled uptake of Na^+, Cl^-, and water through the epithelium [cf. Section 2.3].
- Communication through odorous substances.
- Chemical defence by antimicrobial peptides and poisons secreting glands [cf. Section 4.8].
- Mechanical protection by a tough, flexible and slippery wrapping.
- Development of specialised mechanical devices, such as warts and claws.
- Protection by camouflage, i.e. change of colour and/or colour pattern.
- Protection (e.g., against parasites) by the regular shedding of the outermost cell tissue.

(Lindemann & Voûte, 1976). Amphibians shed their skin spontaneously irregularly, in response to certain chemical stimuli even suddenly.

1.1. Declining amphibian populations

Amphibians are an important part of the ecological balance of many habitats. Amphibians thus are environmental sentinels (Roy, 2002). In some habitats, the biomass of amphibians makes them significant prey items for, and predators of, other species. Amphibians are also important for ecological and biodiversity studies, giving information on the ecological impact of both local and global changes, sometimes with implication for humans (e.g., Heyer et al., 1994; Daszak et al., 2000; Daszak & Cunningham, 2003). Many populations of amphibians are declining on all six continents on which they occur (e.g., Kiesecker et al., 2001; Pounds, 2001). Some causes of declines – habitat destruction, application of xenobiotics, introduction of predators or competitors – are attributable to human activities. As pointed out by the Declining Amphibian Population Task Force (DAPTF), over the last 50 years, many species of amphibians (frogs, toads, salamanders, and newts) throughout the world have declined marked by in numbers. Some species have even become extinct. The reasons for the declines are, on the one hand, a direct response to the impact of human activities (habitat destruction, pollution) acting on local level. On the other hand, there may be several global factors that are adversely affecting amphibians. Strategies for assessing the implications of malformed frogs for environmental health are discussed by Burkhart et al. (2000). Possible causes of declining amphibian populations are, for example:

- Increase in ionising radiation resulting from ozone layer depletion (eggs of frogs and toads can be damaged by UV-B).
- Chemical contamination (e.g., oestrogenic effects of pesticides and precipitation, effects of fertilizers and herbicides).
- Contamination by natural fertilizers (e.g., leading to an increase in the population of parasites, such as the trematode worm *Ribeiroria ondatrae* whose larvae – developing in the snail *Planorbella* – affect tadpoles and disturb the normal development of the extremities during metamorphosis). For effects of trematode infection see also Johnson et al. (1999) [cf. also Section 3.4].
- Fungal infectious diseases, e.g., chytridiomycosis (Berger et al., 1998; see also Section 3.4).
- Introduction of exotic competitors and predators (e.g., fish introductions, aqua-cultural practices; see also Chivers et al., 2001).
- Pathogens (e.g., environmental stress reduces the ability of amphibians to resist diseases, such as those caused by poxvirus-like particles).

Results of long-term studies on fluctuations observed in natural amphibian populations are presented by Meyer et al. (1998). Several incidences where sentinel species have responded to effects of chronic exposure to ambient levels of environmental contaminants are discussed by LeBlanc & Bain (1997). The need for water quality criteria for frogs is pointed out by Bover & Grue (1995). Contaminated ground water, for example, poses a significant health hazard and may also impact wildlife, such as amphibians, when it surfaces (Bruner et al., 1998). Decreases in water quality may be associated with frog embryo mortality and malformations (Boyer & Grue, 1995). Wastewater effluent-irrigated ponds may affect amphibian populations by reducing the survival of amphibian eggs and larvae (Laposata & Dunson, 2000; Harris et al., 2000). Acidification of breeding ponds has been identified as a potential threat to the survival and health of North American amphibian populations. Exposure of embryonic or larval *Bufo americanus* to moderately acidic water (pH 6.0) disrupts the nitrogen balance by increasing nitrogen loss as ammonia, with no compensatory decrease in urea excretion (Tattersall & Wright, 1996; cf. also Hatch et al., 2001; Marco et al., 2001). Amphibian larvae are commonly exposed to low levels of pesticides during their development. Effects of the insecticide Carbaryl are different at different life stages. Any delay in metamorphosis or decrease in size at metamorphosis can impact the population, potentially leading to declines or local extinction (Bridges, 2000). For effects of pesticide exposure at various life stages of leopard frogs see Bridges (2000) and Harris et al. (2000; cf. also Kiesecker et al., 2001; Sparling et al., 2001). Effects of the herbicide Diuron on survival and growth of tree frogs are described by Schuytema & Nebeker (1998; cf. also Normile, 1999; Withgott, 1999; Renner, 2003). Antimicrobial peptide defences against pathogens, too, are associated with global amphibian declines (Rollins-Smith et al., 2002).

Another cause of amphibian decline may be crankcase oil that leaks from motor vehicles and washes into ponds. Ponds containing oil and silt produce salamanders of reduced size and weight. Silt results in reduced growth, earlier metamorphosis and increased susceptibility to *Saprolegnia parasitica* (Lefcort et al., 1997). The impact of lead (Pb) was seldom considered in declines of amphibian populations due to man-made changes in the environment (Vogiatzisch & Loumbourdis, 1999). Under laboratory conditions, concentrations of boron and nitrate within the range measured in wastewater effluent (likely due to rainwater dilution) reduced the hatching process in *Bufo americanus* and produced deformed off-springs in *Rana sylvatica*, *Ambystoma jeffersonianum* and *A. maculatum* (Laposata & Dunson, 1998). It is suggested (Rouse, 1999) that nitrate concentrations in some watersheds in North America are high enough to cause death and developmental anomalies in amphibians and impact other animals (amphibian prey) in aquatic ecosystems (see also Laposata & Dunson, 1998; cf. also Nebeker & Schuytema, 2000; Johansson et al., 2001). For strategies for assessing the implications of malformed frogs for environmental health see Burkhart et al. (2000).

The mortality in amphibians is positively correlated with solar UV radiation (Bruner et al., 1998; see also Ankle et al., 2002; Diamond et al., 2002; Peterson et al., 2002). Differential sensitivity to UV-B radiation among species may be one factor contributing to population declines in amphibians (Phalli et al., 2003). In some species, ambient levels of UV-B cause embryonic mortality in nature or cause deformities in amphibian embryos (Blustering et al., 1997; cf. also Hays et al., 1996). The detrimental effect of UV-B alone or with other agents may ultimately affect amphibians at the population level. For example, synergistic effects between UV-B and a pathogenic fungus increase significantly the mortality of amphibian embryos (Diesinker & Blustering, 1995). The eggs, the most UV-B-sensitive stages, contain photolyase the important enzyme for repair of the major UV photoproducts. Hatching success correlates strongly with photolyase. Different species display (100-fold) different levels in photolayse. Low-egg-photolyase species decline. High-egg-photolyase species may be either robust or are showing declines rarely. The latter effect seen in some species may be due to low-skin-photolyase levels in the developing tadpoles (Hays et al., 1996; for defence against UV-B radiation see also Blaustein & Belden, 2003).

Besides the causes clearly attributable to human activities, infectious diseases appear to be a direct cause of mass amphibian die-offs in relatively undisturbed areas of the world (Daszak et al., 1999). In these cases, it is not yet clear whether these epizootics result from the natural evolution of new pathogens from environmental changes that promote the emergence of pathogenic forms and/or that weaken the immune defence of amphibians (Carey, 1999, 2000). With the amphibian metamorphosis, the immune system becomes reorganized. Most amphibians probably survive the temporary immune-suppression associated with metamorphosis with no deleterious effects (see also Flajnik, 1996). However, if environmental stressors result in the induction of metamorphosis at a less than optimal body size and state of immune maturation, the amphibians could be at a greater risk of infection and death (Rollins-Smith, 1998; see also Denver, 1997). Urodele amphibians show relatively weak and slow immune responses compared to anuran amphibians (Salvadori & Tournefier, 1996).

Future goals in this context should include the establishment of further activities of scientific research that cover all areas of the world where amphibians live, in order to discover which species are rare or declining and to investigate the reasons behind these declines. The DAPTF, established in 1991 by the Species Survival Commission (SSC) of the World Conservation Union (IUCN), can be reached:

http://www.open.ac.uk/daptf/about_daptf.htm - 5k -
On protected amphibian species, see:
http://www.CITES.org

Where possible, amphibians used for experimental or other scientific purposes should be bred and reared in captivity. Purpose-bred animals should be used in preference to animals taken from the wild.

A main problem to be addressed with a view to revising Appendix A to the Convention ETS 123 concerns the declining amphibian populations, on the one side, and the consumption of amphibians for the use in experimental or other scientific procedures, on the other side. One way to tackle this problem is a selection of species under the aspects of protection and breeding programs that maintain the population of amphibians in captivity.

1.2. Selection of species

1.2.1. Examples of species from the four main habitats

Table I.1 lists the four main habitats of amphibians and examples of species of each habitat frequently used for experimental and other scientific purposes. The following proposals provide details on the basic accommodation and care conditions to be covered for species of these habitats. Specific procedures may require the use of certain other species which do not fall into the four habitat categories. Further advice on requirements for these and other species (or if behavioural or breeding problems occur) should be sought from expert specialists and care staff to ensure that any particular species needs are adequately addressed. Additional background information on less commonly used species and habitats is available in the background information document elaborated by the Group of Experts (see paragraph 4, Introduction, General Section).

Table I.1: Main habitat categories and examples par habitat of species frequently used

Habitat	Amphibian species	Size	Original geographic distribution/ Biotope	Optimal temperature	Relative humidity	Main period of activity
Aquatic Urodeles	Ambystoma mexicanum (Axolotl)	24-27 cm	Mexico / Channels of the former sea of Xochimilco	15-22°C	100%	Twilight
Aquatic Anurans	Xenopus laevis (Clawed frog)	6-12 cm	Central and South Africa/ ponds, ground water and spring-fed	18-22°C	100%	Twilight/ Night
Semi-aquatic Anurans	Rana temporaria (Common frog)	7-11 cm	Europe (middle and north) to Asia (without southern Balkan) / Near ponds, lakes, streams (shores, meadows)	10-15°C	50-80%	Day/night
Semi-terrestrial Anurans	Bufo marinus (Marine toad)	12-22 cm	Central and South America/ Mangrove, woods	23-27°C	80%	Night
Arboreal Anurans	Hyla cinerea (Green tree frog)	3-6 cm	Southeast USA / Open bushy borders of cypress swamps, flat country, forest	18-25°C	50-70%	Day/night

1.2.2. Recommended species

The variety of adaptation and the diversity in environmental requirements among amphibian species and subspecies lead to restrictions in establishing proper general conditions for their long-term maintenance in captivity. This is one point suggesting a selection of species of amphibians used for experimental or other scientific procedures.

Another restrictive point concerns the experience that certain European ranid frogs will often not feed in captivity and are, therefore, not suitable for long-term maintenance. One reason is that they hardly accept artificial or semi-natural environments and do not tolerate the disturbances of human traffic, such as even slight vibrations caused by steps at long distances.

A further problem concerns the fact that – unlike fish, birds, and mammals – there are only a few strains of amphibians (genetically adapted to captive conditions) available commercially [for an instruction of breeding *Bombina orientalis* see Section 3.3]. Therefore, most amphibians suitable for holding under laboratory conditions must be collected from the wild. However, consumptive use of significant numbers of wild caught animals from a species or population, which is rare, endangers its extinction or local extirpation. This aspect together with ecological constraints in connection with the progressively endangered natural biotopes of amphibians strongly calls for species protection.
Weighing all these aspects together, it is advisable to consider amphibian species for experimental or other scientific purposes which can be bred and reared easily under laboratory conditions, such as

Ambystoma mexicanum	(Urodela neotenic larvae, aquatic)
Xenopus laevis	(Anura, aquatic)
Xenopus tropicalis	(Anura, aquatic)
Bombina orientalis	(Anura, semi-terrestrial),

and amphibian species whose populations grow progressively, such as

Bufo marinus	(Anura, semi-terrestrial)
Rana catesbeiana	(Anura, semi-aquatic).

Although there may be health concerns over obtaining *Xenopus* from the wild, views have been expressed that these animals obtained from the wild are in general more fecund than their lab-bred counterparts and as a result, scientists use fewer animals to obtain the amount of biological material that is required. *Xenopus laevis* is not an endangered species, and there is no indication that numbers of this species are decreasing in the wild. The same holds true for the European common frog *Rana temporaria* and the American green tree frog *Hyla cinerea*.

The use of certain foreign, not endangered species may become problematically. The UK government, for example, has put severe restrictions on the importation of certain species capable of breeding in the wild in the UK, including the bullfrog *Rana catesbeiana.* The UK government spent about €50,000 for removing a bullfrog population in 1999-2001. In Germany (Rhine area) bullfrogs propagate increasingly and in France the situation seems to be almost out of control. In Spain, on the other hand, bullfrogs belong to anuran species most frequently used in research.

Suggested reading for:

Ambystoma mexicanum [Armstrong & Malacinski, 1989; Brandon, 1989; Smith, 1989]
Xenopus spp. [Kobel & Tinsley, 1996; Elepfand, 1996a,b; Elepfand et al., 2001]
Bombina orientalis [Zimmerman, 1986; Mattison, 1987; Plesner, 2002]
Bufo marinus [Alexander, 1964; Duellman Trueb, 1985; Zug, 1979; 1993; Wachowitz & Ewert, 1996]
Rana catesbeiana [Korschgen & Moyle, 1955; Hayes & Jennings, 1986; Stinner et al., 1994; Bee, 2002]
Rana temporaria [Savage, 1961; Aertsen et al., 1986; Miaud & Guyetant, 1998; Miaud et al., 1995, 1999]
Hyla cinerea [Capranica & Moffat, 1983; Gerhardt, 1981; Moulton et al., 1996; English & English, 2000]

Specific procedures may require the use of certain other species (except those listed in CITES). The permission to use such species for investigations will depend on the applicant's justification for the use and on the decision of the respective authorities of the country in which these investigations are conducted.

1.2.3. Current research

An internet MEDLINE search comparing the number of publications (original research papers) on amphibians in relation to those on reptiles and mammals (rhodents, such as rats and mice) shows that amphibians and reptiles are much less frequently used (Fig.2). Among amphibians, *Xenopus* spp. is the most frequently used genus. An ISI search for all publications on *Xenopus* spp. even shows some kind of exponential increase from 1980 to 2000. The frequent use of *Xenopus* spp. as research subject explains the dominance in the number of research papers on amphibians compared to those on reptiles.

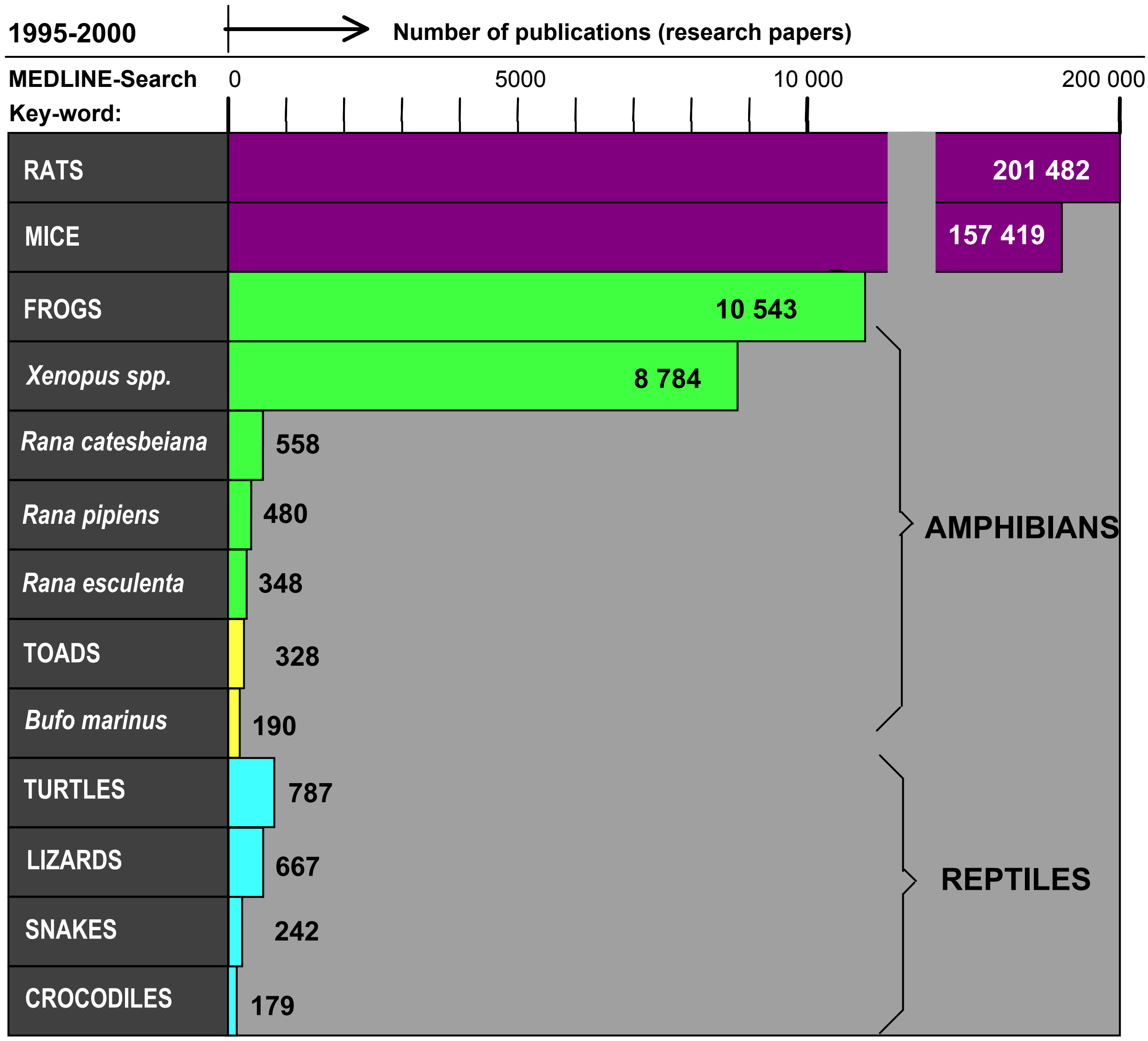


Fig. 2. Internet search for scientific publications related to rhodents, amphibians, and reptiles.

For the time segment 1995 to 2000, a MEDLINE search shows the following research activities in different scientific disciplines (key words: amphibia, amphibians):

Research Discipline 1995-2000	Publications (%) on amphibians
Cellbiology	**17.0**
Vegetative Physiology	**16.9**
Genetics	**14.7**
Morphology	**14.2**
Brain Research	**11.3**
Ecology	6.4
Sensory Physiology	6.1
Biochemistry	5.2
Parasitology	4.9
Pharmacology	2.0
Ethology	1.0
Housing	0.3

By setting a 10-% level, it is evident that studies in Cellbiology, Vegetative Physiology, Genetics, Morphology, and Brain Research preponderate. In functional Morphology, for example, basic research on regeneration capabilities is of medical interest. Urodele amphibians are unique among adult vertebrates in their ability to regenerate organs or parts of it. Regenerated limbs are often indistinguishable from the developmentally produced original (Christensen & Tassava, 2000; see also Crews, 1995; Tsonis et al., 1995; Brockes, 1997; Imokawa & Yoshizato, 1998; Carlson et al., 1998; Torok et al., 1998; Gardiner et al., 2002; Nye et al., 2003). Urodeles also regenerate, for example, the tail (Benraiss et al., 1996) and parts of the spinal cord (Chernoff, 1996; Echeverry & Tanaka, 2003; Chernoff et al., 2003; Zhang et al., 2003).

Obvious deficits in research emerge, for example, in studies on housing and captive breeding amphibians in view of declining populations [see also Section 1.2].

2. The environment and its control

First of all, experience from *good laboratory practice* (GLP) should be considered. In 1990 Greendale Laboratories UK became the first dedicated laboratory to achieve full GLP compliance for all its disciplines. The GLP scheme became administered by the Medicines Control Agency of the Department of Health. Having GLP means that every aspect of the laboratory from automatic analysers right down to the smallest piece of equipment has calibration, maintenance and test methodologies rigorously controlled and checked both prior and during their working lives. It is internationally recognized that GLP is one of the highest quality assurance accreditations that a laboratory can attain. GLP is concerned with the organisational processes and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported. Adherence by laboratories to the principle of GLP ensures the proper planning of studies and the provision of adequate means to carry them out. It facilitates the proper conduct of studies, promotes their full and accurate reporting, and provides a means whereby the integrity of studies can be verified.

Cooper (1984) working on Exotic, Zoological and Wildlife species, is a Consultant Pathologist with Wildlife Health Services UK (see also Cunningham et al., 1996; Cooper & Cooper, 2003).

Persons carrying out scientific procedures on amphibians will maximise the overall benefit for animals as individuals and as a group. Laboratory amphibians must be provided with accommodation and care appropriate to their health and welfare, with consideration of their physiological and ethological needs (e.g., see Dickerson, 1931; Matz, 1983; Duellman & Trueb, 1985; Zimmerman, 1986; Mattison, 1987; Stebbins & Cohen, 1995; Conant & Collins, 1998; O'Rourke, 2002; Matz & Weber, 2002; Matz & Vanderhaege, 2003; Bolhuis & Giraldeau, 2004).

Laboratory amphibians are accommodated in a *vivarium* (e.g., terrarium, aqua-terrarium, tank). A vivarium is any room, building or other facility in which live, vertebrate animals are housed for periods of time exceeding 24 hours. The housing of animals should conform to the *NIH Guide for the Care and Use of Laboratory Animals in Research* (NIH publication No. 85-23, 1985 or succeeding editions). A new vivarium to be constructed should conform to the recommendations of the NIH Guide. Although desirable, it is impractical to require older vivaria to meet all of these standards. Remodels of older facilities, however, should attempt to bring the facilities more nearly in compliance with these standards. Animals may be held in laboratories or other locations outside vivaria for periods of time not to exceed 12 hours, after which time they should be returned to a vivarium. Exceptions to this policy need to be justified. Vivaria should be located in close proximity to the laboratories.

2.1. Ventilation

Enclosures for amphibians should be adequately ventilated. The water in enclosures of aquatic caged amphibians should be filtered, circulated, and aerated (see also Section 4.3.1).

Most amphibians cover reasonable amounts of their oxygen consumption by lung (or gill) and by skin respiration. About 80% of respiratory needs are met by pulmonary gas exchange (rather than cutaneous). With increasing body weight, lung respiration dominates. In order to guarantee sufficient fresh air and to keep down the level of noxious gases, the holding rooms must be adequately ventilated. Unventilated moist "frog cellars" are inadequate. It is generally considered adequate in practice for humidity and temperature control to change the air by means of a correctly engineered air-flow system. The water of the aquatic area in the terraria of terrestrial and semi-terrestrial amphibians should be renewed at least twice a week. In aquatic caged animals, there should be preventive measures to avoid larvae be trapped by filters. *Xenopus* spp. rarely inhabits highly oxygenated (flowing) water in the wild. Therefore, air stones are not necessary for *Xenopus.* If the purpose of air stones is to reduce the fouling of the water, then this should be tackled by more frequent water changes.

2.2. Temperature

Amphibians are ectothermic. Areas of different temperature and humidity are beneficial, to allow amphibians to seek their preferred micro-environment. Amphibians exposed to frequent fluctuations in temperature and humidity may be severely stressed and may be more prone to health problems. Room and water temperatures should be controlled.

Hibernation in amphibians may be induced or interrupted by regulating light-dark rhythm and room temperature. Before inducing hibernation in captivity, animals should be in good health and body condition. In animals used for breeding, a state of near winter torpor (for example dim light to darkness and 8-10°C room temperature) may be simulated where appropriate. Under these conditions, the animals can be kept without feeding for as long as four to five months. Restoration of pre-hibernation environmental conditions will induce activity and mating behaviour.

Prevention of hibernation in a laboratory environment will not cause major welfare problems.

Amphibians are ectotherms ("cold-blooded" animals); unlike endotherms ("warm-blooded"animals) their body temperature is dependent on the ambient environment. The advantage of ectothermy is that the resting metabolic rate and general energy requirements are less than those for mammals or birds of comparable size since no metabolic energy is spent on warming or cooling the body, and less energy is spent on food intake because less food is required to meet the body's low energy demands. The disadvantage of ectothermy, however, is that the ambient temperature determines the animal's metabolic processes and behaviour. The animal must actively seek temperatures that will allow it to feed, digest food, hibernate, etc. Amphibians adapt their body temperatures by finding the appropriate thermal environment through burrowing, hiding under logs or leaves, or entering water.

In many respects ectothermic animals are more interactive with their environments than endotherms. At the same time, they tend to have greater problems adapting to changes in their species-typical environment. Therefore, the design of their artificial habitats demands special care, since research-biasing stress and distress responses to species-inadequate environmental conditions are to be avoided.

Depending on the conditions in the natural biotope, the optimal temperatures of amphibians vary markedly among the species and also within a species with the divergent functional states as feeding and digestion, on the one hand, and torpor on the other hand. In amphibians, body temperature varies with environmental temperature which in turn is associated with changes in metabolic activity (Lillywhite et al., 1999). With decreasing temperature, the processes of respiration and circulation gradually slacken speed. Changes in temperature also modify the transport of body fluids (Pelster, 2000). Effects of temperature on size and developmental time are reported by Gilloly et al. (2002).

Both tadpoles and adults of frogs acclimate their locomotor system to different temperature, i.e., maximum swimming performance of cold-acclimated animals is greater at cool temperatures and lowest at warm temperatures in comparison with the warm-acclimated animals (Wilson & Franklin, 2000; Wilson et al., 2000).

Hibernation in amphibians may be induced or interrupted by regulating light-dark rhythm and room temperature. Before inducing hibernation in captivity, animals should be examined and assessed to be in good health and body condition. In animals used for breeding, a state of near winter torpor (for example dim light to darkness and 8-10°C room temperature) should be simulated. Under these conditions, they can be held without feeding for as long as four to five months. Restoration of pre-hibernation environmental conditions will induce activity and the mating behaviour.

Amphibians can endure an astonishing amount of cold. Torpor like sleep is induced in specimens at varying temperatures below 10°C. In autumn, frogs or toads creep away into some protected place or burrow into the soil into a greater depth, depending on whether the place chosen is at the bottom of a pond (frogs), under logs or stones, or in the earth (common toad). This hibernation lasts until a return of higher temperatures beginning in spring. Many amphibians encounter conditions each winter when their body temperature is so low that normal activities are suspended and the animals enter into a state of torpor. In ice-covered ponds or lakes, oxygen levels may become limiting, thereby forcing animals to endure prolonged periods of severe hypoxia or anoxia. Certain frogs (e.g., *Rana temporaria*) can dramatically suppress their metabolism in anoxia but are not as tolerant to prolonged periods of complete O_2 lack as other facultative vertebrate anaerobes (e.g., turtle, goldfish). Many over-wintering amphibians do, however, tolerate prolonged bouts of severe hypoxia, relying exclusively on cutaneous gas exchange (Boutilier et al., 1997). Various amphibians, which survive at temperatures several degrees below the freezing point of their body fluids, apply adaptive mechanisms that promote freeze avoidance by: (1) colligative depression of the blood freezing point, (2) super-cooling of the body fluids, and (3) biosynthesis of unique antifreeze proteins that inhibit the propagation of ice within body fluids. Freeze tolerance can be achieved by biosynthesis of permeating carbohydrate cryo-protective substances and extensive dehydration of tissues and organs (Costanzo et al., 1995; cf. also Storey et al., 1996).

Most amphibians can endure a greater degree of cold than of heat. In water, death may occur at about 40°C. Certain species of tree frogs – sitting in the sun – can endure temperatures up to 45°C because of the moisture secreted by their skins, in combination with surface cooling by evaporation. In South America, in savannah-like vegetation, during the dry season amphibians burrow into the mud or soil, and either form a cocoon or increase the osmotic concentration of body fluids to reduce evaporative water loss. Some tree frogs coat their body surface with skin secretion and excrete uric acid to minimize water loss. Tadpoles of desert amphibians evolved traits which allow successful development in unpredictable environments. As their pond dries, this environmental stress (involving the corticotropin releasing hormone) sets the cue to accelerate metamorphosis, thus escaping mortality in larval habitat (Denver, 1997). Estivating reed frogs (*Hyperolius viridiflavus*) are extraordinarily resistant to adverse climate conditions in their African savannah habitats during the dry season: air temperatures up to 45°C; solar radiation load up to 1,000 W/m²; no water replenishment possible for up to 3 months. To avoid lethal heat stress, solar heat load is reduced by an extraordinarily high skin reflectivity for solar radiation. Furthermore the half cylindrical body shape allows the animal to expose only a small surface area to direct solar radiation (Kobelt & Linsenmair, 1995). For estivation in South American amphibians see also Abe (1995).

Different species of urodele and anuran amphibians living in different biotopes are listed in the Appendix.

2.3. Humidity

Amphibians do not drink but absorb moisture through their skin. Water loss is an especially critical problem in captive terrestrial and semi-terrestrial amphibians, as a properly hydrated integument is essential to the normal function of the amphibian skin. Areas of different humidity within the enclosure are beneficial. Even desert-adapted amphibians should have access to a humid environment.

Amphibians have developed mechanisms to minimize cutaneous dessication (Castillo & Orce, 1997; Schmuck & Linsenmair, 1997). They absorb water osmotically across their skins and rely on chemosensory information from the skin to assess the suitability of hydration sources. Chemosensory transfer through the skin involves both trans-cellular and para-cellular pathways (Sullivan et al., 2000). Terrestrial amphibians take up water by abducting the hind limbs and pressing a specialized portion of the ventral skin to a moist surface; this behaviour is called the "water absorption response" (Hillyard, 1998, 1999). It can be influenced by barometric pressure and is avoided on hyper-osmotic substrates (Hillyard et al., 1998). Toads are able to detect hyper-osmotic salt solutions in their environment due to chemosensory function of the ventral skin (Nagai et al., 1999). Some amphibians tolerate high ambient salinities (e.g., *Bufo calamita*). In response to high salinities and water shortage, these species accumulate urea (osmo-regulatory urea synthesis) (Jorgensen, 1997a,b; cf. also Pozzi et al., 2002). The hormone angiotensin II − an inducer of thirst-related behaviour in many vertebrate species − increases the water absorption response behaviour in amphibians, such as the spade foot toad, *Scaphiopus couchii* (Propper et al., 1995; see also Slivkoff & Warburton, 2001; Viborg & Rosenkilde, 2001). Whereas the osmo-regulatory system in the tadpole comprises three organs − gut, kidney and gills − the adults involve gut, kidney, urinary bladder and skin. Amphibians may use the urinary bladder as a reservoir from which water is reabsorbed on land (Jorgensen, 1997a,b). Aside from vasotocin anti-diuretic activity in the nephron, the substance hydrin-2 (most common in amphibians except hydrin-1 in *Xenopus*) acts on water channel recruitment (re-hydration) mechanisms in the body skin and urinary bladder (Acher et al., 1997). Frogs have distinct hydro-osmotic receptors for vasotocin and hydrins (Rouille et al., 1995). For hormonal effects on the osmotic, electrolyte and nitrogen balance in terrestrial amphibians see also Warburg (1995).

In the laboratory, the humidity is adjusted by appropriate levels of moisture and temperature. The water area of a terrarium will allow animals to submerge. However, if a toad or frog has escaped from its terrarium, it is advisable to put a wet towel on the floor in the corner of the holding room. Over night, the animal will detect it and approach it and can thus be carefully caught and transferred back to its cage.

Different species of urodele and anuran amphibians living in different biotopes are listed in the Appendix.

2.4. Lighting

Photoperiods reflecting the natural cycle from where the animals originate should be used. Light levels in the enclosures should be consistent with that expected to be encountered under natural conditions. Both semi-terrestrial and aquatic caged animals should have the opportunity to withdraw to shaded areas within the enclosure.

Regarding photoperiods, the recommended laboratory lighting for *Xenopus,* for example, is 14h light and 10h dark, which corresponds roughly to nature. Different anuran species may exhibit preferences for light or dark. *Rana pipiens*, for example, is photopositive while *Leptodactylus pentadactylus* is photonegative (Kicliter & Goytia, 1995). Forest mediated light regime is linked to amphibian distribution and performance (Halverson et al., 2003). Comparing the energy spectra in woods of moderate and tropic climates, it is interesting to note that the UV proportion is about the same [8.5%:8.0%, 320-500 nm]. Differences exist in the green proportion and [15.5%:22.0%, 470-590 nm] particularly in the red [22.0%:45.0%, 600-710 nm]. Studies on spectral and polarization sensitivity of amphibian photoreceptors in the visible and ultraviolet are provided by Palacios et al. (1998).

2.5. Noise

Amphibians are very sensitive to noise (airborne stimuli) and vibration (substrate-borne stimuli) and are disturbed by any new, unexpected stimulus. Therefore, such extraneous disturbances should be minimised.

In neuroethological experiments, recordings from neurones of the auditory centres of the midbrains of various amphibian species have shown sensitivities to specific sound spectra, signalling different events, such as rival, mate, and threat (Feng et al., 1976; Wilczynski & Capranica, 1984; Mudry & Capranica, 1987a,b; Walkowiak et al., 1999; Murphy & Gerhardt, 2000; Bee & Gerhardt, 2002; Hobel & Gerhardt, 2003). Within and close to these brain regions, uni- and multimodal neurones were recorded which are monitoring stimuli of different modality, such as acoustic, vibratory, cutaneous, and visual. Vibration sensive neurones, for example, respond to steps on a floor from a distance of 10 m and even more. In the field, this allows frogs to detect potential predators when they are far out of vision and, eventually, to jump early enough into a pond. The visual receptive field of multimodal neurones may encompass the entire field of vision of both eyes. This massive convergence of different sensory channels toward single neurones allows multi-event detection (Ewert, 1984). Such neuronal "alarm systems" operate rapidly and highly sensitively, because information via one sensory channel (e.g., vibration) lowers the response threshold to information obtained via another sensory channel (e.g., vision), and vice versa.

Xenopus spp., being fully aquatic, lives permanently in a noisy and vibrating environment, – water (cf. Elepfand 1996a,b; Elepfand et al., 2001). Nevertheless, it was observed that populations of laboratory-bred *Xenopus* remaine completely “unmoved” by sudden sharp loud noise. When kept in clear wall tanks, they habituate to researchers moving frequently in the room and are also not necessarily startled by a human appearing overhead.

2.6. Alarm systems (See paragraph 2.6. of the General section)

Adequate alarm systems are recommended if circulation systems are used and/or aeration is required.

It is recommended that the housing facility is equipped with devices to detect fires and the intrusion of unauthorised persons and to maintain support systems such as current and water supplies. For general considerations in the care of captive amphibians see Freye (1977).

3. Health (See paragraph 4.1. of the General section)

Any effort should be made to keep animals under optimal conditions. High quality of scientific results obtained in experimental or other procedures depends largely on the physiological state of an organism. Therefore, animals should be routinely inspected by the authority responsible for the housing facility.

The physiological state of amphibians and, along with this, their behavioural activities rely largely on the time of the day depending on the animal's activity period, day or twilight/night. It could be shown that in the main activity period, heart rates, respiratory ventilations, and neuronal responses to sensory stimuli are higher than in the resting period. The season of the year, too, strongly influences physiological states and behavioural activity patterns. Even in laboratory amphibians whose winter torpor is prevented by constant light/darkness and temperature conditions, these changes are manifest due to an internal rhythm, at a lower level, so that in laboratory jargon names like "summer-frogs" and "winter-frogs" are used occasionally [cf. also Section 2.2]. Under constant laboratory conditions, food consumption is low during winter and spring, and relatively high during summer and autumn. During spring – in the mating season – the cutaneous, auditory, and visual sensitivities are addressed to the sexual partner [cf. Section 4.1].

3.1. Animal supply

In the appropriate season of the year, amphibians should be ordered from commercial dealers or institutions, so that records about the number of individuals are available for book keeping [see also Section 4.10]. New amphibians need to be acclimated to the novel environment. Cages should be set up with all necessary accessoires and enclosures in advance. Amphibians will be often first stressed and will not eat. The temperature and humidity should be checked to be sure the range is right and there is adequate water.

3.2. Animal transportation

Amphibians should be obtained from those commercial institutions which follow the recommendations of adequate animal transportation: the *European Convention on the Protection of Animals during International Transport*, the *International Air Transport Association, and the Animal Air Transport Association*. Semi-terrestrial amphibians should be singly packed in boxes of adequate size and provided sufficiently with air and moisture. Transport of tropic species – depending on the local climate – requires an accommodation with appropriate heating devices. After arrival, animals must be unpacked without delay. Animals which arrived in a sick condition, and which do not have a chance to recover, should be sacrificed at once by a human method [Section 4.9]. The commercial sender should be informed.

3.3. Reproduction

Most amphibians appear to lack morphologically distinguishable sex chromosomes. Nevertheless, amphibians employ a genetic mechanism of sex determination (Hayes, 1998; Schmidt & Steinlein, 2001). Cases of spontaneous and experimental sex reversal (induced by temperature, hormones or surgery) are reviewed by Wallace et al. (1999). The few studies suggesting that temperature influences sex determination – as observed among reptiles – have been conducted at temperatures outside the range normally experienced by the species under study (Hayes, 1998).

Amphibian eggs are mostly laid in the water and develop into tadpoles which after metamorphosis mature to the adults. Unlike most salamanders and gymnophiona, most anurans are external fertilizers. Sperm storage in the oviduct of internal fertilizing frogs is reported by Sever et al. (2001; cf. also Sever, 2002).The developmental biology of amphibians after Hans Spemann is reviewed by Grunz (2001).

In most amphibians, laboratory bred strains are lacking for two reasons: (i) difficulties in establishing suitable environmental conditions to elicit sexual maturity and mating behaviour in captivity; (ii) difficulties in rearing amphibians successfully through their early larval and transformation stages (Nace, 1974). With metamorphosis, the immune system becomes reorganised involving a temporary immune-suppression (Flajnik, 1996). If the laboratory conditions result in the induction of metamorphosis at a less than optimal body size and state of the immune system, the animal runs a risk of infection and death (Rollins-Smith, 1998; cf. also Denver, 1997) [see also Sections 1.1 and 3.4].

Temperatures close to torpor (hibernation) during the winter period and re-warming in spring are often preconditions of successful breeding.

Xenopus laevis shows no seasonal changes in its physiological behaviour. Mating can be easily triggered in adult animals through a temporary UV radiation applied to the tank [aquatic caging see Section 4.3.1]. Alternatively, the gonadotrophic hormone chorionic gonadotrophin may be injected intralymphatically. Three to eight hours thereafter, animals are ready for mating which is indicated by the croaking advertisement calls produced by the males. The male, when ready for mating, also shows a brown velvet-type pigment on the front legs. Females have a fuller developed abdomen and are often twice as big as males. Copulation and spawning should take place in tanks with gravel and artificial water plants on the ground. Copulation lasts for about 24 hours and eggs are laid singly, often fastened on the plants. In most amphibians – especially anurans – eggs are fertilized outside of the female reproductive tract. Female sperm storage in amphibians is reported by Sever et al. (2001) and Sever (2002).

After spawning, animals should be removed from the tank. The hatching tadpoles are filter feeders and can be maintained on nettle powder (dried leaves of *Herba urticae*), egg-powder and dried yeast (the latter two in small amounts) suspended into the water. Under optimal conditions, maturity of the animals may be reached in the first year (Müller, 1976) [feeding adult amphibians see Section 4.4].

Great progress is being made in developing large scale breeding programmes for *Xenopus tropicalis* for genetic studies. The fact that *Bombina orientalis* can be easily maintained and bred in the laboratory under long-term conditions is a relatively new experience. The following list provides a short breeding instruction elaborated in the Department of Neurobiology, Faculty of Natural Sciences, at the University of Kassel, Germany.

Instruction for breeding *Bombina orientalis*

Adult *Bombina orientalis*

Males size: ca. 5.5 cm
Females size: ca. 4.5 cm
Colour: body green in shadings, belly carroty to red depending on vitamin A containing food.

Preparation for hibernation
November
↓
Vivarium: 95x60x30 cm³ in size arranged like the one described in Section 4.3.2, in addition green artificial plants that allow animals to climb up or to shelter
Watering: temperature of 8 to 10°C, renewing carefully once a week
Lighting: reduction of photoperiod and light intensity like outside, without artificial light
Feeding: mini-crickets, small pale mealworms (*Tenebrio molitor*) fruit flies (*Drosophila vestigial*), fly larvae (*Lucilia caesar*), red mosquito larvae (*Chironomus spec*), twice a week until the middle of December
Noise: the room must be absolutely quite, careful renewing the water
↓
Hibernation
December to February
↓
March
Watering: simulating rain by spraying warm water of ca. 18-20°C, renewal 4-5 times a weak
Lighting: elongation of photoperiod and light intensity like outside, without artificial light
Feeding: see above

↓
Mating and spawning
April to August:
↓
Release of eggs
↓ ca. 3 days
Tadpoles
To be separated from adults immediately

about 30 Tadpoles
Tank: 30x60x30 cm³
Watering: temperature 18-20° C, renewal 3-4 times a weak
Lighting: like outside
Feeding: toy fish food, dried mosquito larvae, daily
↓ 4-5 weeks
Beginning of metamorphosis
↓
Vivarium: 95x60x30 cm³ in size arranged like the one above, including stones that will allow juveniles to approach the land area; tadpoles in pool area
Watering: temperature 18-20°C, renewed 4-5 times a week; water level must be adjusted, so that juvenile animals can approach the land area of the vivarium; after metamorphosis, water renewed 3 times a week
Feeding: daily after metamorphosis with leaf-lice, fruit-fly larvae, mini-crickets, mosquito larvae, for about 6 month; later food diet like for adults
↓ ca. 2 years
Adult *Bombina orientalis*

3.4 Diseases

Comprehensive investigations on diseases in amphibians are relatively scarce (e.g., Reichenbach-Klinke & Elkan, 1965; Cosgrove, 1977; Siegmund, 1979; Marcus, 1981; Cunnigham et al., 1996; Mutschmann, 1998; Nichols, 2000; Davies & Johnston, 2000; Williams et al., 2002) in comparison to reptiles (Marcus, 1977; Bush et al., 1980; Murphy & Collins, 1980; Davies, 1981; Jackson & Cooper, 1981; Holt, 1981; Cooper, 1985; Miller, 1996; Mermin et al., 1997 Beynon & Cooper, 1994, 1999; etc). Ippen & Zwart (1996) discuss infections and parasitic diseases of captive amphibians with special emphasis of husbandry practices which prevent or promote diseases (cf. also Cunnigham et al., 1996).

Fungal infections play a prominent role in amphibians. An overview on fungal diseases in amphibians is provided by Pare (2003). Food toxicity associated with the presence of uneaten, partially decomposed food is often caused by growing fungal hyphae. Therefore, it is strongly recommended that overfeeding is avoided and that after each feeding session uneaten food particles are removed soon.

Superficial fungus *(Saprolegnia)* infections, especially among larval salamanders, appear as an opaque, usually fuzzy, white area of skin on an extremity, fin or external gill. Various treatments in segregated infected animals are recommended, such as:

- Solution of 2-3% calcium propionate for 1 min and immediately returned to fresh water, once daily;
- topical painting of infected areas with 2% mercurochrome solution, followed in a few minutes by washing in flowing water;
- dips at potassium permanganate at 1 : 5,000 for 5 min;
- solution of Trypoflavin for three weeks, changed every fourth day.

A newly discovered emerging fungal disease, chytridiomycosis, was found to be associated with amphibian mass mortality events and population declines in Panama and Australia. Since then, the disease has been reported also in North America, South America, Africa, and Europe (see Berger et al., 1998; Daszak et al., 1999; Boyle et al., 2003; cf. also Daszak et al., 2002, 2003; cf. Section 1.1). Various hypotheses are currently tested on what factors drive its emergence and how it causes death in amphibians. Recently, Daszak and co-workers have provided evidence that "pathogen pollution", the anthropogenic introduction of non-native hosts or parasites to new locations, is a major factor contributing to this disease's emergence. The bullfrog, *Rana catesbeiana*, is globally traded as a food item and appears to be relatively resistant to chytridiomycosis. It may serve as an efficient carrier host.

Zygomycosis refers to an angiotropic (blood vessel-invading) fungal infection produced by the various Zygomycetes. This disease is also sometimes referred to as mucormycosis, but the term zygomycosis is preferred. For a review on zygomycosis due to *Basidiobolus ranarum* see Gugnani (2000). Myositis associated with fungal infection by *Ichthyophonus*-like organisms was diagnosed in 35 of 260 wild amphibians (such as *Rana clamitans, R. sylvatica, R. palustris, R. catesbeiana*) collected in Quebeck Canada (Mikaelian et al., 2000). Ichthyophonosis is enzootic in amphibians from Quebeck. Infections with the fungus *Ichthyophonus hoferi* as well as the Gram negative bacterium *Aeromonas hydrophila* were diagnosed in some of the samples. For infections by iridoviruses see Hyatt et al. (2000).

Aeromonas hydrophila is a species of bacterium that is present in all freshwater environments and in brackish water. Some strains of *A. hydrophila* are capable of causing illness in amphibians as well as in humans who may acquire infections through open wounds or by ingestion of a sufficient number of the organisms in food or water. In captive toads and frogs the *Aeromonas* infection (red leg) is the most common disease. Signs of red leg include cutaneous ulcers and characteristic pinpoint hemmorrhages over the abdomen, leg, and tongue, with lethargy and emaciation. This external bacterial infection begins with mechanically caused abrasions or wounds to the skin. Toads and frogs must therefore be inspected for such signs, any diseased animal removed immediately, kept in segregation and treated with antibiotics such as Tetracycline. For amphibian skin diseases see also Reavill (2001).

Amphibians are hosts of nematodes (Goldberg et al., 1996; Moravec & Skorikova, 1998; Barton, 1999). Most nematode species are not host-specific. About 40 families infect amphibians and reptiles; 68 genera infect anurans, of which only 22 are specific to frogs, 4 are shared with salamanders, 11 with fish, 6 with mammals or birds and 37 are shared with reptiles. Systemic infection of frogs with the trematode *Alaria* (Fernandes et al., 1976) may be fatal also to humans handling those frogs (Freeman et al., 1976) [see also Section 4.5]. Alarm over increasing reports of deformed amphibians has intensified since the early 1990s. Over the last decade, abnormalities have been reported in 36 species of amphibians in the USA. The trematode *Ribeiroia* was isolated from deformed frogs in the field, was employed at realistic concentrations in experimental exposures, and produced the same range and frequency of amphibian limb abnormalities as observed at field sites [cf. also Section 1.1]. Ectoparasites in amphibians include leeches (Hirudinea) and helminths. For regulation of worm burdens in amphibians see Tinsley (1995).

Xenopus laevis in captivity is sometimes found turning around its sagittal body axis while swimming, a behaviour that usually "recovers" after two to three days without any therapy. However, if such an animal is swimming with uncoordinated hind legs, it should be isolated, since in a few hours it will become bloated and the lymphatic sacks appear to be extremely filled. This unknown disease is lethal in a few days.

4. Housing, enrichment and care

4.1. Social behaviour

In most amphibians, social behaviour is mainly restricted to the mating season. However, group-housing of amphibians is advisable, for instance to improve feeding and reduce fear responses. For example, in Xenopus spp. group feeding promotes feeding frenzies inducing all animals to feed. At very low stocking densities such frenzies do not occur and food is frequently not eaten.

To avoid cannibalism in certain species (particularly among larval Ambystoma spp. and Scaphiopus spp.), these animals should be maintained in small groups. Cannibalism in groups can be reduced by size grading.

Due to tigmotaxic effects, (semi-)terrestrial and (semi-)aquatic amphibians get their skin in touch with the surface of the hiding place. Terraria should make some allowance for that. Especially in captivity, *Bufo* spp. and *Xenopus* spp. are searching for body contact to conspecifics. This may be also a response to lack of refuges in most terraria where animals attempt to hide under another because of allround illumination. When maintained under natural conditions, *Xenopus* do not live in contact with each other. Nevertheless, group housing is recommended for the stimuli (sight, sound or smell) that conspecifics create within a group [cf. Section 4.3.1]. Group housing is also required for procedures which investigate breeding behaviour or are concerned with captive breeding programmes that maintain the population of laboratory animals.

Mating plays a prominent role in social behaviour of amphibians. The mating season is introduced with the migration over long distances towards the pond in which the animal was born. It is suggested that toads (*Bufo japonicus*) use a local map to orient to the breeding pond. Probably, the local map was memorized by the newly metamorphosed toads at their terrestrial trip from the pond. Since blinded toads – but rarely anosmic toads – could reach the pond, olfaction seems to be an important cue in this context (Ishii et al., 1995). Sexual and seasonal differences in the vomeronasal epithelium were observed in the red-backed salamander (Dawley & Crowder, 1995).

In spring, the males of terrestrial and semi-terrestrial anurans attract their females by species-specific mating calls (e.g., Wilczynski & Capranica, 1984; Mudry & Capranica, 1987a,b; Walkowiak et al., 1999; Murphy & Gerhardt, 2000; Bee & Gerhardt, 2002; Hobel & Gerhardt, 2003). Vocalization behaviours of anuran amphibians are universally sexually dimorphic. Usually, only males give an advertisement call, while female frog calls are limited to a soft and simple release call which is specifically suppressed during mating. In a very few species, however, female frogs also give mating vocalizations (Emerson & Boyd, 1999). Certain visual and/or tactile stimuli release in the males clasping behaviour that is addressed to conspecific females. Most anurans are external fertilizers, whereas most salamanders are internal fertilizers. Among the species *Ambystoma*, there are bisexual and parthenogenetic subspecies.

4.2. Environmental enrichment

The terrestrial habitat of amphibians should be structured, including, for example, branches, leaves, pieces of bark and stones. Amphibians benefit from such environmental enrichment in different ways: for example, such inclusions allow animals to hide, and provide labels for visual and spatial orientation. The side walls of the terraria should be textured to provide a structured surface.

The provision of hiding places/shelters that are appropriate to the amphibian's needs is recommended, because they can reduce stress on captive amphibians. For example, in Xenopus spp. a tube of ceramic or plastic may be provided. Refuges should be inspected regularly for sick or injured animals. A dark floor to the tank may enhance the sense of security in the animals.

Materials used for enrichment devices should not be detrimental to the health of the amphibians. Enclosures and enrichment structures should have smooth surfaces and rounded edges to minimise the risk of injury to the amphibian's skin.

Behavioural studies under laboratory conditions have shown that the presence of background textures is necessary for the accuracy of visual functions (Ewert, 2004a). These capabilities require an evaluation of the visual target in relation to its background. The impact of this experience on the holding conditions is important, particularly in captive terrestrial and semi-terrestrial amphibians. The opaque side walls of the terraria should be textured. Frogs and toads become adapted to their environment based on textural patterns and configurations. Transferred into a different environment (such as an optical homogeneous cage), the animals are scared and will often neglect prey.

Terrestrial amphibians feel more comfortable if a hiding place is available that allows them to get their skin in touch with the shelter's surface [see Section 2.1]. The provision of shelter places for *Xenopus* is advisable, but should not be prescribed. Observation of a large number of laboratory environments has shown that laboratory-bred *X. laevis* and *X. tropicalis* are rarely found to use shelters placed in their tanks. They appear to prefer more lighted environments, "light twilight" to bright light.

4.3. Enclosures – dimensions and flooring

The most practical way of providing suitable holding conditions for amphibians within an animal facility is, first, to establish a set of general environmental conditions for the rooms as a whole and, secondly, for each tank or terrarium to be established as an environmental chamber for one or several individuals. This involves structuring the cage space to allow an individual's activity-related use as well as introducing appropriate stimuli and materials.

Xenopus laevis [Photo: W. Leonard]

4.3.1. Enclosures for aquatic amphibians

Aquatic amphibians, such as Xenopus laevis, or amphibian larvae are housed in tanks and aquaria. These may be equipped with a gentle flow-through water system for the circulation of uncontaminated (for example, dechlorinated) water, a heating device to maintain suitable temperatures, and a compressed air supply and air stones for aeration. Care is needed to ensure that aeration does not cause injury to the animals. Unless a proper flow system is in place, the water in the enclosures should be renewed with water of an appropriate quality about twice a week.

For Xenopus spp., systems with regular changes of water (fill-and-dump systems) are sufficient for maintaining appropriate water quality (such as minimising levels in ammonia). Airstones are not required for Xenopus.

Furthermore, too long, narrow enclosures should be avoided since they may restrict locomotor activity and social behaviour such as feeding frenzies.

Most tadpoles are herbivores or/and detritivores, so that cannibalism will not be a problem. Due to growth inhibition in tadpoles, the population density of the larvae must be kept relatively low (Rose & Rose, 1961). Tadpoles sort out species-specific factors into the water which inhibit growth (Aikin, 1966). This problem can be reduced or eliminated if the water is changed 4 to 5 times a week. Water temperatures for larvae are in the range of 10-12°C (salamanders) or 18-22°C (most species of tadpoles, including the neotenic larvae of *Ambystoma mexicanum)*. Temperatures of 10-25°C are tolerable, whereas 1-10°C lead to torpor.

From the species *Ambystoma mexicanum*, individuals are accommodated in tanks of the recommended size at a dim place. The aerated, circulating filtered, water should have a temperature of 10-25°C; pH=8.5, and 6-16° dH. The bottom of the tanks contains tubes of clay, gravel and stones, so that individuals can select their own territory. *Ambystoma mexicanum* stays for the whole life (up to 13 years) in larval stage (neotenic) due to a dysfunction of its thyroid gland. The larvae reach sexual maturity with an age of one year. Each releases up to 500 eggs. Juvenile larvae (ca. 1 cm in size) are hatching after about four weeks.

Table I.2. Aquatic urodeles,* e.g.*, Ambystoma spp: Minimum enclosure dimensions and space allowances

Body length*) (cm)	***Minimum water surface area (cm^2)***	***Additional minimum water surface area for each animal in group holding (cm^2)***	***Minimum water depth (cm)***
≤10	***262.5***	***50***	***13***
11 – 15	***525***	***110***	***13***
16 – 20	***875***	***200***	***15***
21 – 30	***1837,5***	***440***	***15***
31 – 40	***3150***	***800***	***20***

****) measured from snout to tail***

The recommendations for minimum space requirements for aquatic urodeles, e.g. *Ambystoma* spp. differ from the guide of the Swedish Board of Agriculture (Department for Animal Production and Health, Animal Welfare Division) that suggests more space. The Axolotl is a native of permanent lakes near Mexico City where extinction is imminent due to loss of original habitat. Virtually all information on life history of the Axolotl has been obtained from laboratory colonies as it has been reported that no ecological study of a wild population has been conducted (Armstrong & Malacinski, 1989). This lack of basic information from natural populations contributes to current uncertainties about how best to maintain animals within the laboratory situation. Therefore the proposed recommendations have taken into consideration the wealth of practical experience (good laboratory practice, GLP) gathered from the maintenance of Axolotl colonies in the laboratory situation within the UK, Europe and the United States. The Indiana University having received the first Axolotl's in 1956 from Robert Briggs who pioneered the study of amphibian tumours and nuclear transplantation (Armstrong & Malacinski, 1989). This practical expertise has achieved excellent captive breeding results from Axolotl colonies, ensuring the survival of the species in captivity. Moreover, it has enabled the supply of fertilised embryo's essential for ongoing research programmes that include investigations into development of germ cells and blood cells (Johnson et al., 2001, 2003a, 2003b). The proposed minimal space requirements enable the Axolotl's freedom of movement and importantly satisfy the physiological and behavioural needs as follows:

1. The behavioural repertoire of Axolotl's appears relatively limited. It is clear from numerous observations that the animals have a sedentary lifestyle when maintained under optimum environmental conditions. The animals move in a slow and gentle manner unless startled. If stimulated, they are able to perform tight twists and turns with ease. Therefore tank sizes that are around 5cm wider and 1.75 times longer than body length for single housed animals (see Table below), enable the Axolotl's to move relatively freely within the tanks and without need to resort to tight twists and turns. Increasing tank size does not appear to alter movement significantly. It is recognised that further more detailed studies are required, but that in the absence of evidence to the contrary, tank sizes based upon current successful housing strategies are recommended.

Body length * (cm)	Minimum water surface area(cm^2)	Minimum tank width (cm) 5cm wider than axolotl	Minimum tank length (cm) 1.75* longer than axolotl	Minimum additional water surface area for each additional animal	Minimum water depth (cm)
<10 single 3 animals	262.5 362.5	15 15	17.5 24.2	50	13
11-15 single 3 animals	525 745	20 20	26.3 37.3	110	13
16-20 single 3 animals	875 1275	25 25	35 51	200	15
21-30 single 3 animals	1837.5 2717.5	35 35	52.5 77.6	440	15
31-40 single 3 animals	3150 4750	45 45	70 105.6	800	20

2. The proposed tank sizes also allow the introduction of environmental enrichment materials such as plastic tubing and artificial weed. The use of which is being evaluated in the UK.

3. Females appear gregarious, and do not actively seek to maintain a distance between themselve. When housed together, even in larger tanks, they frequently position so that individual limbs are in contact with each other while resting on the floor of the tank. Only limited additional surface area is required when animals are group housed. The minimum additional surface area for each animal (group holding) has therefore been calculated to give enough extra space to allow the animal to lie diagonally across the additional surface area provided for it. The proposed tank sizes allow animal's space to achieve this.

4. When anticipating being fed, the animals raise their snouts to the surface but maintain contact with the floor of the tank using their hind-limbs and tail. The proposed water depth will achieve this. Increasing water depth to 20cm for animal's of 16 cm body length would not allow this behaviour to occur.

5. The proposed tank sizes should not lead to increased stress for animals housed. Measurable stress factors are: health status, high quality ovulatory response, and successful metamorphosis and development of juveniles. All of which are achieved using the proposed tank sizes. For example at the University of Nottingham the colony of 22 males and 34 females produced 27 successful matings in 13 months with an average embryo yield two to three hundred per month. 50 young axolotls are currently being reared with a mortality level of 3.8% to date. Note: in respect to the development of juveniles, a surface area of around 20 square cm and a depth of around 7cm has been used successfully to raise young animals to around 5cm body length. Given that around 100 offspring are produced from a single spawning, it is highly likely that high densities of juvenile offspring would occur in the wild. However as no information appears to be available about the situation in the wild, and that the density used above proves successful, then until further detailed studies can be undertaken, successful breeding and rearing strategies should be used to maintain this endangered species.

6. "Footprint" area and stocking denstities. Axolotl's like *Xenopus* live in a three-dimensional environment. For *Xenopus* (see below) it has been argued successfully that the minimum water surface area of the animal should be a constant function of the "footprint" area of the animal within a given species. To the first approximation, the animal footprint area is related to the square of any linear dimension such as length. Thus if one plots a graph of body length against the ratio {tank surface area}:{body length squared}, then one should expect to see a flat straight line parallel to the x-axis. Such a plot would indicate that on a proportional basis, one is not discriminating against either larger or smaller animals, in terms of the amount of space allocated to them. The proposed tank sizes do indeed give such a plot indicating minimal discrimination against larger or smaller animals. Nevertheless it is recognised that lack of scientific data makes it difficult to make categoric recommendations. The proposals are therefore based wholly on practical experience in successful husbandry methods in rearing Axolotl's. It is therefore appropriate that interim tank sizes are adopted until scientific data can be obtained to allow informed decisions on tank sizes to be made.

Table I.3. Aquatic anurans,* e.g., *Xenopus spp: Minimum enclosure dimensions and space allowances.*

*Body length** (cm)*	*Minimum water surface area (cm²)*	*Minimum water surface area for each additional animal in group-holding (cm²)*	*Minimum water depth (cm)*
< 6	160	40	6
6 - 9	300	75	8
10 - 12	600	150	10
13 - 15	920	230	12.5

** these recommendations apply to holding (i.e., husbandry) tanks but not to those tanks used for natural mating and super-ovulation for reasons of efficiency, as the latter procedures require smaller individual tanks. Space requirements determined for adults in the indicated size categories; juveniles and tadpoles should either be excluded, or dimensions altered according to the scaling principle*
*** measured from snout to vent*

All species of clawed frogs *Xenopus* are so-to-speak "technically" aquatic amphibians [quality of water see Section 4.5]. However, they live completely aquatic and die very quickly out of water. In the laboratory, *Xenopus laevis* are accommodated in small groups in a tank of the recommended size, covered by a perforated metal plate. All walls, except the front, should be painted grey or black with visual textures. It is advisable that water depth should not be so great throughout the holding area as to preclude the opportunity to surface breathe with hind toes in contact with the base.

The regularly changed water should have room temperatures up to 22°C. In the wild, *Xenopus* spp flourishes in stagnant ponds. Thus, aeration in the tank may be even detrimental to the health, for example, egg production can be reduced. At the bottom of the tank, stones and tubes of clay provide the individuals shelter. However, there is no scientific evidence that such shelters reduce stress in *Xenopus* spp. Furthermore, it is not advisable in these species to enrich the environment with natural or artificial plants, since – in the wild – they live in plantless ponds.

The recommendations for minimum space requirements for aquatic anurans, e.g. *Xenopus* spp. (Table 3), too deviate from the Guide of the Swedish Board of Agriculture [Department for Animal Production and Health, Animal Welfare Division] that suggests more space. The present recommendations consider the ample experimental experience based on good laboratory practice, GLP, by the users, such as the British Xenopus Group. This group is based in about 20 institutions across the UK, has published over 1,000 papers to date and has considerable independent GLP experience in the use of *Xenopus* – ranging from up to 30 years, altogether 500-man years.

In their natural environment, *Xenopus* spp. lay large numbers of offspring and have a relatively fast developmental programme. This results in huge numbers of individuals and densities in ponds in the wild. It is reasonable to expect that *Xenopus* are well adapted to this existence, and there is considerable evidence to suggest that this is indeed the case [cf. "The Biology of *Xenopus*", Kobel & Tinsley, 1996, Oxford Science Publications]. Furthermore, it is known that the behavioural repertoire of *Xenopus* is relatively limited. It can be largely, if not completely, recapitulated using the recommended conditions. Group housing has a variety of advantages in the welfare of *Xenopus*:

(i) Body contact reduces stress. *Xenopus* live in very high densities in small pools in the wild. Irrespective of density and illumination frogs periodically lie on each other, swim into and out of groups and frequently collide with each other. Experience with raising *Xenopus tropicalis* in the laboratory over several generations shows that animals kept at low densities grow more slowly than those kept more densely and are subject to additional stress up to the point of lethality. Furthermore, it can be shown that absence of body contact by conspecifics is an important stress factor. Measurable stress factors are: health status, high quality ovulatory response, and successful metamorphosis and development of the juveniles.

(ii) Feeding frenzies. Another aspect of holding *Xenopus* in groups concerns feeding. Efficient group feeding normally involves feeding frenzies, whereby the feeding activity is initiated by one animal inducing similar behaviour in other animals in the close vicinity. At low densities, feeding frenzies do not occur and food is frequently not eaten.

(iii) Reduced protective arousal. In the wild, group-behaviour minimises the risk of predation by reducing the likelihood that any one individual will be taken by the predator. Consistent with this notion, it was demonstrated that *Xenopus* accommodated in densities according to the recommendations shown in Table 3 show reduced protective arousal responses.

(iv) "Footprint" area and stocking densities. Since *Xenopus* lives and operates in a three-dimensional environment with highly varied dynamics, understanding and optimising *Xenopus* in captivity is therefore dependent on four-dimensional analysis, with dynamics being the fourth dimension. In view of differing depth of water in which *Xenopus* are held, it has become accepted to consider "footprint" as well as quantities of water.

The minimum water surface area per animal should be a constant function of the "footprint" area of the animal, at least within a given species. To the first approximation, the animal footprint area is related to the square of any linear dimension such as length. Thus if one plots a graph of body length against the ratio {tank surface area}:{body length squared}, then one should expect to see a flat straight line parallel to the x-axis. Such a plot would indicate that on a proportional basis, one is not discriminating against either larger or smaller animals, in terms of the amount of space allocated to them.

4.3.2. Enclosures for semi-aquatic and semi-terrestrial amphibians

Semi-aquatic and semi-terrestrial amphibians are kept in enclosures consisting of a terrestrial part and an aquatic part. The water area of the terrarium should allow animals to submerge. Unless a flow-through system is used, water should be renewed at least twice a week.

Each terrarium should be covered to prevent escape. It is advisable to paint or otherwise cover the outside of transparent walls to minimise damage to the animal. Additions to the interior design can include: soft-foamed plastic material on the floor near the pool area, stones, pieces of artificial bark material, artificial branches and leaves, and shelves. Fine sawdust and any other related small-particle substrate should be avoided, as it affects the sensitive body skin, harbours pathogens and is difficult to clean and re-use.

Table I.4. Semi-aquatic anurans, e.g., Rana temporaria: Minimum enclosure dimensions and space allowances

Body length* (cm)	Minimum enclosure area** (cm^2)	Minimum area for each additional animal in group holding (cm^2)	Minimum enclosure height*** (cm)	Minimum water depth (cm)
= 5.0	1500	200	20	10
5.5 - 7.5	3500	500	30	10
> 7.5	4000	700	30	15

** measured from snout to vent*
*** one third land division, two thirds water division sufficient for animals to submerge*
**** measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures should be adapted to the interior design*

Table I.5. Semi-terrestrial anurans, e.g., Bufo marinus: Minimum enclosure dimensions and space allowances

Body length* (cm)	Minimum enclosure area** (cm^2)	Minimum area for each additional animal in group-holding (cm^2)	Minimum enclosure height*** (cm)	Minimum water depth (cm)
= 5.0	1500	200	20	10
5.5 - 7.5	3500	500	30	10
> 7.5	4000	700	30	15

** measured from snout to vent*
*** two-thirds land division, one-third water division sufficient for animals to submerge*
**** measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures should be adapted to the interior design*

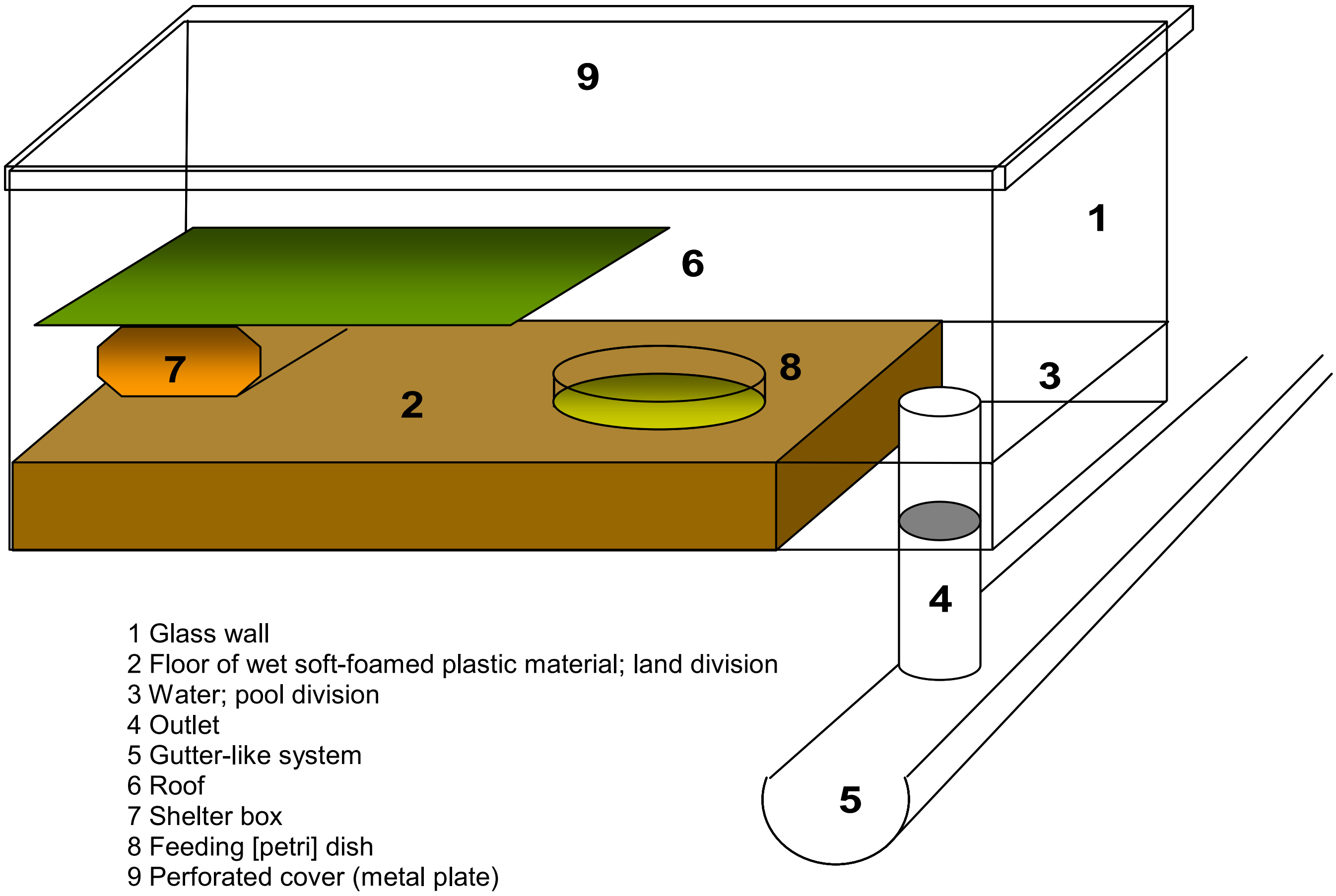


Fig.3. Examples of enclosures of a cage for housing semi-terrestrial anurans. (Department of Neurobiology, Faculty of Natural Sciences, University of Kassel, Germany).

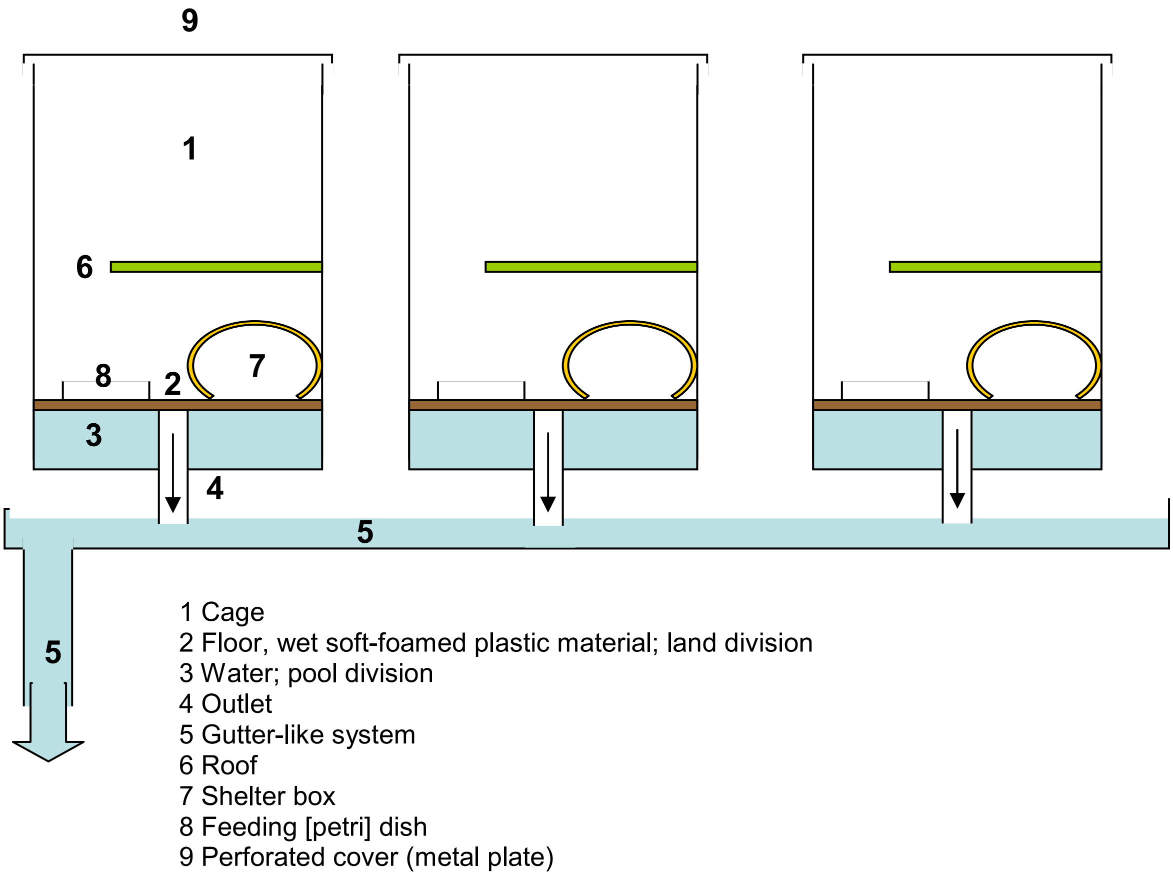


Fig.4. Example of an arrangement of several cages (cf. Fig.3) for housing semi-terrestrial amphibians.

Unlike many "show terraria", laboratory terraria – which accommodate reasonable numbers of individuals – should not be equipped in a manner similar to natural conditions, that is with fine gravel, earth, sand, peat, and living plants. GLP experience has shown that such substrates are contra-indicated for laboratories since it is difficult to remove dirt, excrements, and uneaten partially decomposed food particles to the extent required for scientific standards. As a result, mortality in such-semi-natural environments can be very high due to infections after mechanical injury or food toxicity [see Section 3.4].

Based on GLP experience and local initiatives for improving the housing of toads in captivity, the following standard terraria provide holding conditions that keep the mortality rate very low (Ewert & Ewert, 1981). Experimental animals, e.g., *Bufo marinus*, are kept in an animal facility (amphibian room). Terraria are made of glass. Each one is covered by a removable perforated metal plate that prevents toads from climbing out. Except of the front side, the remaining glass walls are painted dark green with textures. Behavioural studies under laboratory conditions have shown that the presence of visual background textures is necessary for the accuracy of visual functions, such as depth estimation, judging absolute object sizes, and spatial orientation (Ewert, 2004). A portion of grey structured soft-foamed plastic material covers about two thirds of the terrarium floor.

Fig. 5. Arrangement of several cages for housing semi-terrestrial amphibians (cf. Figs.3 and 4) [Source: Department of Neurobiology, Faculty of Natural Sciences at the University of Kassel].

Fig. 6. View into a terrarium (cf. Fig. 5) showing the land division of wet soft-foamed plastic material (right) and the pool division with a bathing cane toad, *Bufo marinus* (left). Examples of enclosures: artificial bark; a feeding dish with mealworms; a half piece of plastic tube; stones; artificial branches with leaves; a piece of pottery.

Artificial bark and tubes of clay or plastic material offer shelter. The remaining part of the terrarium is filled with water and serves as a pool. The bottom has an outlet that is closed by a plug. A gutter-like system underneath the terrarium collects the outlets of a row of several terraria. In this arrangement, the toad's skin is kept moist by the wet foam material, thus allowing the "water absorption response" behaviour [cf. Section 2.3]. Furthermore, these terraria are easy to clean. After feeding toads once or twice a week, uncontaminated (dechlorinated) water is supplied to the terrarium by a hose. This cleans the foam from excrements and food particles that pass the pool through the outlet into the gutter system.

Bombina orientalis can be accommodated in a comparable manner. Artificial branches and leaves, as well as smaller tubes and stones will allow the animals to climb or to take shelter, respectively.

4.3.3. Enclosures for arboreal amphibians

Having regard for the behaviour of different arboreal species, every effort should be made to allow for this by the provision of appropriate structures for climbing and resting by arboreal species (see section 4.3.2). In addition, it is necessary to provide water in which they can submerge themselves or seek greater humidity. If water dishes are used, they should be arranged in such a way that they are easy for the amphibians to enter or to leave.

Table I.6. Minimum space requirements for arboreal anurans, e.g., Hyla cinerea

Body length* (cm)	***Minimum enclosure area** (cm²)***	***Minimum area for each additional animal in group-holding (cm²)***	***Minimum enclosure height*** (cm)***
= 3.0	***900***	***100***	***30***
> 3.0 - 6.0	***1500***	***200***	***30***

**** measured from snout to vent***
***** two-thirds land division, one-third pool division sufficient for animals to submerge***
****** measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures should be adapted to the interior design including, e.g., shelves, large artificial branches, and structures for climbing***

4.4. Feeding

The majority of amphibians are carnivores with food preferences for living small invertebrates (such as larvae, insects and worms). Captive animals should be maintained on their natural foods or on foodstuffs approximating those of their natural diets. However, captive aquatic amphibians can successfully be maintained on pieces of fish fillet or scrapings from frozen liver and heart. The feeding frequency should be related to environmental conditions, such as temperature and light intensity. Daily feeding is not advisable for adult animals, but once to three times weekly to satiation at each feeding is recommended.

4.4.1. Food suitable for aquatic amphibians

It has been shown experimentally that in aquatic amphibians configurational aspects of the shape of prey do not play a role for prey-catching, since the water turbulences do not allow animals to define an object as prey, e.g., by its orientation to its direction of movement. Observation has shown that laboratory-held *Xenopus* have no difficulty in finding their food, as these aquatic frogs do not use their sight primarily, rather often use their limbs, sense of touch and their lateral-line-system to localize and gather food (Elepfand, 1996a,b; Elepfand et al., 2001).

Aquatic adult newts will feed on various aquatic invertebrates, such as snails, crustaceans, aquatic insect larvae and *Tubifex*. *Ambystoma mexicanum* can be fed with *Daphnia*, earthworms, mosquito larvae, and scrapings from frozen heart. Captive aquatic amphibians can be also fed with frozen shrimps, frozen whole minnows, pieces of fish fillet. Aquatic frogs, such as *Xenopus laevis*, will readily take scrapings from frozen liver and heart.

Special food diet is recommended for breeding programs, such as the one proposed for *Bombina orientalis* in Section 3.3.

4.4.2. Ethological studies on prey-catching releasers in terrestrial amphibians

The prey-catching behaviour of toads and frogs (Ewert, 1984, 1997, 2003, 2004a) and salamanders (Roth, 1987) is well investigated in experiments using prey dummies [see also movie on *Image Processing in the Visual System of the Common Toad – Behaviour, Brain Function, Artificial Neuronal Net.* IWF-Film C1805, Video or CD-ROM; IWF Wissen und Medien gGmbH, Institut für den Wissenschaftlichen Film, Nonnenstieg 72, D-37075 Göttingen, Germany, vertrieb@iwf.de].

These amphibians respond preferably to living prey; thus, movement is an essential component of the prey sign stimulus (Ewert, 2004a,b). Jerky movements make prey especially attractive. In common toads, *Bufo bufo*, the speed of prey dummies eliciting maximal prey-catching activity is 30-60 deg. of visual angle per sec [°/sec]. Prey stimuli moving less than 3°/sec are still detected and responded. The minimal detectable speed for movement specific ganglion cells of the retina is even less than 0.1°/sec. Another important component of the prey stimulus is size. In response to square or disc-shaped prey dummies, contrasting with the background, those of an edge length of 5 to 10 mm are optimal prey-catching releasers. Moving small objects of 0.3 mm will still elicit prey-capture. Investigations on different anuran species have shown that the preferred prey size s [mm] correlates with the width w [mm] of the animal's jaw, $s=0.43w$. Moving big objects elicit avoidance behaviour, especially those traversing the animal's dorsal visual field, thus simulating a bird of prey. Configurational aspects of prey, too, play an important role for the release of prey-catching behaviour. Experiments using small moving bars of card board of different length and different orientation, either parallel or across the direction of their movement, have shown: elongation of the edge of a bar parallel to the direction of movement ("worm" configuration) increases the similarity to prey, whereas elongation of the edge across the direction of movement ("anti-worm" configuration) strongly reduces the prey value. From the toad's midbrain roof, responses of neurones were recorded which show preferences to prey-like stimuli. In fact, natural prey objects of the toad's biotope move in direction of their longitudinal body axes, such as earthworms, millipedes, wood lice, and slugs (Ewert, 1974, 1998).

The "anti-worm" stimulus resembles configurational components of moving snakes, the arch-enemies of toads. Certain snakes (e.g., hognose snakes *Heterodon* spp.) are primarily toad predators in nature. Hognose snakes are opisthoglyphous (having fangs at the back of the mouth) and they use this feature to "deflate" toads which may puff themselves up with air to unswallowable proportions. If, under captive conditions, a hognose snake will not accept frogs (which are easier to obtain than toads) they may be "scented" by rubbing with fresh toad skin before feeding. If a hungry hognose refuses mice, rubbing a mouse against a toad, so that the mouse smells like a toad, will often "trick" them into eating mice.

4.4.3. Laboratory food

The preference to worm-like moving objects justifies feeding toads on mealworms (larvae of *Tenebrio molitor),* which can be obtained from mealworm farms. Laboratory experience has shown that for large numbers of toads mealworms are a suitable long-term diet. In this case, however, it is advisable to maintain mealworms on mouse diet pellets which contain the essential nutrients and are enriched with vitamins and trace elements. In this food chain, toads develop no nutritional deficiencies. Nevertheless, the diet of anurans and salamanders should be enriched by earthworms, crickets, collemboles, beetles, woodlice, millipedes, fly larvae, etc.

In many frog species, the preference for worm-shaped prey animals is much less developed than in common toads. Water frogs, especially *Rana esculenta*, prefer various flying insects, attracted by their irregular movements. These frogs are difficult to maintain in captivity. Their terraria must be covered with fine screens to insure that the insects offered as food do not escape. Very large frog and toad species, such as *Rana catesbeiana* or *Bufo marinus* need large amounts of food. They take even live young mice. By conditioning, they can be induced also to take dead food items like liver, heart, cray fish, or small fish. *Bufo marinus* can be conditioned by smell to take even dog food.

4.4.4. Food conditioning and consequences of hand-feeding

During feeding, laboratory toads and frogs quickly associate the prey odour with prey. As a result, in the presence of familiar prey odour, the toad's prey-catching motivation increases to such an extent that non-prey items are included in their prey schema, such as a moving conspecific, or even the moving hand of the experimenter. In the absence of familiar prey odour the prey-selective behaviour is normal. The neurobiological basis of this phenomenon was investigated quantitatively (Ewert et al., 2001). Since in the presence of familiar prey odour, the toad's prey-selecting behaviour is strongly reduced, it is possible to feed toads with mealworms that are filled in a dish. Attracted by the prey odour, the toads approach the dish and – sitting around it – will catch the worms. This arrangement has the advantage that prey items are not scattered over the terrarium. Efficient group feeding involves feeding frenzies, whereby the feeding activity is initiated by one animal inducing similar behaviour in other animals in the close vicinity [cf. also Section 4.3.1].

Toads can be conditioned also in another way, namely by hand-feeding. If a prey object is presented to the toad with the experimenter's hand, for several days once, the toad associates the moving hand (previously a threatening stimulus) with prey. Consequently, the toad treats the hand like a prey and snaps towards it. This effect is generalized to such an extent that almost every moving object (in the absence of prey odour!) releases prey-catching behaviour. Here, too, the neurobiological basis of this type of conditioning was studied in detail (Ewert et al., 2001). It should be emphasized that hand-feeding is unsuitable for procedures in which conditioning would have a negative impact on the experimental results.

Most of the salamander species, too, not only possess a highly efficient visual system, but can orient themselves almost as effectively by means of olfaction or vibration sense. Furthermore, it has been shown that at least part of their behaviour – especially that concerned with feeding – is influenced by individual experience (e.g., see Roth, 1987).

4.5. Water quality

For aquatic and semi-aquatic amphibians water quality, including the concentration of ammonia and the pH level in water, should be regularly monitored.

In order to avoid diseases, the land and pool areas in the terraria must be carefully cleaned from dirt, excrements and food particles [cf. Section 3.4]. Good hygiene improves the health of the laboratory animals.

For aquatic species it is advisable to apply re-circulated biologically filtered water re-use systems, because such systems permit most easily the controlling of water quality. Consistent water quality is of paramount importance, because it reduces stress and contributes to animal health, which in turn permits the user to perform successful science.

It is obviously important to keep *Xenopus laevis* in clean water, least of all to prevent disease. *Xenopus* are adapted to slow-moving, turbid water. However, there is no substantive evidence that circulating flowing systems are more healthful for this particular species of frog in the long run. In the UK, about 70% of institutional facilities operate so-called “fill and dump” systems. Many of these have been in place for 10 years or more and none report any persistent, detrimental welfare problems.

A key issue with fully aquatic species is the volume of water available to them and the frequency with which it is changed. The reason for this is that conditions leading to poor health (and presumably suffering) are those in which the ammonia level rises too high. Ammonia is excreted by amphibians, and its concentration will depend on the water handling regime. A level of 5 mg/lit would be cause for concern, although the degree of potential harm increases steeply with the pH of the water, as there is more ammonia and less $NH4^{+}$ at higher pH. All of the fill and dump facilities in the UK utilise a minimum of 2.5 litres of water per *Xenopus laevis* (1 lit for *Xenopus tropicalis*), and water changes occur at least two times per week with no apparent ill effects. Therefore, a system with regular water changes achieves the goal of maintaining appropriate water quality very successfully. Given the success which individuals have achieved with these systems, they should therefore be considered as well as flow-through water systems. In fact, it is clearly worthwhile to develop good re-circulating systems which utilise continuous purification regimes, since these can generate extremely low levels of ammonia and mimic the condition of an infinite water supply for each animal.

4.6. Substrate, litter, bedding and nesting material (See paragraph 4.8 of the General Section)

4.7. Cleaning

In order to avoid diseases, the terrestrial and aquatic areas in the terraria shall be carefully cleaned to remove dirt, excrement and food particles.

In order to avoid diseases, the land and pool areas of the terraria must be carefully cleaned to remove dirt, excrements, and food particles. The same holds for the tanks of aquatic animals; in these tanks the water should circulate. It should be avoided to use aggressive detergents. Amphibians do explore their cages and will choose a favourite place (e.g., a stone or piece of bark) drinking from and sleeping on. They become used to the enclosures of a cage. After cleaning the cage, therefore, it is best to place each stone and all other enclosures in the cage in the same positions they were before cleaning [see also Section 4.8].

4.8. Handling

The skin of amphibians can be easily damaged. Care is required during handling, which should be kept to a minimum.

When handled roughly, most toads and frogs squirt out from the urinary bladder a transparent odourless and relatively harmless fluid. For studies on stress and adrenocortical modulation in amphibians see Moore & Jessop (2003). Due to various kinds of secretions by the skin of amphibians, persons handling these animals should always wear suitable gloves. There is some dispute over the requirement to use plastic gloves. Although there may be circumstances where the use of gloves is warranted, there are reports of some types of gloves causing severe skin reactions, for example, in *Xenopus*.

Persons dissecting freshly killed frogs should note that these animals may be infected with the trematode *Alaria* which may infect humans too [cf. Section 3.4]. For *Salmonella* infection in amphibians see Pfleger et al. (2003). Appropriate personal protective equipment should be used to protect the handler.

A peculiarity of many amphibian species – especially members of the Salientia – concerns the character of skin secretions (e.g., Summers & Cough, 2001; Chen et al., 2003). In threatened toads, a secretion is given out in minute quantities from glands of the body skin. The poison varies in amount and intensity with the species. Skin glands may be small, so that the skin appearing smooth (frogs). They are large at the warts and parotoid glands of many toads and at the lateral fields of some frogs. Slime glands assist in the processes of respiration by the skin and its alkaloid simultaneously acts protective as a narcotic. The secretion of the parotoids is milky, acid, and thought to act protective as a convulsive to the heart and central nervous system of an aggressor. For example, the poison secreting glands of the common toad *Bufo bufo* contain digitalis. The poison bufotenin is related chemically to the hallucinogenic psilocin of the Mexican fungus *Psilocybe mexicana*. The tolerance to these drugs of certain species of snakes which prefer toads as food [Section 4.3.2] is relatively high. More specifically, four categories of compounds are found in the granular or poison glands: (1) biogenic amines, (2) bufodienolides (bufogenins), (3) alkaloids and steroids, (4) peptides and proteins (Clarke, 1997). Over 300 alkaloids have been identified in the amphibian skin, many of which have unique profiles of pharmacological activity and therapeutic potential (Daly, 1995a). Interestingly, amphibian skin secretions also contain peptide antibiotics (Gallo & Huttner, 1998). Antimicrobial peptides are considered the effector molecules of innate immune acting as a first line of defence against bacterial infections (Simmaco et al., 1998; cf. also Duda et al., 2002; Rinaldi, 2002). Two groups of South African frogs have skin secretions that are potentially lethal to humans and animals (Pantanowitz et al., 1998). For the chemistry of poisons in the amphibian skin see also Daly (1995b). Amphibian skin collagens are identified by Sai & Babu (2001). Doyle et al. (2003) studied anticancer activities of the amphibian skin peptide citropin 1.1.

4.9. Anaesthesia and humane killing

Invasive, potentially painful procedures should be accompanied both by analgesia and anaesthesia. As amphibians' skin accounts for a significant portion of normal gaseous exchanges, in anaesthetised animals, in which lung respiration is reduced or interrupted, the body skin should always be kept moist, for example with a wet tissue.

Pain perception in amphibians is likely analogous to that in mammals. Since response thresholds to chemical (topical acetic acid), thermal, and mechanical stimuli are significantly elevated after spinal opioid administration (effects were abolished by prior systemic administration of the opioid antagonist naltrexone), these sensory modalities are measures of nociceptive activity in amphibians (Willenbring & Stevens, 1997). Amphibians possess neural systems for transmitting pain from peripheral receptors to the brain and anti-nociceptive mechanisms to modulate pain (Downes et al., 1999; Machin, 1999). Invasive, potentially painful procedures should, therefore, be accompanied both by analgesia and anaesthesia.

Whereas opioids in mammals act by interactions with three distinct types of receptors (mu, delta, kappa), in amphibians mu, delta, and kappa opioids mediate anti-nociception via a single type of opioid receptor, the uni-receptor in the spinal route (Stevens & Rothe, 1997; Stevens & Newman, 1999; Newman et al., 2002). Furthermore, analgesia is mediated in frogs by an alpha2-adrenoceptor mechanism, since it was shown that epinephrine and norepinephrine – e.g., released by stress – have analgetic potencies (Stevens et al., 1995; Willenbring & Stevens, 1996). For analgesia in amphibians see also Wright (2000) and Machin (2001).

4.9.1. Anaesthesia

Suitable drugs for local analgesia are, e.g. Novocain®, Meaverin®, or Xylocain®. Suitable anaesthetics commonly used are Tricain (ethyl-2-aminobenzoat), also called MS 222®. This drug should be used as a solution (e.g., 150 mg/l) in combination with sodium-bicarbonate (300 mg) for neutralizing in order to avoid skin irritability. It is important to consider that the tolerance to drugs may depend on the season. Contrasting effects of anaesthetics in tadpole bioassays are reported by Downes & Courogen (1996).

4.9.2. Humane killing

Various methods are suitable for killing amphibians after experiments have been completed. Methods which provide least stress and no pain to the animal are an overdose of an appropriate anaesthetic.

Neuronal activity, and thus pain, depends on metabolic activity which depends on temperature. Some anaesthetic agents, such as ether or halothane, may affect the animal's sensitive skin, release strong secretions of the skin glands, and produce initial violent movements by the animal which can be interpreted as signs of distress. The use of such agents should be avoided.

All methods used must be in conformity with the principles set by the EC guidelines on the human killing of laboratory animals: *European Commission's Publication Euthanasia of Experimental Animals*.

4.10. Records (See paragraph 4.12. of the General Section)

The person responsible for an animal facility should keep a diary in which all events and activities are noticed: feeding, watering, cleaning, actual count of animals per tank or terrarium; admissions, loss by death; cases of disease; unusual disturbances; identification and marking of experimental animals.

4.11. Identification

Where animals need to be identified individually, there are a number of suitable methods such as transponders; tank labels for individually housed animals; monitoring pigment or wart configurations; small labels by coloured thread. Chemical markings should not be used, since substances are absorbed through the skin, possibly causing toxic effects. Toe clipping is deleterious and should not be carried out.

5. Transport

During transport, amphibians should provided with sufficient air and moisture and, if necessary, appropriate devices to maintain the required temperature and humidity.

In the appropriate season of the year, amphibians should be ordered from dealers which follow the recommendations of: the *European Convention on the Protection of Animals during International Transport*, the *International Air Transport Association*, and the *Animal Air Transport Association*. Semi-terrestrial amphibians should be singly packed in boxes of adequate size and provided sufficiently with air and moisture. Transportation of tropic species – depending on the local climate – requires an accommodation with appropriate heating devices. After arrival, animals must be unpacked without delay. Animals which arrived in a sick condition, and which do not have a chance to recover, should be sacrificed at once by a human method [Section 4.9]. The commercial sender should be informed.

Appendix: Examples of amphibians and their biotopes*)

*) Source: Swedish Board of Agriculture, Department of Animal Production and Health, Animal Welfare Division

Biotope	**1** – trees (arboreal) **2** – ground (terrestrial/semi-terrestrial) **3** – half in water (semi-aquatic) **4** – fully in water (aquatic)

Species	
Agalychnis callidryas	1
Agalychnis spurelli	1
Alytes obstetricans	2-3
Ambystoma annulatum	2
Ambystoma cingulatum	2
Ambystoma gracile	2
Ambystoma jeffersonianum	2
Ambystoma laterale	2
Ambystoma macrodactylum	2
Ambystoma maculatum	2
Ambystoma mexicanum (metam.)	2
Ambystoma mexicanum (neoten)	4
Ambystoma opacum	2
Ambystoma talpoideum	2
Ambystoma tigrinum	2
Amphiuma means	4
Aneides ferreus	2
Anotheca spinosa	1
Atelopus varius	2
Batrachoseps attenuatus	2
Bolitoglossa altamazonica	1-2
Bolitoglossa mexicana	1
Bombina bombina	2-3
Bombina orientalis	2-3
Bombina variegata	3
Bufo alvarius	2
Bufo americanus	2
Bufo blombergi	2
Bufo boreas	2
Bufo bufo	2
Bufo carens	2
Bufo marinus	2
Bufo mauretanicus	2
Bufo melanostictus	2
Bufo paracnemis	2
Bufo quercicus	2
Bufo viridis	2
Caecilian thompsoni	2
Ceratophrys aurita	2
Ceratophrys cornuta	2
Ceratophrys cranwelii	2
Ceratophrys ornata	2
Chioglossa lusitanica	2-3
Chiromantis xerampelina	1
Colostethus trinitatus	1-2
Cryptobranchus alleganiensis	4
Cynops ensicauda	3
Cynops orientalis	3
Cynops pyrrhogaster	3
Dendrobates spp.	1-2
Desmognathus auriculatus	2-3
Desmognathus fuscus	2
Desmognathus ochrophaeus	2-3
Desmognathus quadramaculatus	2-3
Desmognathus wrighti	2
Discoglossus pictus	2-3
Dyscophus antongilii	2
Eleutherodactylus spp.	2
Ensatina eschscholtzii	2
Epipedobates tricolor	2
Euproctus plarycephalus	3
Eurycea bislineata	2-3
Eurycea longicauda	2-3
Gastrotheca spp.	1
Hyla arborea	1
Hyla cinerea	1
Hyla ebraccata	1
Hyla gratiosa	1
Hyla vasta	1
Hyla versicolor	1
Hymenochirus boettgeri	4
Hynobius spp.	2-3
Hyperolius marmoratus	1
Ichthyophis glutinosus	2
Ichthyophis kohtaoensis	2
Kaloula pulchra	2
Kassina senegalensis	1-2
Kassina weali	1-2
Lepidobatrachus laevis	2
Leptopelis macrotis	1-2
Litoria caerulea	1
Litoria infrafrenata	1
Manculus quadridigitatus	2-3
Mantella aurantiaca	2
Mantella betselio	2
Mantella cowani	2
Mantella madagascariensis	2
Megophrys spp.	2
Mertensiella luschani	2-3
Necturus maculosus	4
Neurergus spp.	3
Notophthalmus meridionalis	3
Notophthalmus perstriatus	3
Notophthalmus viridescens	3
Occidozyga lima	3

Osteopilus septentrionalis	1
Pachytriton brevipes	3
Paramesotriton caudopunctatus	3
Paramesotriton chinensis	3
Paramesotriton hongkongensis	3
Pedostibes hosi	1-2
Pelobates spp.	2
Pelodytes punctatus	2
Peurodeles waltlii	3-4
Phrynomerus microps	1-2
Phyllobates spp.	2
Phyllomedusa lemur	1
Pipa parva	4
Pipa pipa	4
Plethodon cinereus	2
Plethodon glutinosus	2
Plethodon jordani	2
Plethodon vehiculum	2
Polypedates leucomystax	1
Pseudacris crucifer	1
Pseudacris regilla	1,3
Pseudis paradoxus	3-4
Pseudotriton ruber	2
Pyxicephalus adspersus	3
Rana catesbeiana	3
Rana clamitans	3
Rana iberica	3
Rana pipiens	3
Xenopus muelleri	4

Rana ridibunda	3
Rana temporaria	2-3
Rhacophorus reinwardtii	1
Salamandra atra	2
Salamandra salamandra	2
Salamandrella keyserlingii	2-3
Salamandrina terdigitata	2-3
Scaphiopus spp.	2
Siren intermedia	4
Taricha granulosa	2-3
Taricha torosa	2-3
Triturus alpestris	2-3
Triturus boscai	2-3
Triturus carnifex	2-3
Triturus cristatus	2-3
Triturus dobrogicus	2-3
Triturus helveticus	2-3
Triturus italicus	2-3
Triturus karelini	2-3
Triturus marmoratus	2-3
Triturus montandoni	2-3
Triturus vittatus	2-3
Triturus vulgaris	2-3
Tylototriton spp.	2-3
Typhlonectes natans	4
Xenopus tropicalis	4
Xenopus laevis	4

References

Abe A.S. (1995) Estivation in South American amphibians and reptiles. Braz. J. Med. Biol. Res. (11-12): 1241-1247

Acher R., Chauvet J., Rouille Y. (1997) Adaptive evolution of water homeostasis regulation in amphibians: vasotocin and hydrins. Biol. Cell. 89(5-6): 283-291

Aertsen A.M., Vlaming M.S., Eggermont J.J., Johannesma P.I. (1986) Directional hearing in the grassfrog (*Rana temporaria* L.). II. Acoustics and modelling of the auditory periphery. Hear Res. 21(1): 17-40

Aikin G.C. (1966) Self-inhibition of growth in *Rana pipiens* tadpoles. Physiol. Zool. 39: 341

Alexander T.R. (1964) Observations on the feeding behavior of *Bufo marinus* (Linne). Herpetologica 20(4): 255-259

Ankley G.T., Diamond S.A., Tietge J.E., Holcombe G.W., Jensen K.M., Defoe D.L., Peterson R. (2002) Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hind limb mal-formations in the northern leopard frog (*Rana pipiens*). Environ. Sci. Technol. 36(13): 2853-2858

Armstrong J.B., Malacinski G.M. (eds.) (1989) Developmental Biology of the Axolotl. Oxford University Press, New York

Barton D.P. (1999) Ecology of helminth communities in tropical Australian amphibians Int. J. Parasitol. 29(6):921-926

Barton D.P., Pichelin S. (1999) *Acanthocephalus bufonis* (Acanthocephala) from *Bufo marinus* (Bufonidae: Amphibia) in Hawaii. Parasite. 6(3): 269-272

Bee M.A. (2002) Territorial male bullfrogs (*Rana catesbeiana*) do not assess fighting ability based on size-related variation in acoustic signals. Behavioral Ecology 13: 109-124

Bee M.A., Bowling A.C. (2002) Socially-mediated pitch alteration by territorial male bullfrogs, *Rana catesbeiana*. Journal of Herpetology 36: 140-143

Bee M.A., Gerhardt H.C. (2002) Individual voice recognition in a territorial frog (*Rana catesbeiana*). Proc. R. Soc. Lond. B. Biol. Sci. 269(1499): 1443-1448

Beetschen J.C. (1996) How did urodele embryos come into prominence as a model system? Int. J. Dev. Biol. 40(4): 629-636

Benraiss A., Caubit X., Arsanto J.P., Coulon J., Nicolas S., Le-Parco Y., Thouveny X. (1996) Clonal cell cultures from adult spinal cord of the amphibian urodele *Pleurodeles waltlii* to study the identity potentialities of cells during tail regeneration. Dev. Dyn. 205(2): 135-149

Berger L., Speare R., Daszak P., et al. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences, USA 95: 9031-9036

Beynon P.H., Cooper J.E. (1994) Manual of Exotic Pets. Iowa State University Press

Beynon P.H., Cooper J.E. (1999) Bsava: Manual Animals Exotics. Elsevier Science

Blaustein A.R., Belden L.K. (2003) Amphibian defenses against ultraviolet-B radiation. Evol. Dev. 5(1): 89-97

Blaustein A.R., Johnson P.T. (2003) Explaining frog deformities. Sci. Am. 288(2): 60-65

Blaustein A.R., Kiesecker J.M., Chivers D.P., Anthony R.G. (1997) Ambient UV-B radiation causes deformities in amphibian embryos. Proc. Natl. Acad. Sci. USA 94(25): 13735-13737

Blum S., Basedow T., Becker N. (1997) Culicidae (Diptera) in the diet of predatory stages of anurans (Amphibia) in humid biotopes of the Rhine Valley in Germany. J. Vector. Ecol. 22(1): 23-29

Bolhuis J.J., Giraldeau L.-G. (eds.) (2004) Principles of Animal Behavior. Blackwell Publ.

Boterenbrood E.C., Verhoeff-De Fremery R. (1976) Urodeles. In: UFAW Handbook (5th Edn.). Churchill Livingston, Edinburgh, UK, pp. 525-537

Bover R., Grue C.F. (1995) The need for water quality criteria for frogs. Environmental Health Perspectives. 103(4): 352-357

Boyle D.G., Hyatt A.D., Daszak P., Berger L., Longcore J.E., Porter D., Hengstberger S.G., Olsen V. (2003) Cryo-archiving of *Batrachochytrium dendrobatidis* and other chytridiomycetes. Diseases of Aquatic Oranisms 56: 59-64

Branco L.G. (1995) Interactions between body temperature regulation and blood acid-base status in anuran amphibians. Braz. J. Med. Biol. Res. 28(11-12): 1191-1196

Brandon R.A. (1989) Natural history of the axolotl and its relationship to other Ambystomatid salamanders. In Developmental Biology of the Axolotl (J.B. Armstrong, G.M. Malacinski, eds.). Oxford University Press, NewYork, pp. 13-21

Bridges C.M. (2000) Long-term effects of pesticide exposure at various life stages of the Southern leopard frog (*Rana sphenocephala*). Arch. Environ. Contam. Toxicol. 39(1): 91-96

Brockes J.P. (1997) Amphibian limb regeneration: rebuilding a complex structure. Science. 276(5309): 81-87

Bruner M.A., Rao M., Dumont J.N., Hull M., Jones T., Bantle J.A. (1998) Ground and surface water developmental toxicity at a municipal landfill: description and weather-related variation. Ecotoxicol. Environ. Saf. 39(3): 215-226

Burkhart J.G., Ankley G., Bell H., Carpenter H., Fort D., Gardiner D., Gardner H., Hale R., Helgen J.C., Jepson P., Johnson D., Lannoo M., Lee D., Lary J., Levey R., Magner J., Meteyer C., Shelby M.D., Lucier G. (2000) Strategies for assessing the implications of malformed frogs for environmental health. Environ. Health. Perspect. 108(1): 83-90

Bush M., Custer R.S., Smeller J.M., Charache P. (1980) Recommendations for antibiotic therapy in reptiles. In: Reproductive Biology and Diseases of Captive Reptiles (J.B. Murphy, J.T. Collins, eds.). Symp. Soc. for the Study of Amphibians and Reptiles, Oxford, OH

Calevro F., Campani S., Ragghianti M., Bucci S., Mancino G. (1998) Tests of toxicity and teratogenicity in biphasic vertebrates treated with heavy metals (Cr^{3+}, Al^{3+}, Cd^{2+}). Chemosphere 37(14-15): 3011-3017

Capranica R.R., Moffat J.M. (1983) Neurobehavioral correlates of sound communication in anurans. In: Advances in Vertebrate Neuroethology (J.-P. Ewert, R.R. Capranica, D.J. Ingle, eds.). Plenum Press, New York

Carey C. (2000) Infectious disease and worldwide declines of amphibian populations, with comments on emerging disease coral reef organisms and in humans. Environ. Health. Perspect. 108 Suppl. 1: 143-150

Carey C., Cohen N., Rollins-Smith L. (1999) Amphibian declines: an immunological perspective. Dev. Comp. Immunol. 23(6): 459-472

Carlson M.R., Bryant S.V., Gardiner D.M. (1998) Expression of Msx-2 during development, regeneration, and wound healing in axolotl limbs. J. Exp. Zool. 282(6): 715-723
Castillo G.A., Orce G.G. (1997) Response of frog and toad skin to norepinephrine. Comp. Biochem. Physiol. A. Physiol. 118(4): 1145-1150
Chen T., Farragher S., Bjourson A.J., Orr D.F., Rao P., Shaw C. (2003) Granular gland transcriptomes in stimulated amphibian skin secretions. Biochem. J. 371(Pt 1): 125-130
Chernoff E.A. (1996) Spinal cord regeneration: a phenomenon unique to urodeles? Int. J. Dev. Biol. 40(4): 823-831
Chernoff E.A., Stocum D.L., Nye H.L., Cameron J.A. (2003) Urodele spinal cord regeneration and related processes. Dev. Dyn. 226(2): 295-307
Chivers D.P., Wildy E.L., Kiesecker J.M., Blaustein A.R. (2001) Avoidance response of juvenile Pacific tree frogs to chemical cues of introduced predatory bullfrogs. J. Chem. Ecol. 27(8): 1667-1676
Chivian, E. (ed.) (2002) Biodiversity: Its importance to human health. Harvard Medical School
Christensen R.N., Tassava R.A. (2000) Apical epithelial cap morphology and fibronectin gene expression in regenerating axolotl limbs. Dev. Dyn. 217(2): 216-224
Clarke B.T. (1997) The natural history of amphibian skin secretions, their normal functioning and potential medical application. Biol. Rev. Camb. Philos. Soc. 72(3): 365-379
Cochran D.M. (1961) Living Amphibians of the World. Doubleday and Co., Toronto, Ont.
Conant R.M. (1975) A Field Guide to Reptiles and Amphibians of Eastern and Central North America (2nd Edn.). Houghton Mifflin Co., Boston, M.A.
Conant R., Collins J.T. (1998) Reptiles and Amphibians. Houghton Mifflin Company, New York
Constanzo J.P., Lee R.E. Jr., DeVries A.L., Wang T., Layne J.R. Jr. (1995) Survival mechanisms of vertebrate ectotherms at subfreezing temperatures: applications in cryomedicine. FASEB. J. 9(5): 351-358
Cooper J.E. (1984) The First *Edward Elkan Memorial Lecture*. International Colloquium on the Pathology of Reptiles and Amphibians. Nottingham UK
Cooper J.E. (1985) Manual of Exotic Pets. Revised edn. J.E. Cooper, M.F. Hutchison, O.F. Jackson, R.J. Maurice. BSAVA British Small Animal Veterinary Association, Cheltenham
Cooper J.E., Cooper M.E. (2003) Wildlife Health Services. Wellingborough, Northants UK
Cosgrove G.E. (1977) Amphibians Diseases. In: Current Veterinary Therapy (VI) Small Animal Practice (R.W. Krik, ed.). W.B. Saunders Co., Philadelphia, P.A., pp. 769-772
Crews L., Gates P.B., Brown R., Joliot A., Foley C., Brockes J.P., Gann A.A. (1995) Expression and activity of the newt Msx-1 gene in relation to limb regeneration. Proc. R. Soc. Lond. B. Biol. Sci. 259(1355): 161-171
Cunningham A.A., Sainsbury J.E., Cooper J.E. (1996) Diagnosis and treatment of a parasitic dermatitis in a laboratory colony of African clawed frogs (*Xenopus laevis*). Vet. Rec. 29: 640-642
Cunningham A.A., Daszak P., Rodríguez J.P. (2003) Pathogen pollution: defining a parasitological threat to biodiversity conservation. The Journal of Parasitology 89: S78-S83
Daly J.W. (1995a) Alkaloids from frog skins: selective probes for ion channels and nicotinic receptors. Braz. J. Med. Biol. Res. 28(10): 1033-1042
Daly J.W. (1995b) The chemistry of poisons in amphibian skin. Proc. Natl. Acad. Sci. USA. 92(1): 9-13
Daszak P., Cunningham A.A. (2003) Anthropogenic change, biodiversity loss, and a new agenda for emerging diseases. The Journal of Parasitology 89: S37-S41
Daszak R., Berger L., Cunningham A.A., Hyatt A.D., Green D.E., Speare R. (1999) Emerging infectious diseases and amphibian population declines. Emerg. Infect. Dis. 5(6): 735-748
Daszak P., Cunningham A.A., Hyatt A.D. (2000) Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287: 443-449
Daszak P., Cunningham A.A., Hyatt A.D. (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78: 103-116
Daszak P., Cunningham A., Hyatt A. (2003) Infectious disease and amphibian population declines. Journal of Diversity and Distributions 9: 141-150
Davies P.M. (1981) Anatomy and physiology. In: Diseases of the Reptilia Vol.1 (J.E. Cooper, O.F. Jackson, eds.). Academic Press, London, pp. 9-67
Davies A.J., Johnston M.R. (2000) The biology of some intraerythrocytic parasites of fishes, amphibia and reptiles. Adv. Parasitol. 45: 1-107
Dawley E.M., Crowder J. (1995) Sexual and seasonal differences in the vomeronasal epithelium of the red-backed salamander (*Plethodon cinereus*). J. Comp. Neurol. 359(3): 382-390
Delfino G., Brizzi R., Alvarez B.B., Taddei L. (1999) Secretory polymorphism and serous cutaneous gland heterogeneity in *Bufo granulosus* (Amphibia, Anura) Toxicon. 37(9): 1281-1296
Denver R.J. (1997) Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. Horm. Behav. 31(2): 169-179
Deuchar E.M. (1972) *Xenopus laevis* and developmental biology. Biol. Rev. 47: 37
Diamond S.A., Peterson G.S., Tietge J.E., Ankley G.T. (2002) Assessment of the risk of solar ultraviolet radiation to amphibians. III Prediction of impacts in selected Northern Midwestern wetlands. Environ. Sci. Technol. 36(13): 2866-2874
Dickerson M.C. (1931) The Frog Book. Doubleday, Doran, and Company, Inc., Garden City, New York
Donoghue S. (1996) Veterinary nutritional management of amphibians and reptiles. J. Am. Vet. Med. Assoc. 208(11): 1816-1820
Downes H., Courogen P.M. (1996) Contrasting effects of anesthetics in tadpole bioassays. J. Pharmacol. Exp. Ther. 278(1): 284-296
Downes H., Koop D.R., Klopfenstein B., Lessov N. (1999) Retention of nociceptor responses during deep barbiturate anesthesia in frogs. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 124: 203-210

Doyle J., Brinkworth C.S., Wegener K.L., Carver J.A., Llewellyn L.E., Olver I.N., Bowie J.H., Wabnitz P.A., Tyler M.J. (2003) nNOS inhibition, antimicrobial and anticancer activity of the amphibian skin peptide, citropin 1.1 and synthetic modifications. The solution structure of a modified citropin 1.1. Eur. J. Biochem. 270(6): 1141-1153.

Duchamp V.P., Palouzier P.B, Duchamp A. (1996) Odor coding properties of frog olfactory cortical neurons. Neuroscience. 74(3): 885-895

Duda T.F. jr., Vanhoye D., Nicolas P. (2002) Roles of diversifying selection and coordinated evolution in the evolution of amphibian antimicrobial peptides. Mol. Biol. Evol. 19(6): 858-864

Duellman W.E., Trueb L. (1985) Biology of Amphibians. McGraw-Hill, New York

Echeverry K., Tanaka E.M. (2003) Electroporation as a tool to study in vivo spinal cord regeneration. Dev. Dyn. 226(2): 418-425

Ehret G., Capranica R.R. (1980) Masking patterns and filter characteristics of auditory nerve fibers in the green treefrog *(Hyla cinerea)*. J. Comp. Physiol. 141: 1-12; 13-18

Elepfandt A. (1996a) Sensory perception and the lateral line system in the clawed frog, *Xenopus*. In: The Biology of *Xenopus* (H.R. Kobel, R. Tinsley, eds.). Symp. Zool. Soc. London 68: 97-120

Elepfandt A. (1996b) Underwater acoustics and hearing in the clawed frog, *Xenopus*. In: The Biology of *Xenopus* (H.R. Kobel, R. Tinsley, eds.). Symp. Zool. Soc. London 68: 177-193.

Elepfandt A., Eistetter I., Fleig A., Günther E., Hainich M., Hepperle S.,Traub B. (2001) *Hearing threshold and frequency discrimination in the purely aquatic frog Xenopus laevis* (Pipidae)*: determination by means of conditioning.* J. Exp. Biol. 203: 3621-3629.

Emerson S.B., Boyd S.K. (1999) Mating vocalizations of female frogs: control and evolutionary mechanisms. Brain Behav. Evol. 53(4): 187-197

English R., English A. (2000) Geographic distribution, *Hyla cinerea.* Herpetol. Rev. 31:251

Epstein P.R., Defilippo C. (2001) West Nile virus and drought. Global Change and Human Health 2(2): 2-4

Ewert J.-P. (1974) The neural basis of visually guided behavior. Scientific American 230: 34-42

Ewert J.-P. (1980) Neuroethology. An Introduction to the Neurophysiological Fundamentals of Behavior. Springer, Berlin, Heidelberg, New York

Ewert J.-P. (1984) Tectal mechanisms that underlie prey-catching and avoidance behaviors in toads. In: Comparative Neurology of the Optic Tectum (H. Vanegas, ed.). Plenum Press, New York, London, pp. 247-416

Ewert J.-P. (1985) The Niko Tinbergen Lecture 1983: Concept in vertebrate neuroethology. Animal Behaviour 33: 1-29

Ewert J.-P. (1997) Neural correlates of key stimulus and releasing mechanism: a case study and two concepts. Trends Neurosci. 20: 332-339

Ewert J.-P. (1998) Neurobiologie des Verhaltens. Huber Verlag, Bern

Ewert J.-P. (2003) Instinct, evolving concept: toad model. In: Elsevier's Encyclopedia of Neuroscience. CD-ROM, 3rd Edn. (G. Adelman, B.H. Smith, eds.), Elsevier Science BV

Ewert J.-P. (2004) Motion perception shapes the visual world of amphibians. In: Complex Worlds from Simpler Nervous Systems (F.R. Prete, ed.), MIT Press, Cambridge

Ewert J.-P. (2005) Stimulus perception. Chapter 2; in: The Behavior of Animals (J.J. Bolhuis, L.-A. Giraldeau, eds.). Blackwell Publ.

Ewert J.-P., Ewert S.B. (1981) Wahrnehmung. Biologische Arbeitsbücher 35, Quelle & Meyer, Heidelberg

Ewert J.-P., Buxbaum-Conradi H., Dreisvogt F., Glagow M., Merkel-Harff C., Röttgen A., Schürg-Pfeiffer E., Schwippert W.W. (2001) Neural modulation of visuomotor functions underlying prey-catching behaviour in anurans: Perception, attention, motor performance, learning. Comp. Biochem. Physiol. A. Physiol. 128(3): 417-461

Feller A.E., Hedges S.B. (1998) Molecular evidence for the early history of living amphibians. Mol. Phylogenet. Evol. 9(3): 509-516

Feng A.S., Gerhardt H.C., Capranica R.S. (1976) Sound localization behavior of the green treefrog (*Hyla cinerea*) and the barking treefrog *(H. gratiosa).* J. Comp. Physiol. 107: 241-252

Ferretti P. (1996) Re-examining jaw regeneration in urodeles: what have we learnt? Int. J. Dev. Biol. 40(4): 807-811

Flajnik M.F. (1996) The immune system of ectothermic vertebrates. Vet. Immunol. Immunopathol. 54(1-4): 145-150

Frazer J.F.D. (1976) Anura (Frogs and Toads). In: UFAW Handbook (5th Ed.) Churchill Livingston, Edinburgh, UK., pp. 516-524

Freeman R.S., Stuart P.F., Cullen J.B. (1976) Fatal human infection with mesocercariae of the trematode *Alaria Americana*. Amer. J. Trop. Med. Hyg. 25: 803

Freye F.L. (1977) General considerations in the care of captive amphibians. In: Current Veterinary Therapy VI Small Animal Practise (R.S. Kirk, ed.). W.B. Saunders, Philadelphia, P.A., pp. 772-778

Funk W.C., Tallmon D.A., Allendorf F.W. (1999) Small effective population size in the long-toed salamander. Mol. Ecol. 8(10): 1633-1640

Gallo R.L., Huttner K.M. (1998) Antimicrobial peptides: an emerging concept in cutaneous biology. J. Invest. Dermatol. 111(5): 739-743

Gardiner D.M., Endo T., Bryant S.V. (2002) The molecular basis of amphibian limb regeneration: integrating the old with the new. Semin. Cell Dev. Biol. 13(5): 345-352

Gerhardt H.C. (1981) Mating call recognition in the green treefrog (*Hyla cinerea*). J. Comp. Physiol. 144: 9-16

Gillooly J.F., Charnov E.L., West G.B., Savage V.M., Brown J.H. (2002) Effects of size and temperature on developmental time. Nature. 417(6884): 70-73

Goleman W.L., Uruquidi L.J., Anderson T.A., Smith E.E., Kendall R.J., Carr J.A. (2002) Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in *Xenopus laevis*. Environ. Toxicol. Chem. 21(2): 424-430

Goldberg S.R., Bursey C.R., Gergus E.W., Sullivan B.K., Truong Q.A. (1996) Helminths from three treefrogs *Hyla arenicolor, Hyla wrightorum*, and *Pseudacris triseriata* (Hylidae) from Arizona. J. Parasitol. 82(5): 833-835

Grunz H. (2001) Developmental biology of amphibians after Hans Spemann in Germany. Int. J. Dev. Biol. 45: 39-50

Guyetant R., Miaud C., Battestyi Y., Nelva A. (1994) Caractéristiques de la reproduction de la Grenouille rousse *Rana temporaria* L. (Amphibiens, Anoures) en altitude (Massif de la Vanoise, Alpes du nord, France). Bull. Soc. Herp. Fr. 71-72: 13-21

Halverson M.A., Skelly D.K., Kiesecker J.M., Freidenburg L.K. (2003) Forest mediated light regime linked to amphibian distribution and performance. Oecologia. 134(3): 360-364

Harris M.L., Chora L., Bishop C.A., Bogart J.P. (2000) Species- and age-related differences in susceptibility to pesticide exposure for two amphibians, *Rana pipiens* and *Bufo americanus*. Bull. Environ. Contam. Toxicol. 64(2): 263-270

Hartup B.K. (1996) Rehabilitation of native reptiles and amphibians in DuPage County, Illinois. J. J. Wildl. Dis. 32(1): 109-112

Harvell C.D., Mitchell C.E., Ward J.R., Altizer S., Dobson A.P., Ostfeld R.S., Samuel M.D. (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296: 2158-2162

Hatch A.C., Belden L.K., Scheessele E., Blaustein A.R. (2001) Juvenile amphibians do not avoid potentially lethal levels of urea on soil substrate. Environ. Toxicol. Chem. 20(10): 2328-2335

Hayes M.P., Jennings M.R. (1986) Decline of ranid frog species in western North America: are bullfrogs (*Rana catesbeiana*) responsible? Journal of Herpetology 20: 490-509

Hayes T.B. (1998) Sex determination and primary sex differentiation in amphibians: genetic and developmental mechanisms. J. Exp. Zool. 281(5): 373-399

Hays J.B., Blaustein A.R., Kiesecker J.M., Hoffman P.D., Pandelova I., Coyle D., Richardson T. (1996) Developmental responses of amphibians to solar and artificial UVB sources: a comparative study. Photochem. Photobiol. 64(3): 449-456

Heyer R. et al. (eds.) (1994) Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. Smithsonian Institution Press, Washington and London

Hillyard S.D. (1999) Behavioral, molecular, and integrative mechanisms of amphibian osmoregulation. J. Exp. Zool. 283(7): 662-674

Hillyard S.D., Hoff K.S., Propper C. (1998) The water absorption response: a behavioural assay for physiological processes in terrestrial amphibians. Physiol. Zool. 71(2): 127-138

Hobel G., Gerhardt H.C. (2003) Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). Evolution Int. J. Org. Evolution. 57(4): 894-904

Holt P.E. (1981) Drugs and dosages. In: Diseases of the Reptilia Vol.2 (J.E. Cooper, O.F. Jackson, eds.). Academic Press, London, pp. 551-584

Hyatt A.D., Gould A.R., Zupanovic Z., Cunningham A.A., Hengstberger S., Whittington R.J., Kattenbelt-Coupar B.E. (2000) Comparative studies of piscine and amphibian iridoviruses. Arch. Virol. 145(2): 301-331

Imokawa Y., Yoshizato K. (1998) Expression of sonic hedgehog gene in regenerating newt limbs. Wound. Repair. Regen. 6(4): 366-370

Ippen R., Zwart P. (1996) Infectious and parasitic disease of captive reptiles and amphibians, with special emphasis on husbandry practices which prevent or promote diseases. Rev. Sci. Tech. 15(1): 43-54

Ishii S., Kubokawa K., Kikuchi M., Nishio H. (1995) Orientation of the toad, *Bufo japonicus*, toward the breeding pond. Zool. Sci. 12(4): 475-484

Jackson O.F. and Cooper, J.E. (1981) Nutritional diseases. In: Diseases of the Reptilia Vol.2 (J.E. Cooper, O.F. Jackson, eds.). Academic Press, London, pp. 409-428

Johansson M., Rasanen K., Merila J. (2001) Comparison of nitrate tolerance between different populations of the common frog, *Rana temporaria*. Aquat. Toxicol. 54(1-2): 1-14

Johnson A. D., Bachvarova R. F., Drum M., Masi, T. (2001). Expression of axolotl dazl RNA, a marker of germ plasm: widespread maternal RNA and onset of expression in germ cells approaching the gonad. Dev. Biol. 243, 402-415.

Johnson A.D., Bachvarova R. F., Drum M., Masi, T. (2003a).Regulative Germ Cell Specification in Axolotl Embryos: A Primitive Trait Conserved in the Mammalian Lineage. Philosophical Transactions of the Royal Society

Johnson A.D., Bachvarova R. F., Drum M., Masi, T. (2003b).Evolution of predetermined germ cells in vertebrate embryos: implications for macroevolution. Evol. Dev. 5, 414-431

Johnson P.T.J., Lunde K.B., Ritchie E.G, Launer A.E. (1999) The effect of trematode infection on amphibian limb development and survivorship. Science 284: 802-804

Jorgensen C.B. (1997a) 200 years of amphibian water economy: from Robert Townson to the present. Biol. Rev. Camb. Philos. Soc. 72(2): 153-237

Jorgensen C.B. (1997b) Urea and amphibian water economy. Comp. Biochem. Physiol. A. Physiol. 117(2): 161-170

Kicliter E., Goytia E.J. (1995) A comparison of spectral response functions of positive and negative phototaxis in two anuran amphibians *Rana piepiens* and *Leptodactylus pentadactylus*. Neurosci. Lett. 185(2): 144-146

Kiesecker J.M. (2002) Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? Proc. Natl. Acad. Sci. USA 99(15): 9900-9904

Kiesecker J.M., Blaustein A.R. (1995) Synergism between UV-B radiation and a pathogen magnifies amphibian ambryo mortality in nature. Proc. Natl. Acad. Sci. USA 92(24): 11049-11052

Kiesecker J.M., Blaustein A.R., Belden L.K. (2001) Complex causes of amphibian population declines. Nature 410(6820): 681-684

Kilpatrick A.M. (2002) Variation in growth of Brown-headed cowbird (*Molothrus ater*) nestlings and energetic impacts on their host parents. Canadian Journal of Zoology 80: 145-153

Kilpatrick A.M. (2003) The impact of thermoregulatory costs on foraging behaviour: a test with American Crows (*Corvus brachyrhynchos*) and eastern grey squirrels (*Sciurus carolinensis*). Evolutionary Ecology Research 5: 781–786

Kilpatrick A.M., Ives A.R. (2003) Species interactions can explain Taylor's power law for ecological time series. Nature 422: 65-68

Kobel HR, Tinsley P. (1996) The biology of *Xenopus*. Symp Zool Soc. London, Vol. 66, Oxford University Press

Kobelt F., Linsenmair K.E. (1995) Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environmental VII. The heat budget of *Hyperolius viridiflavus nitidulus* and the evolution of an optimized body shape. 165(2): 110-124

Korschgen L.J., Moyle D.L. (1955) Food habits of the bullfrog in central Missouri farm ponds. Am. Midl. Nat. 54(2): 332-341
Laposata M.M., Dunson W.A. (1998) Effects of boron and nitrate on hatching success of amphibian eggs. Arch. Environ. Contam. Toxicol. 35(4): 615-619
Laposata M.M., Dunson W.A. (2000) Effects of spray-irrigated wastewater effluent on temporary pond-breeding amphibians. Ecotoxicol. Environ. Saf. 46(2): 192-201
LeBlanc G.A., Bain L.J. (1997) Chronic toxicity of environmental contaminants: sentinels and biomarkers. Environ. Health Perspect. 105 Suppl 1: 65-80
Lefcort H., Hancock K.A., Maur K.M., Rostal D.C. (1997) The effects of used motor oil, silt, and the water mold *Saprolegnia parasitica* on the growth and survival of salamanders (genus *Ambystoma)*. Arch. Environ. Contam. Toxicol. 32(4): 383-388
Lillywhite H.B., Zippel K.C., Farrell A.P. (1999) Resting and maximal heart rates in ectothermic vertebrates. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 124(4): 369-382
Lindemann B., Voûte, C. (1976) Structure and function of the epidermis. In: Frog Neurobiology (R. Llinás, W. Precht, eds.), Springer-Verlag, Berlin, Heidelberg, pp. 169-210
Ludolph D.C., Neff A.W., Paerker M.A., Mescher A.L., Smith R.C., Malacinski G.M. (1995) Cloning and expression of the axolotl proto-oncogene skin. Biochem. Biophys. Acta. 1260(1): 102-104
Machin K.L. (1999) Amphibian pain and analgesia. J. Zoo. Wildl. Med. 30(1): 2-10
Machin K.L. (2001) Fish, amphibian, and reptile analgesia. Veterinary Clin. North Am. Exot. Anim. Pract. 4(1): 19-33
Malvin G.M., Macias S., Sanchez M., Dasalla R., Park A., Duran M. (1995) Lymphatic regulation of hematocrit during hypoxia in the toad *Bufo woodhousei*. Am. J. Physiol. 269: R814-821
Marco A., Cash D., Belden L.K., Blaustein A.R. (2001) Sensitivity to urea fertilization in three amphibian species. Arch. Environ. Contam. Toxicol. 40(3): 406-409
Marcus L.C. (1977) Parasitic diseases of captive reptiles. In: Current Veterinary Therapy, Vol.6 (R.W. Kirk, ed.). W.B. Saunders Co., Philadelphia, PA., pp. 801-806
Marcus L.C. (1981) Veterinary Biology and Medicine of Captive Amphibians and Reptiles. Lea and Febiger, Philadelphia, P.A.
Mattison C. (1987) Frogs and Toads of the World. Blandford Press, Poole, New York, Sydney
Matz G. (1983) Amphibien und Reptilien. BLV-Verlagsgesellschaft, München
Matz G., Weber D. (2002) Guide des amphibiens et reptiles d'Europe. Les Guides du Naturaliste. Delachaux & Niestle
Matz G., Vanderhaege M. (2003) Terrarium. Guides du Naturaliste. Delachaux & Niestle
McAllister C.T., Trauth S.E. (1995) New host records for *Myxidium serotinum* (Protozoa: Myxosporea) from North American amphibians. J. Parasitol. 81(3): 485-488
McCall B., Epstein J.H., Neill A.S., Heel K., Field H., Barrett J., Smith G.A., Selvey L.A., Rodwell B., Lunt R. (2000) Potential human exposure to Australian bat *Lyssavirus*, Queensland, 1996-1999. Emerging Infectious Diseases 6: 259-263
McGregor H.C., Uzzell T.M. Jr. (1964) Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. Science 142: 1043
Mermin J., Hoar B., Angula F.J. (1997) Iguanas and *Salmonella marina* infection in children: a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics 99(3): 399-402
Meyer A.H., Schmidt B.R., Grossenbacher K. (1998) Analysis of three amphibian populations with quarter-century long time-series. Proc. R. Soc. Lond. B. Biol. Sci. 265(1395): 523-528
Miaud C., Guyetant R. (1998) Plasticité et sélection sur les traits d'histoire de vie d'un organisme à cycle vital complexe, la Grenouille rousse *Rana temporaria* (Amphibien: Anoure). B. Soc. Zool. Fr. 123(4): 325-344
Miaud C., Guyetant R., Humert A. (1995) Caractéristiques démographiques des populations d'Amphibiens en altitude: exemple des structures d'âges des Tritons alpestres (*Triturus alpestris* Laur.) et des Grenouilles rousses (*Rana temporaria* L.) dans le Parc National de la Vanoise (Alpes du nord). Bull. Soc. Ecophysiol. 1-2: 47-51
Miaud C., Guyetant R., Elmberg J. (1999) Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. J. Zool. (A) (London) 249: 61-73
Mikaelian I., Quellet M., Pauli B., Rodrigue J., Harshbarger J.C., Green D.M. (2000) *Ichthyophonus*-like infection in wild amphibians from Quebec, Canada. Dis. Aquat. Organ. 40(3): 195-201
Miller R.E. (1996) Quarantine protocols and preventive medicine procedures for reptiles, birds and mammals in zoos. Rev. Sci. Tech. 15(1): 183-189
Moore I.T., Jessop T.S. (2003) Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Horm. Behav. 43(1): 39-47
Moravec F., Skorikova B.(1998) Amphibians and larvae of aquatic insects as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea), a swimbladder parasite of eels. Dis. Aquat. Organ. 34(3): 217-222
Moulton C.A., Fleming W.J., Nerney B.R. (1996) The use of PVC pipes to capture hylid frogs. Herpetol. Rev. 27: 186-187
Mrozek M., Fischer R., Trendelenburg M, Zillmann U. (1995) Microchip implant system used for animal identification in laboratory rabbits, guineapigs, woodchucks and amphibians. Lab. Anim. 29(3): 339-344
Mudry K.M., Capranica R.R. (1987a) Correlation between auditory evoked responses in the thalamus and species-specific call characteristics. I *Rana catesbeiana* (Anura: Ranidae). J. Comp. Physiol. [A]. 160(4): 477-489
Mudry K.M., Capranica R.R. (1987b) Correlation between auditory thalamic area evoked responses and species-specific call characteristics. II *Hyla cinerea* (Anura: Hylidae). J. Comp. Physiol. [A]. 161(3): 407-416
Müller H. (1976) The frog as an experimental animal. In: Frog Neurobiology (R. Llinás, W. Precht, eds.), Springer-Verlag, Berlin, Heidelberg, pp. 1023-1039
Mullineaux E., Best D., Cooper J.E., Best R. (2003) Manual of Wildlife Casualties. BSAVA Manuals, British Small Animal Veterinary Association
Mutschmann F (1998) Erkrankungen der Amphibien. Parey-Verlag, Berlin
Murphy J.T., Collins, J.B. (eds.) (1980) Reproductive biology and diseases of captive reptiles. Proc. Symp. Soc. for the Study of Amphibians and Reptiles, Oxford
Murphy C.G., Gerhardt H.C. (2000) Mating preference functions of individual female barking treefrogs, *Hyla gratiosa*, for two properties of male advertisement calls. Evolution Int. J. Org. Evolution. 54(2): 660-669

Nace G.W. (ed.) (1974) Amphibians. Guidelines for the Breeding, Care and Management of Laboratory Animals. National Academy of Sciences, Washington, D.C.

Nagai T., Koyama H., Hoff K.S., Hillyard S.D. (1999) Desert toads discriminate salt taste with chemosensory function of the ventral skin. J. Comp. Neurol. 408(1): 125-136

Nebeker A.V., Schuytema G.S. (2000) Effects of ammonium sulfate on growth of larval Northwestern salamanders, red-legged and pacific treefrog tadpoles, and juvenile fathead minnows. Bull. Environ. Contam. Toxicol. 64(2): 271-278

Newman L.C., Sands S.S., Wallace D.R., Stevens C.W. (2002) Characterization of mu, kappa, and delta opioid binding in amphibian whole brain tissue homogenates. J. Pharmacol. Exp. Ther. 301(1): 364-370

Nichols D.K. (2000) Amphibian respiratory diseases. Veterinary Clin. North. Am. Exot. Anim. Pract. 3(2): 551-554

Normile D. (1999) Are pathogen s felling frogs? Science 284: 728-729

Nye H.L., Cameron J.A., Chernoff E.A., Stocum D.L. (2003) Regeneration of the urodele limb: a review. Dev. Dyn. 226(2): 280-294

O'Rourke D.P. (2002) Reptiles and amphibians as laboratory animals. Lab. Anim. NY 31(6): 43-47

Pahkala M., Laurila A., Merila J. (2003) Effects of ultraviolet-B radiation on behaviour and growth of three species of amphibian larvae. Chemosphere. 51(3): 197-204

Palacios A.G., Srivastava R., Goldsmith T.H. (1998) Spectral and polarization sensitivity of photocurrents of amphibian rods in the visible and ultraviolet. Vis. Neurosci. 15(2): 319-331

Pare J.A. (2003) Fungal diseases of amphibians: an overview. Veterinary. Clin. North. Am. Exot. Anim. Pract. 6(2): 315-326

Parish M.E. (1998) Coliforms, *Escherichia coloi* and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. J. Food. Prot. 61(3): 280-284

Pascolini R., Daszak P., Cunningham A.A., Tei S., Vagnetti D., Bucci S., Fagotti A., Di Rosa I. (2003) Parasitism by *Dermocystidium ranae* in a population of *Rana esculenta* complex in Central Italy and description of *Amphibiocystidium* n. gen. Diseases of Aquatic Organisms 56: 65-74

Pelster B. (1999) Environmental influences on the development of the cardiac system in fish and amphibians. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 124(4): 407-412

Peterson G.S., Johnson L.B., Axler R.P., Diamond S.A. (2002) Assessment of the risk of solar ultraviolet radiation to amphibians. II. In situ characterization of exposure in habitats. Environ. Sci. Technol. 36(13): 2859-2865

Pfleger S., Benyr G., Sommer R., Hassl A. (2003) Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium. Int. J. Hyg. Environ. Health 206(1): 53-59

Plesner K. (2002) Chinese Fire-Bellied Toad, *Bombina orientalis*. Meloissa Kaplan's Herp Care Collection

Porter K.R. (1972) Food Relations of Amphibians and Reptiles. In: Herpetology (K.R. Porter, ed.). W.B. Saunders Co., Philadelphia, P.A., pp. 31-329

Pounds J.A. (2001) Climate and amphibian declines. Nature 410: 639-640

Pozzi A.G., Lantos C.P., Ceballos N.R. (2002) Effect of salt acclimatization on 3 beta-hydroxysteroid dehydrogenase/isomerase activity in the interrenal of *Bufo arenarum*. Gen. Comp. Endocrinol. 126(1): 68-74

Propper C.R., Hillyard S.D., Johnson W.E. (1995) Central angiotensin II induces thirst-related responses in an amphibian. Horm. Behav. 29(1): 74-84

Reavill D.R. (2001) Amphibian skin diseases. Veterinary. Clin. North. Am. Exot. Anim. Pract. 4(2): 413-440

Reichenbach-Klinke H.H., Elkan E. (1965) Diseases of Amphibians. TFH Publications, Neptune, N. J.

Renner R. (2003) More evidence that herbicides feminize amphibians. Environ. Sci. Technol. 37(3): 46A

Rheinlaender J., Gerhardt H.C., Yager D.D., Capranica R.R. (1979) Accuracy of phonotaxis by the green treefrog (*Hyla cinerea*). J. Comp. Physiol. 133: 247-255

Rinaldi A.C. (2002) Antimicrobial peptides from amphibian skin: an expanding scenario. Curr. Opin. Chem. Biol. 6(6): 799-804

Rollins-Smith L.A. (1998) Metamorphosis and the amphibian immune system. Immunol. Rev. 166: 221-230

Rollins-Smith L.A., Doersam J.K., Longcore J.E., Taylor S.K., Shamblin J.C., Carey C., Zasloff M.A. (2002) Antimicrobial peptide defenses against pathogens associated with global amphibian declines. Dev. Comp. Immunol. 26(1): 63-72

Rose S.M., Rose F.C. (1961) Growth controlling exudates of tadpoles Proc. Symp. Soc. Exp. Biol. Med. 15: 207-281

Roth, G. (1987) Visual Behavior in Salamanders. Springer-Verlag, Berlin

Rouille Y., Ouedraogo Y., Chauvet J., Acher R. (1995) Distinct hydro-osmotic receptors for the neurohypophysial peptides vasotocin and hydrins in the frog *Rana esculenta*. Neuropeptides 29(5): 301-307

Rouse J.D., Bishop C.A., Struger J. (1999) Nitrogen pollution: an assessment of its threat to amphibian survival. Environ. Health. Perspect. 107(10): 799-803

Roy D. (2002) Amphibians as environmental sentinels. J. Biosci. 27(3): 187-188

Rugh R. (1962) Experimental Embryology: Techniques and Prodecures (3rd Edn.) Burgess Publ. Co., Minneapolis, M.N.

Salvadori F., Tournefier A. (1996) Activating by mitogens and superantigens of axolotl lymphocytes: functional characterization and ontogeny study. Immunology 88(4): 586-592

Sai K.P., Babu M. (2001) Studies on *Rana tigerina* skin collagen. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 128(1): 81-90

Savage R. (1961) The Ecology and Life History of the Common Frog. Sir Isaac Pitman and sons, LTD., London

Schmidt M., Steinlein C. (2001) Sex chromosomes, sex-linked genes, and sex determination in the vertebrate class amphibia. EXS 91: 143-176

Schmuck R., Linsenmair K.E. (1997) Regulation of body water balance in reedfrogs (superspecies *Hyperolius viridiflavus* and *Hyperolius marmoratus*: Amphibia, Anura, Hyperoliidae) living in unpredictably varying savannah environments. Comp. Biochem. Physiol. A. Physiol. 118(4): 1335-1352

Schuytema G.S., Nebeker A.V. (1998) Comparative toxicity of diuron on survival and growth of Pacific treefrog, bullfrog, red-legged frog, and African clawed frog embryos and tadpoles. Arch. Environ. Contam. Toxicol. 34(4): 370-376

Sever D.M. (2002) Female sperm storage in amphibians. J. Exp. Zool. 292(2): 165-179

Sever D.M., Moriarty E.C., Rania L.C., Hamlett W.C. (2001) Sperm storage in the oviduct of the internal fertilizing frog *Ascaphus truei*. J. Morphol. 248(1): 1-21

Siegmund O.H. (ed) (1979) Diseases of Amphibians. In: Merck Veterinary Manual (5th Edn.). Merck and Co. NJ., pp. 1206-1209

Simmaco M., Mignogna G., Barra D. (1998) Antimicrobial peptides from amphibian skin: what do they tell us? Biopolymers 47(6): 435-450

Slivkoff M.D., Warburton S.J. (2001) Angiotensin II alters blood flow distribution in amphibians. Physiol. Biochem. Zool. 74(4): 576-583

Smith, H.(1989) Discovery of the axolotl and its early history in biological research. In: Developmental Biology of the Axolotl (J.B. Armstrong, G.M. Malacinski, eds.). Oxford University Press, New York, pp. 3-12

Sparling D.W., Fellers G.M., McConnell L.L. (2001) Pesticides and amphibian population declines in California, USA. Environ. Toxicol. Chem. 20(7): 1591-1595

Stebbins R.C. (1966) A Field Guide to Western Reptiles and Amphibians (2nd Edn.). Houghton Mifflin Co., Boston, M.A.

Stebbins R.C., Cohen N.W. (1995) A Natural History of Amphibians. Princeton University Press, Princeton, N.J.

Stevens C.W., Newman L.C. (1999) Spinal administration of selective opioid antagonists in amphibians: evidence of an opioid unireceptor. Life. Sci. 64(10): PL125-130

Stevens C.W., Rothe K.S. (1997) Supraspinal administration of opioids with selectivity for mu-, delta- and kappa-opioid receptors produces analgesia in amphibians. Eur. J. Pharmacol. 331(1): 15-21

Stevens C.W., Sangha S., Ogg B.G. (1995) Analgesia produced by immobilization stress and an enkephalinase inhibitor in amphibians. Pharmacol. Biochem. Behav. 51(4): 675-680

Stewart J.W. (1969) Care and Management of Amphibians, Reptiles and Fish in the Laboratory: In: IAT Manual of Laboratory Animal Practice and Techniques (2nd Edn.) (D.J. Short, D.P. Woodnott, eds.). C. Thomas, Springfield, IL

Stinner J., Zarlinga N., Orcutt S.(1994) Overwintering behavior of adult bullfrogs, *Rana catesbeiana*, in northeastern Ohio. Ohio J. Sci. 94: 8-13

Storey K.B., Mosser D.D., Douglas D.N., Grundy J.E., Storey J.M. (1996) Biochemistry below 0 degrees C: nature's frozen vertebrates. Braz. J. Med. Biol. Res. 29(3): 283-307

Sullivan P.A., von Seckendorff H.K., Hillyard S.D. (2000) Effects of anion substitution on hydration behavior and water uptake of the red-spotted toad, *Bufo punctatus*: is an anion paradox in amphibian skin? Chem. Senses 25(2): 167-172

Summers K., Clough M.E. (2001) The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proc. Natl. Acad. Sci. USA 98(11): 6227-6232

Tattersall G.J., Boutilier R.G. (1997) Balancing hypoxia and hypothermia in cold-submerged frogs. J. Exp. Biol. (Pt 6): 1031-1038

Temple R., Fowler M.E. (1978) Amphibians. In: Zoo and Wild Animal Medicine (M.E. Fowler, ed.). W.B. Saunders, Philadelphia, P.A., pp. 81-88

Tinsley R.C. (1995) Parasitic disease in amphibians: control by the regulation of worm burdens. Parasitology 111 Suppl: S153-178

Torok M.A., Gardiner D.M., Shubin N.H., Bryant S.V. (1998) Expression of HoxD genes in developing and regenerating axolotl limbs. Dev. Biol. 200(2): 225-233

Tsonis P.A., Washabaugh C.H., Del-Rio-Tsonis K. (1995) Transdifferentiation as a basis for amphibian limb regeneration. Semin. Cell. Biol. 6(3): 127-135

Uzzell T.M. Jr. (1964) Relations of the diploid and triploid species of the *Ambystom*a *jeffersonianum* complex (Amphibia Canudada). Copeia 257

Uzzell T.M. Jr., Goldblatt S.M. (1967) Serum proteins of salamanders of the *Ambystoma jeffersonianum* complex, and the origin of the triploid species of this group. Evolut. 21: 345

Viborg A.L., Rosenkilde P. (2001) Angiotensin II elicits water seeking behavior and the water absorption response in the toad *Bufo bufo*. Horm. Behav. 39(3): 225-231

Vogiatzisch A.K., Loumbourdis N.S. (1999) Exposure of *Rana ridibunda* to lead I. Study of lead accumulation in various tissues and hepatic delta-aminolevulinic acid dehydratase activity. J. Appl. Toxicol. 19(1): 25-29

Wachowitz S., Ewert J.-P. (1996) A key by which the toad's visual system gets access to the domain of prey. Physiol. Behav. 60(3): 877-887

Wake D.B., Hanken J. (1996) Direct development in the lungless salamanders: what are the consequences for the developmental biology, evolution and phylogenesis? Int. J. Dev. Biol. 40(4): 859-869

Wake M.H., Dickie R. (1998) Oviduct structure and function and reproductive modes in amphibians. J. Exp. Zool. 282(4-5): 477-506

Walker C.H. (1998) Biomarker strategies to evaluate the environmental effects of chemicals. Environ. Health. Perspect. 106 Suppl 2: 613-620

Walkowiak W., Berlinger M., Schul J., Gerhardt H.C. (1999) Significance of forebrain structures in acoustically guided behavior in anurans. Eur. J. Morphol. 37(2-3): 177-181

Wallace H., Badawy G.M., Wallace B.M. (1999) Amphibian sex determination and sex reversal. Cell. Mol. Life. Sci. 55(6-7): 901-909

Warburg M.R. (1995) Hormonal effect on the osmotic, electrolyte and nitrogen balance in terrestrial amphibia. Zoolog. Sci. 12(1): 1-11

Warburg M.R., Rosenberg M., Roberts J.R., Heatwole H. (2000) Cutaneous glands in the Australian hylid *Litoria caerulea* (Amphibia, Hylidae) Anat. Embryol. Berl. 201(5): 341-348

Wilczynski W., Capranica R. R. (1984) The auditory system of anuran amphibians. Prog. Neurobiol. 22(1): 1-38

Williams E.S., Yuill T., Artois M., Fischer J., Haigh S.A. (2002) Emerging infectious diseases in wildlife. Rev. Sci. Tech. 21(1): 139-157

Willenring S., Stevens C.W. (1996) Thermal, mechanical and chemical peripheral sensation in amphibians: opioid and adrenergic effects. Life. Sci. 58(2): 125-133

Willenbring S., Stevens C.W. (1997) Spinal mu, delta and kappa opioids alter chemical, mechanical and thermal sensitivities in amphibians. Life Sci. 61(22): 2167-2176

Wilson R.S., Franklin C.E. (2000) Thermal acclimation of locomotor performance in tadpoles of the frog *Limnodynastes peronii*. J. Comp. Physiol. B. 169(6) 445-451

Wilson R.S., James R.S., Johnston I.A. (2000) Thermal acclimation of locomotor performance in tadpoles and adults of the aquatic frog *Xenopus laevis*. J. Comp. Physiol. B. 170(2): 117-124

Wilt F.H., Wessels N.K. (eds) (1967) Methods in Developmental Biology. Thomas Y. Crowell, New York, N.Y.

Withgott J. (1999) Ubiquitous herbicide emasculates frogs. Science 296: 447-448

Wright K.M. (2000) Surgery of amphibians. Veterinary Clin. North. Am. Exot. Anim. Pract. 3(3): 753-758

Zhang F., Ferretti P., Clarke J.D. (2003) Recruitment of postmitotic neurons into the regenerating spinal cord of urodeles. Dev. Dyn. 226(2): 341-348

Zimmerman E. (1986) Breeding Terrarium Animals. T.F.H. Publications, Neptune, New Jersey

Zug G.R. (1993) Herpetology. Academic Press, San Diego

Zug G.R., Zug P.B. (1979) The Marine Toad, *Bufo marinus*: A Natural History Resume of Native Populations. Smithsonian Contributions to Zoology. Smithsonian Institution Press, Washinton D.C.

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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)

6th Meeting
Strasbourg, 25-27 March 2003

Species-specific provisions for birds

Background information for the proposals presented by the Group of Experts on birds

PART B

revised by the Group of Experts

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Future principles for housing and care of laboratory birds

Report for the revision of the Council of Europe Convention ETS123 Appendix A for birds

Issued by the Council's Working Group for Birds

Penny Hawkins (Co-ordinator), Franz Bairlein, Ian Duncan, Christian Fluegge, Roger Francis, Jorge Geller, Linda Keeling and Chris Sherwin

Part B

Background for the proposals of the Working Group

Preamble

Avian intelligence, behavioural complexity and capacity to suffer physical pain have often been regarded as inferior to those of mammals. There is a growing body of evidence that such assumptions are not justified (Elzanowski 1991, Gentle 1991, 1992, Ristau 1991, Marler 1996, Skutch 1996), but there is comparatively little information available on husbandry refinements including environmental stimulation for laboratory birds (Poole & Stamp Dawkins 1999). The literature largely relates to companion or zoo birds (Coulton *et al*. 1997, VanHoek & King 1997, but see King 1993) or to birds reared for meat or egg production (Bell & Adams 1998, Jones & Carmichael 1998).

The recommendations made by the Expert Working Group are thus based on published literature wherever possible, but where housing and husbandry refinements have not been scientifically evaluated, the Group has based its recommendations on members' own experience as experts in the field, current good practice and the recent expert Working Group report on *Laboratory birds: Refinements in husbandry and procedures* (Hawkins *et al.* 2001).

Species–specific sections – birds

1 Introduction

Birds are used for a broad range of purposes including fundamental research, applied veterinary medical studies and toxicology. Domestic fowl and turkeys are the most common laboratory birds and are often used in developmental studies and for the production of biological materials such as tissue and antibodies. Domestic poultry are also the most commonly used species in bird welfare research. Fowl are used for pharmaceutical safety and efficacy evaluation, whereas quail and other birds are more frequently the subjects of ecotoxicology studies. The other, less commonly used species such as the pigeon and wild birds are generally used in psychology and fundamental physiology or zoology research. Catching wild birds to use as experimental animals should be avoided unless it is necessary for the purposes of the experiment.

All birds are essentially built for flight and so share the same basic body plan despite their extremely diverse range of adaptations for locomotion and feeding. Most species are adapted to range over relatively large, three-dimensional areas by one or more means of locomotion including flying, walking, running, swimming or diving, both while foraging and during migration. Many species of bird are highly social and should be kept in stable groups wherever possible.

Additional details are provided for the commonly bred and used laboratory species. It is essential that the housing and care of less commonly used species not included below pay due regard to their behavioural, physiological and social requirements. Housing, husbandry and care protocols for such species should be researched before birds are obtained or used. Advice on requirements for other species (or if behavioural or breeding problems occur) should be sought from experts and care staff to ensure that any particular species needs are adequately addressed. Information and guidance on less commonly used species is available in the background information document.

Some research using domestic poultry needs to approximate 'farm' conditions for the purposes of the study, e.g*. poultry welfare projects or research into farm bird husbandry or pathologies. It may therefore be necessary to obtain strains with inherent welfare problems, or to house birds using the same space allowance that poultry in commercial units would be given. In such circumstances the project should be justified and directly applicable to commercial poultry production. The housing and husbandry standards should at least be equal to those set out in the standards laid out in the European Convention on the Protection of Animals kept for Farming Purposes and related Recommendations for farm animals..*

As stated in the Introduction, bird behaviour, ecology and physiology are diverse and both behavioural abnormalities and substantial suffering can be caused if housing and care are inappropriate. This is unacceptable for ethical and scientific reasons. All those concerned with the husbandry, care and use of laboratory birds should fully research their behavioural and physiological requirements and use the information gained to design experimental and husbandry protocols that will minimise stress. Best practice for a particular species or strain should be applied to all individuals, regardless of the conditions in which that species or strain may be housed for farming, unless the information obtained can be used to justify improving the lives of farmed birds (see Hawkins *et al.* 2001).

1.1 Potential welfare problems

Many of the potential welfare problems specific to birds are associated with inappropriate pecking behaviour. This can be divided into (i) aggressive pecking; (ii) feather pecking (where individuals either peck at other birds' feathers or pluck and pull at their own); and (iii) pecking at the skin of other birds, which can cause serious suffering and mortality if unchecked. There are a number of measures that should be employed to avoid outbreaks *of injurious pecking wherever possible and to reduce or prevent this behaviour should it occur. Prevention is especially important because fowl are attracted to damaged feathers, such that the presence of a few feather-pecked birds may lead to the rapid spread of injurious pecking.*

The cause of inappropriate pecking is not always clear, but it is often possible to avoid outbreaks by rearing chicks with access to substrate that enables them to forage and peck appropriately. Chicks of all species should therefore be housed on solid floors with litter.

There are a number of measures that can be employed to reduce the incidence of injurious pecking should it occur. These include (i) providing alternative pecking substrates such as foraging substrate, bunches of string, pecking blocks or straw, (ii) providing visual barriers, (iii) periodically or temporarily lowering the light intensity or using red light, and (iv) using light sources that emit UV. Anti-pecking sprays are commercially available and can be used to reduce the incidence of injurious pecking in the short term, but it will still be necessary to address the underlying causes of the behaviour. Some strains of domestic bird have been selectively bred so that inappropriate pecking is reduced and such strains should be researched and used wherever possible.

Methods which cause pain or distress, such as very low lighting (i.e. below 20 lux) for prolonged periods or physical modifications such as beak trimming should not be used.

Feather pecking in domestic fowl is thought to be mis-directed foraging behaviour (ground pecking) rather than aggression (Blokhuis 1986, Blokhuis *et al.* 2001, Anonymous 2001). Likely contributory factors are believed to be large group sizes, grid flooring and bright lighting (Duncan 1999, Bilcik & Keeling 2000). A recent epidemiological study in fowl (Green *et al.* 2000) has indicated an association between injurious pecking and factors that reduce opportunities to forage, such as high stocking density, compacted litter and agoraphobia (*e.g.* resulting in low use of the outdoor range in free-range systems). The birds' genetic background can also be a significant factor. Controlled studies where birds' feathers were artificially damaged indicate that fowl are highly attracted to damaged feathers and this can mediate the spread of feather pecking and cannibalism (McAdie & Keeling 2000).

It would therefore appear to be important to increase foraging opportunities and reduce competition and frustration when housing domestic birds. Early exposure to sufficient, suitable pecking substrates such as wood shavings or straw has been demonstrated to reduce the incidence of feather pecking in adulthood (Huber-Eicher & Sebö 2001, Nicol *et al.* 2001), and so it is essential to rear chicks on solid floors with litter and continue to provide it throughout birds' lives. Current research suggests that bunches of white string (*e.g.* polypropylene baling twine) are preferred to many other pecking items and that the interest is sustained in the long term (Jones *et al.* 1997, 2000, Jones & Rayner 2000, Jones 2001). Furthermore, the bunches of string have been shown to be more attractive to birds than damaged feathers (McAdie & Keeling 2000).

Although feather pecking is less likely in small groups with access to litter and other pecking substrates, it may still develop for seemingly reasons that are relatively poorly understood at the time of writing. There are a number of measures that have been reported as alleviating the problem in various circumstances and these should be researched and trialled as appropriate, with the emphasis on using a combination of techniques to provide an appropriate environment for the birds. Temporarily lowering the light intensity or changing the light colour to red, providing supplementary ultraviolet (UV) light, and providing alternative pecking substrates may reduce the incidence of feather pecking once it has developed (Sherwin *et al.* 1999a). Blood from peck wounds cannot be seen in red light, but housing birds under red light should be regarded as an emergency measure to prevent more serious injury. Using UV light, preferably from hatch, is a more constructive way or preventing or alleviating feather pecking. Many components of the avian diet, such as berries and seeds, are highly reflective of UV light and so it has been speculated that light sources that do not include UV might make the environment appear more barren than it really is to birds. This could result in foraging pecks becoming redirected to the feathers of other birds (Sherwin *et al.* 1999a, Lewis *et al.* 2000).

Visual barriers, *e.g.* free-standing, plywood boards, have also been trialled in studies aiming to help reduce injurious pecking. It is thought that barriers enable subordinate birds to retreat and also prevent other birds from seeing and copying injurious pecking behaviour, but results have been variable (Sherwin *et al.* 1999a, Wechsler & Schmid 1998, Lewis *et al.* 2000). It is advisable to use barriers in conjunction with other techniques such as providing UV light and pecking substrate. Commercially available anti-pecking sprays may be useful in the short term but, like low intensity or red light, should only be regarded as a temporary measure to prevent acute suffering. If animals are performing injurious behaviours, simply preventing the behaviours will not address their underlying causes and the welfare problems will remain. This will cause the animals avoidable suffering and is not an appropriate course of action.

If, for experimental reasons, birds are to be kept in an environment where severe feather pecking is likely to occur, it is advisable to use strains that have been selected to show little feather pecking (Craig & Muir 1993, Kjaer & Sorensen 1997, Duncan 1999; see also de Jong *et al.* 2001, Preisinger 2001). Some strains have also been demonstrated to show less cannibalism. It is therefore important to research each strain thoroughly when planning projects.

Commercially, some birds (*e.g.* turkeys) are usually housed under very low light intensities to reduce feather pecking, sometimes in conjunction with long (23 hours) or continuous photoperiods. However, such lighting conditions might cause great concern for welfare as they can result in retinal detachment, buphthalmia (distortions of the eye morphology) and subsequent blindness (Ashton *et al.* 1973, Siopes *et al.* 1984, Davis *et al.* 1986, Manser 1996). Behavioural studies have shown that turkeys prefer light intensities (*e.g.* 20 lux, RSPCA 1997) higher than those generally provided under commercial conditions (Sherwin 1998). In addition, very low intensities make it difficult for humans to detect colours, thus making it almost impossible to adequately inspect the birds. No birds should be housed in very low light intensities for prolonged periods.

Beak trimming or tipping are commonly used, or ‘spectacles’ fitted, if feather pecking or cannibalism become a problem in commercial situations. These procedures can cause both acute and chronic pain regardless of the age at which they are carried out (Duncan *et al.* 1989, Gentle *et al.* 1990) and should never be undertaken without compelling justification; appropriate anaesthesia and analgesia must also be administered (Hawkins *et al.* 2001). If pecking problems persist, beak trimming is preferable to debeaking (Hawkins *et al.* 2001).

Metal anti-pecking rings (‘bits’) pass through the nasal septum and between the mandibles so that birds cannot fully close their beaks. This inhibits normal behaviour more than debeaking, and neither method is desirable (Hawkins *et al.* 2001). In the case of quail, housing males and females together in appropriately composed groups before sexual maturity should render debeaking unnecessary (Gerken & Mills 1993), and providing sufficient space and environmental stimulation for the birds is also likely to reduce aggression. The beaks of ducks are richly innervated and very well supplied with sensory receptors such that beak trimming can cause acute and chronic pain (Gentle 1992, Hawkins *et al.* 2001). Inappropriate pecking in ducks should therefore be countered by reviewing husbandry and care.

Birds housed in a poor quality environment that does not permit them to forage, exercise or interact with conspecifics will experience chronic distress that may be indicated by stereotypic behaviour, for example autophagia (self-pecking), feather pecking, and pacing. Such behaviours should be regarded as indicative of serious welfare problems and should *lead to an immediate review of housing, husbandry and care.*

Chronic distress in birds is often indicated by stereotypic behaviour. A stereotypy has been defined as a repeated pattern of movements which shows little or no variation and has no obvious function (Mason 1991, Manser 1992), such as circling, pacing or pecking at one spot. Stereotypies are generally associated with poor welfare (inappropriate husbandry or environment) and are regarded as indicators of inability to cope with physiological or psychological stressors. Stereotypies may have different causes (Keiper 1969) but most can usually be greatly reduced or eliminated by improving animals' environments, for example by providing better quality and quantity space and companions where appropriate. Any abnormal behaviours should be taken seriously as indicative of a welfare problem and regarded as unacceptable (Hawkins *et al.* 2001).

2 The environment in the animal enclosures and its control.

2.1 Ventilation

Many species are especially susceptible to draughts. Measures should therefore be in place to ensure that individuals do not become chilled. Accumulation of dust and gases such as carbon dioxide and ammonia should be kept to a minimum.

See Kirkwood (1999a).

2.2 Temperature

Where appropriate, animals should be provided with a range of temperatures so that they can exercise a degree of choice over their thermal environment. All healthy adult quail, pigeon and domestic ducks, geese, fowl and turkeys should be housed at temperatures between 15 and 21 ^{o}C. It is essential to take account of the interaction between temperature and relative humidity, as some species will suffer from heat stress within the prescribed temperature range if relative humidity is too high. For species where there are no published guidelines on temperature and humidity, the climate experienced in the wild throughout the year should be researched and replicated as closely as possible. Higher room temperatures than those indicated or a localised source of supplementary heat such as a brooder lamp may be required for sick or juvenile birds (see Table below).

Recommended temperatures and relative humidities for juvenile domestic fowl and turkeys, G. gallus domesticus and Meleagris gallopavo

Age (days)	*Under lamp (^{o}C)*	*Ambient temperature in room (^{o}C)*	*Relative humidity (%)*
Up to 1	*35*	*25 to 30*	*70 ± 10*
1 to 7	*32*	*22 to 27*	*70 ± 10*
7 to 14	*29*	*19 to 24*	*40 to 80*
14 to 21	*26*	*18 to 21*	*40 to 80*
Over 21	*-*	*15 to 21*	*40 to 80*

The chicks' behaviour should be used as a guide when setting brooder lamp temperature. Chicks of all species should be evenly spread and making a moderate amount of noise; quiet chicks may be too hot and chicks making noisy distress calls may be too cold. Where brooder lamps are used, chicks will huddle directly under the lamp if they are cold, in which case the lamp should be lowered, or will form a circle around the periphery of the heated area if they are too hot, in which case the lamp should be raised.

If birds are subjected to the physiological stress of attempting to adapt to inappropriate climates, both welfare and experimental results are likely to be affected (Hawkins *et al.* 2001). The Group has therefore stressed the importance of researching the climate to which each species is adapted and providing a choice of temperatures wherever possible. The temperature requirements of hatchlings can be very different to those of adult birds and we strongly recommend that the new Appendix A draws attention to this.

Proposals for different temperature ranges for different species were initially based on Duncan (1999), Mills *et al.* (1999), Hutchison (1999) and Hawkins *et al.* (2001) and were consolidated in response to comments from the Netherlands (Document GT123 (2001) 32).

2.3 Humidity

Relative humidity should be maintained within the range of 50 to 70 % for healthy, adult, domestic birds.

2.4 Lighting

Light quality and quantity are critically important for some species at certain times of the year for normal physiological functioning. Appropriate light:dark regimes for each species, life stage and time of year should be researched before animals are acquired.

Lights should not be abruptly switched off, but should be dimmed and raised in a gradual fashion, and dim 'night lights' should be provided. This is especially important when housing birds capable of flight.

Normal fluorescent tubes, which flash at 100 Hz, may well be perceived as flickering to some birds. Although it is not known whether flickering is always aversive, high-frequency fluorescent tubes, or incandescent lighting, should be used wherever possible.

Light quality, levels and duration are all extremely important to birds. The eyes and optic region of the avian brain are highly developed, which reflects their adaptation for vision during flight. The avian retina is considerably more complex than that of mammals (Bowmaker *et al.* 1997) and so photoreception and vision in birds are very different from humans. Birds have excellent colour vision; the visual acuity of some species (*e.g.* raptors) exceeds that of old world primates, and some species also have specialised areas of the retina for different visual tasks.

It is essential to research appropriate light:dark regimes because photoperiod directly influences bird development and physiology. A photoreceptor within the thalamus co-ordinates photoperiodic responses to changing day lengths in birds, and is activated when light passes through the thin avian skull (Follett 1984). Consequently, light quality and quantity may be critically important for some species at certain times of the year for normal physiological functioning (*e.g.* CCAC 1984, Hutchison 1999, Mills *et al.* 1999). Life stages must also be taken into consideration because the requirements of juveniles may differ from those of more mature animals (Mills *et al.* 1999).

The welfare implications of other aspects of light quality are less well researched, but a logical case can be made from what is known of avian vision. The 'critical flicker fusion frequency', or frequency at which a strobe light is no longer perceived as flashing, is notably higher for birds than humans (reviewed by D'Eath 1998). It would seem likely that normal fluorescent tubes, which flash at 100 Hz, would be perceived as flickering to a bird such as a starling *Sturnus vulgaris*. High-frequency fluorescent tubes, or incandescent lighting, would therefore seem preferable on these grounds._Under some circumstances, however, birds do not find this flicker aversive, and may even prefer fluorescent light; possibly because of its spectral properties (Sherwin 1999, Widowski *et al* 1992).

- Further research is needed to study the impact of light quality (*i.e.* flicker frequency) on the behaviour and welfare of a range of bird species.

Most diurnal birds can also see ultraviolet (UV) light and many species have UV reflecting plumage (Bennett & Cuthill 1994); it has been hypothesised that such markings may be related to feather pecking among groups of turkeys (Sherwin & Devereux 1999). It has been suggested that UV colouration may turn out to be an important component of bird communication (Manning & Stamp Dawkins 1998).

Most commercially available artificial light sources have considerably less ultraviolet (UV) light than full daylight, so that their colour balance would be likely to appear unnatural to birds. There is experimental evidence that some species make different mate choice decisions when the UV waveband is not present, most probably because the plumage (which reflects UV as well as human-visible wavelengths) appears an odd colour to the bird (Bennett *et al.* 1997). There is evidence that birds prefer environments that contain a UV component in the lighting (Moinard & Sherwin 1999).

Thus, although birds can be, and have been, kept successfully under artificial lighting, it is possible that any visual tasks based on colour (social signals, displays, foraging) are rendered more difficult. Direct effects of light on stress and welfare in birds are, as yet, little researched. However, if outdoor housing, windows or skylights are not possible, use of special daylight-mimicking fluorescent lighting, running at high frequencies, would seem advisable (Hawkins *et al.* 2001).

- The effects of light sources with and without a UV component on bird behaviour, (including aggression and feather pecking) and welfare need further evaluation.

2.5 Noise

Some birds, e.g. *the pigeon, are thought to be able to hear very low frequency sounds. Although infrasound (sound below 16 - 20 Hz) is unlikely to cause distress, birds should be housed away from any equipment that emits low frequency vibrations whenever possible.*

Most birds can hear sounds between 1 and 5 kHz, with a high frequency hearing limit of about 10 kHz for passerines and 7.5 kHz for non-passerines (Dooling 1992, see also Heffner 1998). Birds do not utilise high frequencies for sound localisation (apart from owls (Strigiformes), who can hear up to 12 kHz) and none studied to date can equal even human high frequency hearing (Heffner 1998), so ultrasound is unlikely to cause welfare problems. Sensitivity decreases gradually below 1 kHz but some birds, *e.g.* the pigeon, are thought to be able to hear very low frequency sounds (Kreithen & Quine 1979). Although infrasound (sound below 16 - 20 Hz) is unlikely to cause distress, birds should be given the benefit of the doubt and housed away from any equipment that emits low frequency vibrations whenever possible (G Sales, pers. comm.).

- Research is needed into the impact of infrasound on birds.

3 Health

Captive bred birds should be used wherever possible. Wild birds may present special problems in terms of their behaviour and health when in a laboratory situation. A longer period of quarantine and habituation to captive conditions is generally required before they are used in scientific procedures.
Careful health monitoring and parasite control should minimise health risks in birds with outdoor access.

Captive bred birds of a suitable health status should be used wherever possible. Wild birds may present special problems in terms of their behaviour and health when in a laboratory situation. A period of 28 days quarantine should normally be allowed for wild caught birds where possible. During this time the birds can become adapted to the laboratory conditions and their health monitored prior to experimental work commencing. Monitoring should be agreed with a veterinary surgeon and may consist of faecal sampling and examination for the presence of parasites and bacteria, including potential zoonoses such as those caused by Salmonellae and *Campylobacter*. During this period birds may be treated for the presence of endo- and ectoparasites on advice from the attending veterinarian (Hawkins *et al.* 2001).

4 Housing and enrichment

Introduction

Birds should be housed in enclosures which facilitate and encourage a range of desirable natural behaviours, including social behaviour, exercise and foraging. Many birds will benefit from housing that allows them to go outdoors and the feasibility of this should be evaluated with respect to the potential to cause distress or to conflict with experimental aims. Some form of cover such as shrubs should always be provided outdoors to encourage birds to use all the available area.

A good standard of well-being and welfare cannot be achieved without appropriate housing, husbandry and care. In common with most other laboratory animals, birds spend the majority of their time in their holding cages or pens, not undergoing procedures. Good housing should make them feel safe, secure and able to exercise, to control their environment to a degree and to express a range of natural behaviours including interactions with conspecifics (Nicol 1995, see also FAWC 1993). Poor quality and quantity of space is likely to lead to boredom and frustration which may be expressed as stereotypic behaviour, which should be regarded as unacceptable and to be avoided (Hawkins *et al.* 2001).

In general, birds should be housed in pens or aviaries as opposed to cages (Coles 1991, also see Kirkwood 1999a). Domestic fowl, for example, can usually be provided with a better and more appropriate environment if they are group housed in large pens (Duncan 1999) and may also be less fearful (Hansen *et al.* 1993). While some birds, *e.g.* small passerines, can be provided with an acceptable quality of life by group housing them in large, enriched cages, larger species will require more space and should be housed in aviaries or pens (Hawkins *et al.* 2001). Many birds will benefit from access to outdoor runs and the feasibility of this should be evaluated case by case, with respect to the potential to cause physiological or psychological stress or to conflict with experimental aims. Cover, such as shrubs, is essential for feelings of safety (*e.g.* Cornetto & Estevez 1999, Newberry & Shackleton 1997) and to reduce aggression (Cornetto *et al* 2002). Birds able to go outdoors will be at some risk of contracting disease from wild populations, although this does not necessarily outweigh the advantages associated with access to the outside, such as experiencing a more stimulating environment and reduced fearfulness (Grigor *et al.* 1995). Careful health monitoring and regular worming should minimise health risks (Hawkins *et al.* 2001). For an example of an housing system for laboratory fowl incorporating an outdoor run, see Fölsch *et al.* (2002).

Standards of husbandry and care in the laboratory should exceed commercial conditions (Duncan 1999, Hawkins *et al.* 2001), unless the project in question has a direct application that aims to alleviate a welfare problem occurring in practice.

4.1 Social housing

Most species of bird are social for at least part of the year and highly sensitive to family relationships so the formation of appropriate, stable, harmonious groups should be given a high priority. Research into the optimal composition of groups and at what stage in the birds' lives these should be created is essential before groups are formed and studies are planned.

The social behaviour of birds and the importance of kin relationships has been reviewed in Marler (1996). The optimum timing and membership of groups should therefore be researched for each species and strain (Hawkins *et al.* 2001). Studies in a number of different fields indicates that interaction with conspecifics is important and meaningful to birds; some examples are set out below.

Birds have a great capacity for social learning, *i.e.* learning by watching the activities of others. It is generally considered that this is an ‘advanced’ form of learning and indicates higher cognitive capacity. Most social learning has been related to foraging or feeding activities learnt by watching parents (Hatch & Lefebvre 1997, Stokes 1971, Sherry 1977), siblings (Tolman 1964, Tolman & Wilson 1965, Johnston *et al.*1998, Nicol & Pope 1999) or models (Turner 1964, Tolman 1967, Fritz & Kotrschal 1999). This form of learning can lead to the rapid spread of novel behaviours such as the opening of milk bottle tops by blue tits *Parus caeruleus* (Fisher & Hinde 1949, Sherry & Galef 1990). Vocal learning has evolved (probably independently) in at least three avian orders; the Passeriformes, Psittacines and Apodiformes (*e.g.* swifts *Apus* spp. and hummingbirds) (Dooling 1992).

Teaching by an animal could indicate that it is aware of the consequences of the ‘student’ animal’s behaviour and so may be capable of identifying with another animal’s thought processes, *i.e.* possess a form of empathy. Nicol & Pope (1996) showed that when a hen saw her chicks eating food which she believed was distasteful (though in reality it was perfectly appetising) she increased her vocalisations and pecking activity, apparently attempting to direct her chicks to a dish containing more palatable food.

4.2 Environmental Enrichment

A stimulating environment is a very important contributor to good bird welfare. Perches, dust and water baths, suitable nest sites and nesting material, pecking objects and substrate for foraging must always be provided for species and individuals that will benefit from them unless there is compelling scientific or veterinary justification for withholding such items. Birds should be encouraged to use all three dimensions of their housing for foraging, exercise and social interactions including play wherever possible.

It is generally accepted that animals may suffer if prevented from carrying out actions that they are strongly motivated to perform, for example if laying hens are prevented from building nests (Duncan & Wood-Gush 1972, Cooper & Appleby 1994). Some behavioural studies using birds have suggested that they possess ‘object permanence’, *i.e.* they can remember objects that are no longer there, so that ‘out of sight’ is not ‘out of mind’. This object permanence would warrant birds with the ability to suffer due to the absence of a valued resource, such as a nest box. Parrots have highly developed object permanence abilities that are comparable to those in 2-year-old humans, and can locate a goal by predicting its concealed movement and position (Pepperberg & Funk 1990). Pigeons (Neiworth & Rilling 1987), domestic fowl (Freire *et al.* 1997) and chicks (Vallortigara *et al.* 1998) are also able to mentally represent the hidden movement and position of objects and thus accurately locate a goal after it has been moved out of sight. This suggests that out of sight is not necessarily out of mind, and so birds’ cognitive capacities should be considered along with behavioural and motivational studies when trying to predict whether frustration and suffering are likely to occur.

The range of cognitive skills now known to exist in birds indicates that birds have a higher mental capacity than has been previously thought. For example, tool use occurs amongst many bird species including thrushes (*Turdus* spp.), finches (Fringillidae), ravens (*Corvus corax*) and vultures (Cathartiformes) (McFarland 1993). Recently, Keas (*Nestor notabilis*) have been used in studies on imitation by giving them the opportunity to open artificial fruit puzzles (Huber *et al.* 1998) and the ability of birds to count has been investigated using an African grey parrot *Psittacus erithacus* (Pepperberg 1994). Locomotory, social and object play have also been observed in birds, particularly corvids (Skutch 1996).

There is thus considerable potential for birds to experience suffering and distress, and so preventative measures should be taken wherever possible. A stimulating environment is likely to be a very important contributor to good bird welfare and should always be provided. Whether birds are kept in cages, aviaries or pens, providing them with an adequate quantity of space is not enough. Good quality space is vital for good welfare. Space can be made more complex and interesting by providing separate areas for different activities such as dust bathing, bathing in water, perching and play as appropriate (Duncan 1999, Hawkins *et al.* 2001). Domestic fowl provided with such accommodation will occupy different areas and carry out a range of activities at different times of day (Channing *et al.* 2001) and the same is likely to be true of other species. Passerines require perches of varying diameters to exercise the feet (Coles 1991, Association of Avian Veterinarians 1999) and perches are also extremely important for sitting during the day and roosting at night for many non-passerines (CCAC 1984, Jacobs *et al.* 1995, Duncan 1999, Hutchison 1999, Kirkwood 1999a). Studies on domestic fowl have found that birds are less fearful when they are perching off the ground and that this is consistent with the retention of perching behaviour as an antipredation strategy (Keeling 1997, Newberry *et al.* 2001).

Furthermore, studies in domestic fowl with experimentally-induced sodium urate arthritis have found that birds subsequently housed in large pens with litter and companions exhibited less pain-related behaviour and lameness than those housed in standard cages (Gentle & Corr 1995, Gentle & Tilston 1999). This suggests that it is especially important to provide a complex environment for birds who may be experiencing discomfort or pain, as mental stimulation will help to divert attention and aid endogenous analgesia.

For further justification for the inclusion of dust and water baths, nesting material, pecking objects and substrate for foraging, see Table legends for individual species below.

- More research is needed objectively to evaluate appropriate environmental stimulation for a range of species of bird. This should examine the impact of enrichment items on both experimental birds and on the results of studies. Particular attention should be given to devising appropriate environmental stimulation for birds used in infectious disease studies.

4.3 Enclosures - dimensions and flooring

Guidelines for enclosure dimensions are set out in the species-specific provisions for domestic fowl, domestic turkeys, quail, ducks and geese, pigeons and zebra finches. All birds, especially species that spend a significant proportion of their time walking, such as quail or fowl, should be housed on solid floors with substrate rather than on grid floors. Birds can be prone to foot problems, e.g. overgrown claws, faecal accumulation and foot lesions such as foot pad dermatitis due to standing on wet litter, on any type of flooring and so frequent monitoring of foot condition is always necessary. In practice, it may be necessary to consider a compromise between solid and grid flooring for scientific purposes. In such cases, birds should be provided with solid floored resting areas occupying at least a third of the enclosure floor. Grid areas should be located under perches if faecal collection is required. To reduce the incidence of foot injuries, slats made of plastic should be used in preference to wire mesh wherever possible. If wire mesh must be used, it should be of a suitable grid size to adequately support the foot and the wire should have rounded edges and be plastic coated.

Wire flooring does not permit the provision of substrate for dust bathing, scratching and pecking and does not permit the scattering of food or treats on the floor to encourage natural foraging behaviour. It has been demonstrated that the foraging behaviour of domesticated Swedish bantams still corresponds to the optimal foraging strategies displayed by wild-type birds. Although the domesticated birds employed less costly behavioural strategies, which was interpreted by the authors as a possible passive adaptation to a domesticated life, they had retained the ability to respond in an adaptive manner to their environment (Andersson *et al.* 2001). Furthermore, domestic fowl have been shown strongly to prefer solid floors with litter rather than grid floors (FAWC 1997) and caged hens to have a high demand for a litter substrate (Gunnarsson *et al.* 2000). Grid floors are therefore unsuitable for housing birds and their use should be discontinued on animal welfare grounds.

Foot lesions can cause problems if species that spend a large proportion of their time walking are housed on unsuitable flooring or substrate. In general, these species (*e.g.* quail, fowl) should be housed on solid floors with appropriate substrate (see 4.6 for examples) to avoid lesionsHousing on solid floors may, however, lead to accumulation of faeces on the feet so monitoring and husbandry must be adequate to prevent this. It is especially important to maintain litter in a dry condition to avoid foot pad dermatitis

In practice, a compromise between solid and wire flooring may be required for scientific purposes. In this case, birds should be provided with solid floored resting areas occupying at least a third of the pen or cage floor (Hawkins *et al.* 2001). All wire mesh areas should be of a suitable size and construction, with rounded edges and plastic coating, as this has been found in practice to reduce the incidence of lesions. This is especially important where large areas of wire flooring are deemed necessary, for example in toxicology or metabolism studies (Hawkins *et al.* 2001).

- More research is needed to investigate optimum flooring for birds; in particular, ratios of solid: grid floor, appropriate mesh or grid sizes and flooring materials.

4.4 Feeding

Feeding patterns of wild birds vary widely and consideration should be given to the nature of the food, the way in which it is presented and the times at which it is made available. Diets that will meet the nutritional requirements of each species and promote natural foraging behaviour should be researched and formulated before any animals are obtained. Part of the diet or additional treats should be scattered on the enclosure floor to encourage foraging wherever appropriate. Dietary enrichment benefits birds, so additions such as fruit, vegetables, seeds or invertebrates should be considered where appropriate even if it is not possible to feed birds on their 'natural' diet. Where new foods are introduced, the previous diet should always be available so that birds will not go hungry if they are unwilling to eat new foods. Some species are more adaptable than others and advice should be sought on appropriate dietary regimes.

Some species, particularly granivores, require grit to digest their food. Appropriately-sized grit must be made available where required. Birds will select grit of the size they prefer if material of various sizes is provided. The grit should be replaced regularly. Dietary calcium and phosphorus should also be provided for birds in an appropriate form and at an appropriate level for each life stage, to prevent nutritional bone disease. Any such requirements should be thoroughly researched and catered for. Food should be supplied in troughs rather than circular feeders, as circular feeders occupy valuable floor area and can hinder effective cleaning and inspection of birds. Chicks of some species (e.g. domestic turkeys) may need to be taught to feed and drink in order to avoid dehydration and potential starvation. Food for all species should be clearly visible and provided at several points to help prevent feeding problems.

Food selection, feeding times and durations ultimately depend on a bird's species, age, the season and which food is currently available (Paulus 1988). Many species, *e.g.* waterfowl, often spend more time feeding than doing anything else (Goudie & Ankney 1986, Paulus 1988, Sedinger 1992) so it is very important to encourage appropriate foraging behaviour (see also Andersson *et al.* 2001). It is essential to ensure that lighting is adequate so that chicks can see their food; UV light may well help with this (see above). Many chicks will copy feeding behaviour, so tapping at their food with a finger or pencil will often encourage them to peck at the food for themselves. Alternatively, housing chicks of different ages together or so that they can see one another will enable younger chicks to copy older birds.

Birds have relatively few taste buds in comparison with mammals (*e.g.* blue tits have 24, fowl 340, mallard ducks *Anas platyrynchos* 375 and rats 1,265) but nevertheless appear to have an acute sense of taste (Welty & Baptista 1988). Taste is thus relevant to many birds (Lint & Lint 1981) and dietary enrichment should be considered (Association of Avian Veterinarians 1999); fowl can taste well (Gentle 1971) and the taste of food appears to be important to the pigeon (Zeigler 1975). Birds also learn to avoid unpalatable substances and chicks learn to associate the consequences of eating foods with their taste. While some species are specialist feeders that are adapted to eat a narrow range of food items, generalists may benefit from dietary enrichment (Hawkins *et al.* 2001). Diet preferences are shaped by early experience, however, so any new foods should be introduced gradually and as an extra option, especially where birds have previously been fed on bland or uniform diets. Some species or individuals may be unwilling to eat new foods as adults, so the diet to which they are accustomed should always be available as well (Association of Avian Veterinarians 1999).

Many species of granivorous and herbivorous birds ingest small pieces of insoluble grit which they retain in their gizzards and which assist in the process of degradation, grinding and breaking up of seeds and other fibrous plant matter prior to chemical digestion. It is essential that appropriately-sized grit is made available to species that require it (Kirkwood 1999a). Birds will select grit of the size they prefer if material of various sizes is provided (Hawkins *et al.* 2001).

Nutritional bone disease is a potential problem in many species of birds maintained in captivity (Kirkwood 1999b). Because of their very rapid rates of skeletal growth compared to mammals (Kirkwood *et al.* 1989) birds tend to have requirements for higher dietary calcium concentrations during growth and can develop skeletal pathology (including poorly mineralised bones and pathological fractures) very rapidly if calcium intake is inadequate. Although the dietary calcium concentrations of the main components of the diets of many species of birds are relatively low (*e.g.* many invertebrates, grains, fruits, and green plants), nutritional bone disease is rare in free-living birds. It is known that some species include calcium-rich items such as fragments of bone or snail shell in the diet they feed to their chicks (*e.g.* Seastedt & Maclean 1977, Kirkwood 1999a) and this behaviour may be more common among birds than has previously been realised. It is important to estimate dietary calcium concentrations carefully because both deficiencies and excesses can cause severe skeletal pathology. This is especially important in high egg production strains of domestic fowl, which are prone to osteoporosis. Where calcium supplementation in the feed is judged to be necessary, quantities should be calculated and administered with precision (Kirkwood 1996). Additional calcium may be offered in the form of crushed oyster shells in a separate container to be taken *ad libitum*.

4.5 Watering

One nipple or cup drinker should be provided for every 3 or 4 birds with a minimum of two in each enclosure. Care should be taken to ensure that chicks cannot become trapped in drinkers as they will become chilled and may drown. Water may also be given in birds' feed if appropriate.

All birds should have access to water at all times, even species that do not normally drink when in good health (Kirkwood 1999a). The number of drinkers is taken from Duncan (1999). Water in the feed has been shown to be a potent reinforcer in domestic fowl (Sherwin 1993). Water should be clearly visible, especially for young chicks. If juvenile birds are not drinking for themselves, they should be individually 'beak dipped' by placing the beak gently in cold water for 1 to 2 seconds.

4.6 Substrate, litter, bedding and nesting material

Suitable substrates for birds should be absorbent, unlikely to cause foot lesions and of an appropriate particle size to minimise dust and prevent excessive accumulation on the *birds' feet. Suitable substrates include chipped bark, white wood shavings, chopped straw or washed sand, but not sandpaper. Litter should be maintained in a dry, friable condition and be sufficiently deep to dilute and absorb faeces. Other suitable floor coverings include plastic artificial turf or deep pile rubber mats. A suitable pecking substrate such as pieces of straw should be scattered over the floor.*

Hatchlings and juvenile birds should be provided with a substrate that they can grip to avoid developmental problems such as splayed legs. Juvenile birds should also be encouraged if necessary, for instance by tapping with the fingers, to peck at the substrate to help prevent subsequent misdirected pecking.

Many species of birds have a high demand for substrate, especially those that spend much time walking and/or inhabit a forest floor habitat in the wild, e.g. domestic fowl, turkeys and quail (Schmid & Wechsler 1997, Gunnarsson *et al.* 2000, see Hawkins *et al.* 2001). All of the substrates listed above have been successfully provided in practice in the experience of Expert Group members. Sandpaper should not be used because it abrades the feet and may be ingested for the grit when faecally contaminated (Coles 1991). It is important to monitor birds' feet regularly for signs of lesions and faecal accumulation when using any type of substrate. Excessive accumulation of faeces mixed with substrate and lesions such as hock burns or pododermatitis can be caused by poor quality litter and/or inadequate husbandry. If problems of this nature occur, birds should not be denied substrate but the type of litter and animal care should both be reviewed.

4.7 Cleaning

See item 4.9 of the General Section of Appendix A.

4.8 Handling

Suitable equipment for catching and handling should be available, e.g. well maintained nets in appropriate sizes and darkened nets with padded rims for small birds.

If the experimental procedure requires adult birds to be handled regularly, it is recommended from a welfare and experimental perspective to handle chicks frequently during rearing as this reduces later fear of humans.

All birds are liable to find restraint and handling extremely stressful, perhaps because handling by humans may be interpreted as a close encounter with a predator. The bird's point of view must be considered at all times before and during handling. Competent handling is thus vital not only for the safety of the human handler but also because attacking birds may be dropped or mishandled, which could result in bruising or broken bones. Even if there is no physical damage, the psychological distress will lead to greater fear, anxiety and aggression the next time the bird has to be caught (Hawkins *et al.* 2001).

There are a number of essential factors that must be addressed during training. These include applying the correct amount and method of restraint and ensuring that respiration is not prevented by incompetent or inappropriate handling (Fowler 1995). Other important considerations are the potential for hyperthermia during handling and the likelihood that birds will employ antipredator strategies such as panting, gaping, closing the eyes or fluffing up the feathers (Redfern & Clark 2001). It is essential that bird handlers are properly trained to recognise signs of genuine distress that could indicate shock, wing sprain, leg or wing fractures, skin damage or heat stress and know the appropriate actions to take (Fowler 1995, Redfern & Clark 2001).

It may be possible to reduce handling stress by habituating birds to human contact and handling from hatch (if possible), using positive reinforcement and rewards (Jones 1994, see Laule 1999). However, this may not be as effective in particularly 'flighty' strains (Murphy &

Duncan 1978).

- More research is needed into the effect of handling chicks from hatch on subsequent handling stress in adult birds.

4.9 Humane killing

The preferred method of killing for juvenile and adult birds is an overdose of anaesthetic using an appropriate agent and route.

Euthanasia by an appropriate anaesthetic agent is preferable to CO_2 *inhalation for birds and embryonic birds, as* CO_2 *may be aversive. As diving birds and some others, e.g. mallard ducks, can slow their heart rates and hold their breath for long periods, care should be taken when killing such species using chemical agents by inhalation to ensure that they do not recover from anaesthesia. Ducks, diving birds and very young chicks should not be killed using carbon dioxide.*

According to the EC Working Party Report (Close *et al.* 1997), the most acceptable method for killing either embryonic or adult birds is an overdose of sodium pentobarbitone. The most commonly used method for killing bird embryos is cooling or freezing, and this was considered by the EC Working Party to be humane provided that death was confirmed by a suitable method afterwards. Disruption of the membranes and maceration (*NB* in a macerator designed for the purpose) were also considered to be acceptable for embryonic birds.

It is the opinion of the Working Party that chilling is likely to be aversive and that the stage in incubation at which it is no longer acceptable is difficult to define (one would not kill a day-old chick by chilling and a chick one day before hatching is little different). Chilling should therefore not be carried out before killing using mechanical means as this may cause avoidable suffering.

For adult birds, an overdose of an appropriate anaesthetic agent was considered to be most acceptable and humane by the EC Working Party and the BVA(AWF)/FRAME/RSPCA/UFAW Joint Working Group on Refinement (Close *et al.* 1997, Hawkins *et al.* 2001).

Diving birds and some other species (*e.g.* the mallard duck *Anas platyrhynchos*) possess physiological mechanisms that enable them to withstand long periods of hypoxia and hypercapnia (reviews in Jones 1976, Butler 1982, see also Butler & Taylor 1983). Ducks and diving birds should therefore not be killed using carbon dioxide (Hawkins *et al.* 2001). Very young chicks are resilient to CO_2 because it accumulates in the air space before hatching (Jaksch 1981) and can take a long time to die (M Raj, unpubl. obs. on domestic fowl and turkey chicks). There is an increasing body of evidence to suggest that a range of species find CO_2 aversive (*e.g.* Leach *et al.* 2002), and there have been a number of behavioural studies of turkeys and domestic fowl exposed to CO_2 and to gas mixtures including CO_2. These describe behavioural responses to such gases including gasping and head shaking, which are interpreted as indicating or causing distress (Raj 1996, Lambooij *et al.* 1999, Webster & Fletcher 2001). On this basis, birds should be given the benefit of the doubt and carbon dioxide euthanasia avoided where possible.

- Further studies are needed to evaluate at which levels and in which proportions in gas mixes CO_2 is aversive to birds.

4.10 Records

See item 4.12 of the General Section of Appendix A.

4.11 Identification

Non-invasive or minimally invasive methods such as noting physical differences, ringing with either closed or split rings and staining or dyeing the feathers are preferable to more invasive techniques such as electronic tagging or wing tagging. Combinations of coloured leg rings minimise handling for identification, although due regard should be paid to any potential impact of colours on behaviour If the use of transponders is required to log how frequently birds are present at different locations, it may be possible to fix them to rings rather than to implant them. When using rings as temporary marking for rapidly growing chicks, regular checking is essential to ensure that the ring is not impeding the growth of the leg.

Highly invasive marking methods such as toe clipping or web punching cause suffering and should not be used.

Birds are commonly identified by several methods, including (from least to most invasive):

- Noting physical differences, *e.g.* plumage colours and patterns, morphological differences.
- Ringing with either closed or split rings. Care must be taken to ensure that leg ring colours and symmetry will not affect behaviour (*e.g.* Swaddle & Cuthill 1994) and that the correct size of ring is fitted for each species and age group. Particular care is needed for young animals that can quickly out-grow rings, *e.g.* domestic turkey chicks.
- Staining or dyeing the feathers. The potential impact of the chosen colour on behaviour must be researched and groups closely monitored following marking. All staining agents must be non-toxic and non-irritant.
- Electronic tagging. The left pectoral muscle is commonly used as an insertion site in birds, although this may not be appropriate for small birds likely to spend a significant amount of time flying. If it is considered that intramuscular implantation could cause pain or impair movement, transponders should be implanted subcutaneously at the base of the neck.

- Wing tagging. Even small wing tags are likely to interfere with normal behaviour to a greater extent than the alternative methods outlined above (provided that they are competently employed), so markers should not be fixed to the wings unnecessarily. Wing badges must be competently fitted, must not impede flight and must always be as small as possible.

The list of identification methods and guidance for using them is taken from Hawkins *et al.* (2001); see also Redfern & Clark (2001). The practical or legal need to mark animals at all should always be questioned. If it is necessary to identify individual birds, the technique that is least invasive, causes least suffering and is compatible with experimental aims should always be used.

II. Species-specific enclosure dimensions and husbandry guidelines

The pen and cage dimensions and husbandry guidelines in this section were based on those in Hawkins *et al.* (2001). These, in turn, were set out according to current good practice and the experience of the JWGR Working Party members.

Guidelines for caging birds in stock and during procedures in user establishments

The Group proposes that there should be separate Tables for domestic fowl, domestic turkeys, quail, ducks and geese, pigeons and zebra finches, with legends as follows:

A standard pen size of $2m^2$ (with the exception of pens for turkeys over 20 kg) was agreed by the Working Group because:

- $2m^2$ is an easily manageable pen size that is already in use at some establishments;
- it provides the flexibility to house a range of different species by altering the number of animals in each pen;
- it provides sufficient space for environmental enrichment to encourage a range of behaviours and also allows for behaviours such as pre-laying pacing, social attraction and repulsion and so on;
- $2m^2$ permits easy access for cleaning, catching and monitoring animals.

Guidelines for housing the domestic fowl, *Gallus gallus domesticus*, in stock and during procedures

Domestic fowl retain much of the biology and behaviour of the Jungle fowl from which they were domesticated. Behaviours that are most important to the species are nesting (in females), perching and using litter for foraging, scratching, pecking and dustbathing. Fowl are social and should be housed in groups of around 5 to 20 birds, with fewer males than females in mixed groups, e.g. a ratio of 1:5. Attempts have been made to select strains of fowl for reduced feather pecking or agonistic behaviour. The existence of appropriate strains of this type, and the feasibility of acquiring them, should be evaluated for each project.

Laying hens should have access to nest boxes from at least 16 weeks of age so that they can investigate them before they come into lay at 18 weeks. Nest boxes should be enclosed and large enough to allow one hen to turn around. A loose substrate such as wood-shavings or straw should be supplied within nestboxes to promote nesting behaviour. Substrate should be regularly replaced and kept clean.

Fowl should always be provided with the opportunity to perch, peck appropriate substrates, forage and dustbathe from one day old. Perches should be 3 to 4 cm in diameter and round with a flattened top. The optimum height above the floor varies for different breeds, ages and housing conditions but perches should initially be fixed at 5 to 10 cm and at 30 cm above the floor. Perch heights can then be adjusted in response to the birds' behaviour by seeing how easily birds can get on and off perches and move between them. Birds should also be briefly observed during dark periods; all individuals should be roosting unless the perches are too high. Each individual should be allowed 15 cm of perch at each level. Suitable materials for dustbathing include sand, soft wood shavings.

Fowl are highly motivated to perform 'comfort behaviours' such as wing flapping, feather ruffling and leg stretching, which help to maintain strong leg bones. Birds should therefore be housed in floor enclosures large enough to permit all of these behaviours whenever possible. Ideally, birds should be housed with outdoor access; appropriate cover such as bushes is essential to encourage fowl to go outside. Flooring for fowl should be solid, as this enables the provision of substrate to encourage foraging and possibly help to reduce

the incidence of feather pecking. If fowl need to be caged for scientific purposes, they should be housed in enclosures designed to address behavioural requirements. If there are scientific reasons for not providing a solid floor, a solid area with loose substrate and items such as bunches of string, pecking blocks, rope, turf or straw should be provided for pecking.
Fowl strains developed for rapid growth rates (broilers) are highly susceptible to lameness and their use should be avoided wherever possible. If broilers are used, individuals should be assessed for lameness at least weekly and grown more slowly than commercially unless growth rate is essential for the study.

The minimum enclosure size for group housed domestic fowl is 1m² for birds less than 600g bodyweight and 2m² for birds greater than 600g.

Body mass (g)	**Area per bird – pair housed (m^2)**	**Area per bird – group housed (m^2)**	**Minimum height (cm)**	**Minimum length of feed trough per bird (cm)**
Up to 300	0.5	0.15	30	3
300 to 600	0.5	0.2	40	7
600 to 1200	1	0.3	50	15
1200 to 1800	1	0.4	50	15
1800 to 2400	1.5	0.5	55	15
Over 2400	1.5	0.6	75	15
Male birds	1.5	0.6	75	15

Behaviour
The domestic fowl is derived from the Burmese Red Jungle fowl *Gallus gallus*. Domestication, probably for cockfighting and as a sacrificial bird, began more than 5000 years ago. Despite the lengthy domestication period, domestic fowl retain much of the biology and behaviour of Jungle fowl, although domestic birds employ more energy saving strategies (Schütz *et al.* 2001, Ito *et al.* 1999) and modern breeds have been successfully re-established in the wild (McBride *et al.* 1969, Anonymous 2001, Andersson *et al.* 2001). Consideration of the ecology and behaviour of Jungle fowl is therefore essential for predicting the requirements of the domestic fowl (Hawkins *et al.* 2001, Fölsch *et al.* 2002).

Jungle fowl are predominantly ground-living in tropical and temperate scrub, forest or jungle habitats with ample overhead cover. The provision of appropriate cover is also very important for domesticated birds (Cornetto & Estevez 1999, Cornetto *et al.* 2002), and studies have suggested that visually discontinuous cover provides the greatest feelings of security (Newberry & Shackleton 1997). The most common social organisation in Jungle fowl is one male with up to four females, though they form larger groups of around 20 birds in more open environments. Other males either are solitary or form unisexual groups of 2 or 3 birds. Mixed groups have a well-defined home range with a regular roosting location. Jungle fowl spend the majority of their time foraging for seeds, fruits and insects (Duncan 1999), while domestic hens provided with concentrated food *ad libitum* may spend around 35 % of their day ground pecking and scratching (Anonymous 2001, Fölsch *et al.* 2002). It is therefore very important that husbandry systems should encourage this foraging behaviour. Maintaining the condition of the plumage through preening and dustbathing is also a time-consuming daily activity which becomes more important to fowl as they mature (Duncan 1999, Anonymous 2001).

Fowl are highly social and will form groups with stable hierarchies under appropriate conditions (see Anonymous 2001). Hens prefer to be with conspecifics (Lindberg & Nicol 1996), prefer familiar birds to an empty cage and should not be housed in isolation (Hawkins *et al.* 2001). There is probably no optimum group size, though it has been suggested that small groups of around 5 to 20 birds are generally favourable as there is less aggression and stress than in larger sized groups (Duncan 1999). Conversely, subordinate hens have been shown to prefer larger groups, possibly because this provides more opportunities to escape dominant birds (Lindberg & Nicol 1996). Mixed sex groups should contain few males to avoid excessive competition between them. Female groups with a small number of males may have lower aggression than groups of females only. However, the addition of males to a group of females may increase social stress if there is insufficient space (Duncan 1999).

Behaviours that are most important to fowl are nesting, perching and using litter for scratching, pecking and dustbathing (FAWC 1997, Duncan 1999, Olsson & Keeling 2000, Widowski & Duncan 2000, Anonymous 2001), so fowl should therefore be housed in floor pens large enough to permit all of these behaviours (Duncan 1999, Fölsch *et al.* 2002). Fowl are also highly motivated to perform 'comfort behaviours' such as wing flapping, feather ruffling and leg stretching, which help to ensure strong leg bones (Knowles & Broom 1990). It is also important to recognise that fowl will synchronise their activities and prefer to carry out specific behaviours together as a flock, so any facilities provided for them should enable them to do this (Duncan 1999, Anonymous 2001, Channing *et al.* 2001).

Domestic fowl in commercial flocks are often housed in extremely barren environments that seriously limit their ability to exercise and to express a range of natural behaviours (Anonymous 2001). The Group believes that this does not justify inadequate housing and husbandry in the laboratory and that fowl should be provided with good quality and quantity space wherever they are kept. (see also Höfner *et al.* 1997, Hawkins *et al.* 2001)*,* Table (i) on page 45 sets out fowl welfare criteria according to the animals' main needs, summarised from Anonymous *et al.* (2001). The third column has been added by the Expert Group.

Flooring

When kept on loose substrate, fowl spend a great deal of time performing foraging behaviour, *i.e.* pecking and scratching the ground (Duncan 1999, Anonymous 2001). Motivation for this behaviour appears to be high, as hens will forage for feed rather than eat identical feed that is freely available (Duncan & Hughes 1972) and, when given the choice, strongly prefer litter to a wire floor (FAWC 1997). Fowl kept on a wire floor or without appropriate pecking substrate may express their motivation to forage by excessive food manipulation with the beak and by feather pecking, a severe welfare problem (Green *et al.* 2000, Anonymous 2001). The incidence of feather pecking is significantly lower when litter is provided (Blokhuis 1989) and fowl have a high demand for a litter substrate (Gunnarrson *et al.* 2000), so fowl should be kept in pens with a loose substrate such as sand or soft wood-shavings (Hughes & Channing 1998). In these conditions it is important to replace the litter frequently to remove droppings and reduce the risk of disease.

Dustbathing is an especially important behaviour for hens (e.g. Zimmerman *et al.* 2000), but is thwarted by many housing systems (Anonymous 2001). 'Sham' or 'vacuum' dustbathing is often performed by fowl housed on wire floors, where animals are strongly motivated to dust bathe but unable to do so. Performing this vacuum dustbathing behaviour does not affect a bird's motivation to dustbathe in a suitable substrate (Lindberg 1999), so is not a substitute for 'real' dustbathing, which is likely to be pleasurable to hens (Widowski & Duncan 2000) and also appears to remove excess feather lipids and help to maintain plumage. Activities such as dust bathing require a considerable amount of space, as birds tend to roam some distance when foraging and bathing, but are important to the birds and so should always be possible (Duncan 1999, Fölsch *et al.* 2002).

A cage is not an appropriate housing environment for fowl (Fölsch *et al.* 2002, see also Hansen *et al.* 1993). If fowl must be kept in cages, it is advisable to provide a solid area with loose substrate covering at least one third of the floor area to allow some expression of foraging behaviour (Hawkins *et al.* 2001). Several cage designs have been developed that incorporate such an area (see Sherwin 1994, Fölsch *et al.* 2002). Many modified cages also provide a perch and nest box that allow much of the natural behavioural diversity of the species and provide an alternative method of housing if very hygienic conditions are required. However, measures that reduce feather pecking may be necessary (see below), such as providing objects for pecking or temporarily lowering the light intensity. It is advisable to use systems that have been extensively developed and tested, as deaths due to trapping are common in many early designs. If there are sound scientific reasons for not providing a solid area with loose substrate, toys or other items such as pecking blocks (*e.g.* Pecka-blocks, Breckland International Ltd, UK), rope, turf or straw should be provided for pecking, as fowl are attracted towards novel stimuli such as pecking items and sustain interest in them provided that they are selected with care (Newberry 1999). Plain bunches of white string are particularly effective (Jones *et al.* 1997, 2000, Jones & Carmichael 1998, Jones & Rayner 2000, Jones 2001).

Table (i) overview of fowl welfare

Need	**Natural behaviour and welfare issues**	**Potential welfare problems in standard cages with no enrichment and grid floors?**
Food, foraging	Pecking, ground scratching, needs litter and variety of food items	YES
Water	Frequent drinking, generally not at night	NO
Rest	Perching close together, usually at night	YES
Thermoregulation	Panting, raising feathers or wings	NO (in general)
Health	*E. coli*, coccidiosis, bone fractures	NO (parasites)/ YES (fractures)
Social contact	Peck order, communication, social recognition, possibility of escape	YES
Nesting	Nesting behaviour	YES
Maternal	Brooding, raising chicks	YES
Exploration	Pecking, scratching, visual investigation	YES
Safety	Fear, hysteria, need cover in open spaces, neophobia, frustration	YES/NO
Movement	Lack of movement, bone atrophy	YES
Body care/comfort	Preening, dust bathing, wing flapping, stretching, raising feathers	YES

Perches

Fowl have feet that are anatomically adapted to close around a perch when they roost, and feral and wild fowl spend a large amount of time perching on branches. In captive environments with limited perch space, hens struggle vigorously to obtain and keep perching space despite severe crowding, which indicates a high motivation to do so. Recent studies indicate that hens will push through heavy doors to gain access to perches at lights-off (Olsson & Keeling 2001) and become frustrated when they cannot perch at lights-off, such that the welfare of hens who cannot perch is reduced (Olsson & Keeling 2000). Providing perches reduces bird density on the floor, allows subordinate birds to escape dominant individuals by day and reduces agonistic interactions (Cordiner & Savory 2001, Pettit-Riley & Estevez 2001). Additional welfare benefits include feelings of safety (Keeling 1997), enhanced spatial awareness (Gunnarsson *et al.* 1999), improved foot and plumage condition and increased leg bone strength. Any deleterious effects of perching, such as bumblefoot or keel deformation, are due to poor perch design or positioning (Baxter 1994).

Fowl should therefore always be provided with the opportunity to perch and so at least 15 cm should be available to each bird (Duncan 1999, RSPCA 1999b). Perches should have a flat top about 3 to 4 cm wide, as round perches can increase the incidence of keel deformation (Duncan *et al.* 1992). The optimum height above the floor varies for different breeds and housing conditions (see also Lambe & Scott 1998), although layers can generally reach higher perches than broilers.

Nest boxes

Pre-laying behaviour occurs between 20 and 120 minutes before oviposition and starts with searching behaviour that leads to selection of a nest site and nest building. Hens are strongly motivated to obtain a suitable nest site (Cooper & Appleby 1994, Freire *et al.* 1997, Fölsch *et al.* 2002) and become frustrated and develop stereotypic pacing if deprived of access to one (Duncan & Wood-Gush 1972). Competition for preferred nest boxes may be a problem so a sufficient number should always be provided to allow subordinate birds access to nesting areas. Physiological stress arising from the failure to find a suitable nest site can lead to the egg being retained and 'dropped' later in the day without pre-laying behaviour. These eggs have a dusted or banded appearance, arising from extended calcification, and are a good indicator of stress during the pre-laying period. Laying hens should therefore have access to nest boxes from at least 16 weeks of age (Rietveld-Piepers *et al.* 1985, Hawkins *et al.* 2001). Although the exact nest site requirements of individual hens vary considerably, an enclosed individual nest box is satisfactory and highly preferable to most hens. Nest boxes should preferably be littered, enclosed and allow one hen to turn around. Hens are motivated to examine nest sites in the weeks before they come in to lay and allowing them to do so increases later use of the nest box (Rietveld-Piepers *et al.* 1985, Sherwin & Nicol 1993). A loose substrate such as wood-shavings or clean straw is also important and allows complete expression of nest building activities. An astroturf floor is also suitable though less preferable to the hens.

Broilers

Lameness is highly prevalent in all broilers grown on commercial (or approximately commercial programmes (Kestin *et al.* 1999; Sanotra 2000) and this is known to be painful (McGeown *et al.* 1999; Danbury *et al.* 2000; Weeks *et al.* 2000). The majority of the lameness is due to the excessively fast growth rates that occur in modern broiler strains. Growth can be influenced by decreasing the day length so that birds have less time to feed and this is the preferred method (Classen 1992). Alternatively, altering the level of protein and energy content or restricting feed, will reduce the incidence of lameness and its attendant welfare problems (Su *et al.* 1999). However, feed restriction can lead to aggressive behaviour, feather pecking and increased competition at around the time when food is delivered. Close observation and adequate management are therefore essential. See also Anonymous *et al.* (2001) for a summary of welfare issues relating to broilers.

Cage and pen sizes

The current European Convention and Directive guidelines for caging fowl (Council of Europe 1986) allow 650 cm^2 per bird for groups of three birds or more with body mass 1800-2400g. Six large fowl could therefore legally be kept in an area approximately 50 cm x 80 cm. This would not be sufficient to permit a range of normal behaviours or the provision of a good quality environment and the minimum pen area and area per bird both therefore need to be increased.

If birds must be caged, *e.g.* where a study requires the collection of faeces, they should be housed in modified cages designed to address behavioural requirements (Sherwin 1994) rather than standard size or ‘battery’ cages (see also Duncan 1999). Standard cages with a height of 40 cm prevent many comfort behaviours, and so cage heights that prevent full extension of the head and wings should not be used. Adult birds will take at least 2000cm^2 for the expression of comfort behaviours (Dawkins & Hardie 1989, Duncan 1999) and male birds require at least 2,500 cm^2 (Duncan 1999). Clearly, exercise, foraging and the inclusion of environmental enrichment will require yet more space.

The requirement for an extensive area appears to be important in two contexts. First, in the early stages of pre-laying behaviour, hens with access to a suitable nest site show increased walking and exploration in larger rather than smaller areas. If access to the nest is denied, hens develop stereotypic pacing suggesting that locomotory motivation is thwarted (Duncan & Wood-Gush 1972). These findings suggest that hens may be motivated to walk and explore the environment during the early stages of pre-laying behaviour. If so, then a confined area such as a cage will not meet these requirements and may account for the unusually high number of nest entries sometimes observed in cages with nest sites. Second, studies on spacing suggest that there are social attraction and repulsion forces and that when hens are given sufficient space, their chosen stocking density can vary considerably (*e.g.* Keeling & Duncan 1989, Channing *et al.* 2001). In small pens, birds may be motivated to achieve appropriate spacing, but be physically prevented from doing so. It is likely that the failure to express this motivation gives rise to social stress (Hawkins *et al.* 2001).

Fowl will walk up to 2.5 km a day and fly to and from elevated places if they have the opportunity to do so (Keppler & Fölsch 2000). They prefer large spaces and appear to find small spaces aversive if they have the opportunity to avoid them (Lindberg & Nicol 1996). Furthermore, in a survey of welfare scientists, summarised in Table (ii) below, space was rated as the most important welfare criterion to domestic fowl (Anonymous 2001).

Table (ii) design criteria for welfare in domestic fowl, ranked in order of importance (1 = most important)

Rank	Design criteria	Consequences if design is not appropriate
1	Space	Bone weakness, restricted or abnormal behaviour, fear
2	Substrate	Restricted or disturbed behaviour
2	Laying nest	Disturbed behaviour, gakel calls
4	Genetic background (strain)	Feather pecking, need to beak trim, fearfulness
5	Social contact (group size)	Abnormal or disturbed behaviour; social stress, aggression, feather pecking
6	Light	Eye abnormalities, reproductive depression
7	Perches	Behavioural restriction

Feeding

Fowl show diurnal rhythms in feeding behaviour with peaks in feeding usually at the beginning and end of the light period. Additionally, the sight and sound of a feeding bird triggers feeding behaviour in others, and so it is likely that at certain times of the day all birds are motivated to feed. The provision of insufficient feeder space for all birds to feed synchronously is likely to be deleterious to the well-being of displaced birds; the resulting competition and feelings of frustration could also lead to outbreaks of feather pecking (see Green *et al.* 2000). A minimum of 15cm of feeder length per bird should thus be provided to allow birds of any strain to feed synchronously (Duncan 1999).

Guidelines for housing the domestic turkey, *Meleagris gallopavo*, in stock and during procedures

Wild turkeys regularly utilise a diverse range of environments and perform a variety of behaviours including dustbathing, foraging and hunting. The social behaviour of the wild turkey is complex, particularly during the breeding season. Domestic turkeys retain many of the characteristics of wild birds but there are some fundamental differences, e.g. domestic turkeys are unable to fly but have retained the ability to run quickly, jump and glide, especially at younger ages.

Domestic turkeys are highly social and should not be singly housed. Stable groups should be formed as soon as birds are acquired and adequate monitoring is essential as injurious feather pecking and head pecking can occur from the first day of life.

Lameness is a common problem and needs to be carefully monitored. A policy for dealing with lameness should be agreed with the attending veterinarian.( )

Turkeys should be provided with perches placed at a height where birds on the ground are not able easily to peck and tug at the feathers of perching birds. However, if birds are older and less agile, the access to perches should be facilitated by special equipment such as ramps. Where this is not possible, perches should be placed at a low height (e.g. 5 cm). The shape and size of the perch should be in accordance with the rapidly growing claws of the birds. Perches should be ovoid or rectangular with smoothed corners and made of wood or plastic.

Substrate for dustbathing should always be provided. Suitable materials are fresh sawdust or sand. Straw bales may be used for enrichment and to provide a refuge from dominant birds, but will need to be frequently replaced and older, heavier birds may need ramps to gain access to them.

The minimum enclosure size for group housed domestic turkeys is 2 m^2. For birds over 20 kg, the minimum enclosure size is 3 m^2 and all enclosure sides should be at least 1.5 m long.

Body mass (kg)	**Area per bird – group housed (m^2)**	**Minimum height (m)**	**Minimum length of feed trough per bird (cm)**
1	0.3	1	15
4	0.35	1	15
8	0.4	1	15
12	0.5	1.5	20
16	0.55	1.5	20
20	0.6	1.5	20
Over 20	1	1.5	20

Behaviour

The domestic turkey is derived from the native wild turkey of North America. The natural habitat used by wild turkeys varies considerably according to the season, climatic conditions and behaviour being performed. Turkeys regularly utilise environments as diverse as open plains, dense woodland, thick scrub, treetops, and can sometimes even be seen wading in lakes. The walking speed of the wild turkey is approximately 5 km/h but birds can run with great manoeuvrability at speeds up to 30 km/h. Although their endurance is not great, wild turkeys are capable of flight - in stark contrast to the domesticated strains. Wild turkeys are not true migrants but will move up to 80 km between winter and summer sites. Typically, daily movement is 2-3 km and the home range covers from 200 to 1,000 acres (Bent 1963, Schorger 1966, Williams 1981).

The social behaviour of the wild turkey is complex. During the breeding season, males congregate in large groups to display to each other, emitting their characteristic 'gobbling' call. In domestic birds this display is readily elicited by the presence of humans. After hatching, the family is a basic social unit with the young firmly imprinted on the hen. The mother apparently teaches the young about the suitability of various foods with a series of displays and distinctive 'clucks'. Several broods usually join together in the spring to form a larger flock with the males leaving in the winter such that during this season there are 4 types of flock; (i) old hens without broods, (ii) brood hens with female offspring, (iii) young males recently separated from mothers and (iv) older males (Schorger 1966, Watts and Stokes 1971).

Wild turkeys perform a wide variety of other behaviours such as dustbathing, anting, foraging, hunting and fighting (which may sometimes last for hours). They are a highly vocal animal with a wide diversity of calls; eight are recognised and used routinely during hunting (Williams 1981).

Social housing

Domestic turkeys are highly social and become very distressed when isolated. Handling or housing birds as individuals should be avoided as this generally makes the birds considerably less tractable. However, turkeys are capable of recognising one another and placing any 'strange' turkey into an established group will almost certainly result in that individual being attacked by several others and possibly killed. Group housed turkeys can be highly aggressive to one another. Intense sparring fights can occur as the birds mature. During such fights, the opponents become almost oblivious of extraneous stimuli - handlers must be cautious if trying to intervene in a fight. The most extreme form of injurious pecking is head pecking in which one individual is incessantly targeted and pecked, sometimes with great force, by several other birds. It tends to become more frequent when the turkeys reach sexual maturity, especially if there is a significant difference in size between birds (Hawkins *et al.* 2001).

Feather pecking and aggression

Injurious feather pecking can occur from the first day of life. Recent evidence (Sherwin *et al.* 1999a) indicates that, at least in relatively small groups (of between 50 and 100 birds), this can be considerably reduced by providing supplementary ultraviolet radiation (turkeys can see in the UV spectrum), pecking substrates (*e.g.* straw) and visual barriers to reduce social transmission of this behaviour. Other pecking substrates which might be used are chains or twine (both at head height to ensure the birds do not become entangled), vegetable matter such as cabbages or scattering food in the substrate.

Environmental stimulation

Like the domestic fowl, the turkey is often housed in extremely barren conditions when kept in a commercial flock. Less has been published on environmental stimulation for turkeys than fowl (see Sherwin *et al*. 1999a and b), but it is equally important to provide sufficient quality space to allow turkeys to express a range of natural behaviours (Berk 1999, Hawkins *et al.* 2001). Enrichment, in conjunction with low stocking density, has also been found to reduce mortality in the BUT Big6 strain (Berk 1999).

As noted above, scattering food such as grain in pecking substrates such as straw promotes foraging behaviour and other vegetable matter, such as brassicas (*e.g.* cabbage leaves) or scattered grain (Crowe & Forbes 1999), can also be provided on the floor of the pen (Sherwin *et al*. 1999b). Straw bales also make the birds' environment more interesting and can provide a refuge from dominant birds, but will need to be frequently replaced (Hawkins *et al* 2001). While enrichment improves walking ability, older, heavier birds may need ramps if they are still to gain access to and benefit from perches and ramps (Berk 1999). Turkeys are particularly attracted to peck at string-like objects or those that are easily manipulated by the beak (Crowe & Forbes 1999).

Space allowances

Turkeys are the largest domesticated gallinaceous bird. They show a variety of 'comfort' behaviours such as wing-flapping, feather ruffling and leg stretching. In addition, they show spontaneous vigorous locomotion ('frolicking') which has all the appearance of 'play' and which decreases in frequency as the birds get older (Sherwin & Kelland 1998). All these activities, particularly locomotion, require a considerable amount of space. The UK Farm Animal Welfare Council noted that a maximum stocking density of 38.5kg/m^2 had been recommended, but provided their own formula which suggested a maximum permissible stocking density of 59.1kg/m^2 (FAWC 1995). This higher density approximates to 3 adult birds each weighing 20 kg being provided with 1 m^2, which is clearly limiting for birds wishing to perform behaviours that require considerable space such as dustbathing and wing-flapping. Good practice would suggest a considerably lower density is maintained to allow comfort behaviours, exercise and environmental enrichment. Fully grown stags may have a wing span of 1.5 m and should at least be able to extend their wings.

Perching and dustbathing

Recommendations on the provision of perches and substrate for dust baths are taken from Hawkins *et al.* (2001).

Guidelines for housing quail (*Coturnix spp.; Colinus virginianis; Lophortyx californica; Excalfactoria chinensis*) in stock and during procedures

Wild quail live in small social groups and devote much of their time to scratching and foraging for seeds and invertebrates on the ground. The preferred habitat of many species is dense vegetation such as grasslands, bushes alongside rivers and cereal fields. Domestication does not appear substantially to have altered quail behaviour, so it is essential to design housing systems that respect this and allow the provision of substrate for scratching, pecking and dustbathing, nestboxes and cover wherever possible. The housing of quail in aviaries or pens as opposed to cages is therefore strongly recommended.

Quail should be group housed in either all female or mixed sex groups. Where the sexes are mixed, the ratio of males to females should be low (e.g. 1:4) to reduce aggression between males and injuries to females. It may be possible to pair house males if stable pairs are formed during rearing. The likelihood of aggressive pecking leading to skin lesions and feather loss is reduced if quail are not kept under intensive conditions and established groups are not mixed.

Quail are capable of extremely rapid, upward escape flights, which can lead to head injuries in captivity. Staff should therefore always approach birds slowly and calmly and quail should be provided with cover and environmental enrichment, especially early in life, in order to reduce fear. Quail chicks should have access to coloured objects such as balls, tubing and cubes to alleviate fear of both human beings and novel stimuli in adult birds. Adult birds should be given pecking objects such as stones, pine cones, balls and branches of vegetation. Sand, wood shaving or straw substrate for foraging and a place to which the birds can withdraw should be provided, with additional dust baths of sand or sawdust if the foraging substrate is not suitable for dust bathing. Laying hens should have access to nest boxes and nesting material, such as hay.

If quail must be housed in cages, consideration should be given to combining enclosures, adding enrichment items and providing a minimum enclosure height of 30 cm (with a roof made of pliant material) to reduce the risk of head injury. Solid enclosure roofs may make birds feel safer, although this could result in unacceptably low light levels in lower enclosures if birds are housed in racks. Birds should be cage housed for the minimum possible period because many welfare problems become more severe with age, especially in birds kept for one year or more.

The minimum enclosure size for group housed quail is 1 m^2.

Body mass (g)	Area per bird – pair housed (m^2)	Area per additional bird-group housed (m^2)	Minimum height (cm)	Minimum length of trough per bird (cm)
Up to 150	0.5	0.1	30	4
150-250	0.6	0.15	30	4

Behaviour
Quail are adapted to inhabit grasslands with a degree of cover (Mills *et al.* 1999, Hawkins *et al.* 2001). The Japanese quail *Coturnix japonica* is omnivorous and the diet of wild birds comprises small seeds, insects and spiders (Kawahara 1967). Although European and Japanese quail are migratory, quail generally only perform short flights to escape predators during the breeding season. Most have short, rounded wings and are capable of extremely rapid, upward flight that enables them to escape from danger.

Domestication does not appear to have substantially altered quail behaviour, so it is essential to design housing systems that respect this. Substrate for scratching, pecking and dustbathing, nestboxes and cover are all important for quail welfare (Johnson & Guthery 1988, Schmid & Wechsler 1997, see Mills *et al.* 1997).

Group composition

Male quail should be housed in stable pairs because (i) aggression and homosexual copulation attempts are frequent in larger all-male groups and (ii) males are aggressive towards unfamiliar males (see Mills *et al.* 1999). In general, the literature recommends low male:female ratios for the quail (Mills *et al.* 1999, Wechsler & Schmidt 1998). A ratio of one male to four females is suggested in Mills *et al.* (1999), and a previous study evaluating a range of sex ratios found that fertility was satisfactory in breeding groups with sex ratios of 1:8 or 1:12 (Wechsler & Schmidt 1998).

Birds introduced into established groups are likely to be attacked, especially where strange birds are introduced into the home cages of groups with established hierarchies. Groups should therefore never be mixed or birds replaced with others (Mills *et al.* 1999).

Aviaries and floor pens

Quail are generally housed in outdoor aviaries, floor pens with deep litter or smaller battery type caging. Aviaries with outdoor access are to be preferred, but where birds must be housed wholly indoors, serious consideration should be given to housing in pens as opposed to cages (Hawkins *et al.* 2001). Current standard size quail cages do not permit environmental enrichment or a range of behaviours and so their use should be discontinued on animal welfare grounds.

Groups of eight Japanese quail housed in semi-natural outdoor aviaries of 19 m^2 containing a substrate of soil and wood chips, herbs, shrubs and artificial shelters have been reported to display a range of natural behaviours including exercise, foraging, flight and dustbathing. These birds had been reared in battery cages up to 5 weeks of age (Schmid & Wechsler 1997). Aviary-housed female Japanese quail have been found to have a strong preference for artificial cover, especially while egg laying, and also to show flight behaviour in response to a frightening stimulus significantly less when under cover (Buchwalder & Wechsler 1997). Cover (either natural or artificial) should therefore be provided to encourage natural behaviour and reduce stress. Quail are thus able to express a range of natural behaviours in aviary housing, so should be housed in pens or aviaries wherever possible (Hawkins *et al.* 2001).

Floor pens are an adaptation of agricultural practice and commonly suggested stocking densities range between 40 to 200 birds/m^2 (Home Office 1989, Hodgetts 1999, Mills *et al.* 1999). Suitable substrates include sand, wood shavings or straw. Higher levels of fertility and hatchability are achieved in floor pens at low stocking densities (Ernst & Coleman 1966). Some welfare problems may still occur when birds are housed in floor pens, however. Quail housed in floor pens are often found to have hardened balls of food, litter and faeces adhering to their feet (Gerken & Mills 1993), which can lead to increased pecking at the toes, injuries and possibly cannibalism. Good husbandry and regular monitoring of birds' feet are therefore essential, whichever type of flooring is used.

Cages

Standard laboratory battery cages are extremely small and in no way allow birds to exercise adequately or permit the provision of environmental stimulation. For example, quail housed in semi-natural aviaries (with cover) were found to spend 24 % of their time walking, running or flying and 8 % pecking and scratching away from their feeder, despite having *ad libitum* access to food (Schmid & Wechsler 1997); neither of these activities are possible in standard size cages. Breeding female quail housed in cages also exhibit pre-laying restlessness

(Gerken & Mills 1993). The justification for housing quail in standard size 'battery' cages should therefore always be questioned, and birds housed in aviaries or pens wherever possible.

If there are compelling scientific or veterinary reasons for keeping quail in cages, serious consideration should be given to modifying them to provide better quality and quantity of space and thereby improving welfare. The typical quail flight response is vertical and this can result in serious injuries when birds are housed in standard cages. Quail are therefore typically kept in cages with insufficient headroom to permit high jumps. However, the welfare of caged quail can be significantly improved by combining cages to give birds more space for exercise, adding enrichment items and by providing a minimum cage height of 30 cm. Although it has been suggested that injuries will be worse if cage height exceeds 20 cm (Mills *et al.* 1999, Gerken & Mills 1993), it is the experience of BVA(AWF)/FRAME/RSPCA/UFAW JWGR members that cages 30 cm high significantly reduce or even eliminate the problem. Consideration could also be given to providing solid cage roofs, as this may make birds feel safer, although this could result in unacceptably low light levels in lower cages if birds are housed in racks (Hawkins *et al.* 2001).

If large areas of wire flooring are deemed to be necessary, *e.g.* for some toxicology studies, a solid resting area should be provided and the wire should be coated with soft plastic to reduce damage to the feet of the birds (Hawkins *et al.* 2001). The duration of studies where birds are housed in cages should be kept to a minimum because many welfare problems become more severe with age, especially if studies last for a year or more (Gerken & Mills 1993).

Environmental stimulation

Providing environmental stimulation for chicks in the form of coloured objects (balls (Sherwin 1995), tubing, cylinders and cubes) alleviates fear of both human beings and novel stimuli in adult birds, perhaps by reducing underlying fearfulness (Jones *et al.* 1991, review in Mills *et al.* 1997). Toys in the form of stones and pine cones may reduce aggression in groups of adult birds (Ottinger & Rattner 1999). Other commonly provided items are balls, tubes, mirrors and branches of vegetation (K Miller, pers. comm.).

Nest boxes

Laying hens will benefit from nest boxes, which can be clipped on to the sides of their cages, and nesting materials. Nest boxes filled with chaff were preferred over those containing hay or turf in a study using Japanese quail (Schmid & Wechsler 1998).

Perches

A study involving continuous observation of Japanese quail in a semi-natural aviary at twilight found that the birds did not roost on perches at night, unlike fowl. They also spent very little time (0.5 %) on elevated structures, *e.g.* on top of shelters (Schmid & Wechsler 1997). It is not currently considered to be necessary to provide perches.

Dustbathing

Dustbathing is important to quail (Schmid & Wechsler 1997); Japanese quail use litter for dustbathing (Mills *et al.* 1999) and exhibit vacuum dustbathing behaviour in its absence (Gerken 1983, Mills *et al.* 1997). Dust baths should therefore always be provided, with suitable substrates such as sand or sawdust.

Guidelines for housing ducks and geese in stock and during procedures

Domestic ducks and geese commonly used in research and testing include Anas platyrhynchos, Anser anser domesticus and Cairana moschata. All waterfowl are primarily adapted for locomotion and feeding in water, which is also very important for 'comfort' behaviours such as bathing and preening. Ducks and geese should be provided with a pond with a mixture of stones and grit on the bottom, both to increase the birds' behavioural repertoire and to encourage adequate maintenance of the feathers. The very minimum that waterfowl should be able to do is immerse their heads under water and shake water over the body. Drinkers and ponds for waterfowl should be located over grid areas with drains beneath to reduce flooding.

Water to a depth of around 1 cm should be provided in a shallow bowl for bathing within 24 hours of hatching. After the first week, a shallow pond (dimensions as in table) with large stones on the bottom should be provided with food or grit scattered among the stones to encourage dabbling or diving as appropriate. Access to ponds for juvenile birds should only be under supervision to ensure that they can leave the water and do not become chilled. This should continue until they are clearly capable of leaving the water unaided and their feathers have begun to emerge. It is not necessary to control the temperature of the water.

Ducks and geese should be housed on solid floors and have sufficient space to permit foraging, walking, running and wing flapping. Grazing geese should be provided with natural plant cover (either in outdoor runs or using potted shrubs indoors) or boxes and straw bales. Ducks and geese should always be kept outdoors or have access to outdoor runs unless there is scientific or veterinary justification for keeping them indoors. Birds housed with outside access should be kept secure from predators and should be supplied with a dry shelter to enable them to rest. They should also have a pond and vegetation for cover and/or grazing as applicable. Serious consideration should be given to supplying other features of the habitat that are likely to be important to each species whether birds are housed indoors or outdoors. This includes shallow water with vegetation for dabbling ducks, turf for geese and deeper water with large stones for species that live along rocky coasts.

Ducks and geese should be housed in appropriately sized groups wherever possible and the amount of time when any individual is left alone should be minimised. Many species of waterfowl become territorial during the breeding season, however, so it may be necessary to reduce group sizes and ensure that there is sufficient enclosure space to reduce the risk of injury, particularly to female birds.

Domestic geese and ducks have been selected for meat and egg production, but all breeds retain most of their 'wild type' behaviour and are generally more nervous and easily upset than other domestic fowl, especially when they are moulting.

The minimum enclosure size for group housed ducks and geese is 2 m^2.

	Area per bird – pair housed (m^2)*	**Area per bird – group housed (m^2)***	**Minimum height (cm)**
Ducks up to 1.2 kg	1	0.33	200
Ducks over 1.2 kg	1.5	0.5	200
Geese	1.5	0.5	200

*This should include a pond (see table below).

- Minimum pond sizes and depths for ducks and geese

	Pair housed[a]		**Group housed[a]**	
	Area (m^2)	**Depth (cm)**	**Area (m^2)**	**Depth (cm)**
Ducks	0.15	30	0.5	30
Geese	0.15	10 to 30	0.5	10 to 30

[a] Pond sizes are per 2 m^2 enclosure. The pond may contribute up to 50% of the minimum enclosure size.

Water

Ducks, geese and swans belong to the family Anatidae, which includes over 140 species distributed world-wide. All are wetland specialists, so are primarily adapted to locomotion and feeding in water and have varying abilities to walk and feed on land. Most species live on or near fresh water ponds, rivers and lakes, although many inhabit or feed at brackish estuaries and some are marine (see *e.g.* Owen & Black 1990). Ponds and lakes are used by ducks and geese for feeding, mating (in large bodied domestic birds) and as a refuge, particularly at night. Water is also very important for 'comfort' behaviours such as bathing and preening (Hawkins *et al.* 2001).

The opportunity to replicate natural wildfowl habitats is limited in the laboratory, but consideration should be given to supplying features of the habitat that are likely to be important to the birds, for example shallow water with vegetation for dabbling ducks, turf for geese and deeper water with large stones for species that live along rocky coasts (Forbes & Richardson 1996). Regular access to water is also important for the integrity of the feet and to help prevent cloacal infections (usually *Pseudomonas* spp) caused by the birds being unable to defecate naturally into water while swimming (Redig 1996). All waterfowl should therefore have a pond with a mixture of stones and grit on the bottom, both to increase the birds' behavioural repertoire and to encourage adequate maintenance of the feathers. The very minimum that waterfowl should be able to do is immerse their heads under water and shake water over the body (RSPCA 1999a). Many species may be nocturnal and rest during the day but still make good use of the pond during the night, especially if they are fed in or under the water (Hawkins *et al.* 2001).

Ducklings and goslings are capable of walking, eating, swimming and diving almost immediately after hatching. They can be introduced to water 1 cm deep for bathing in a shallow bowl within 24 hours of hatching (Forbes & Richardson 1996), but it is important to note that hand-reared chicks can become soaked and chilled if they spend too long in the water and may also have difficulty leaving the water (Hawkins *et al.* 2001). Naturally-reared chicks are brooded by their parents, which keeps them clean and dry (Robinson 1996). It is advisable to allow hand-reared ducklings and goslings two or three short (*e.g.* 15 minutes), supervised swims a day to provide exercise and encourage preening for the first 2 to 3 weeks. A shallow pond with large stones on the bottom will provide extra interest and exercise after the first week; food or grit can be scattered among the stones to encourage dabbling. All bowls and ponds should be emptied or closed to the birds when unsupervised until they are larger and feathers have begun to replace their juvenile 'down', which occurs at 3 to 6 weeks of age, depending on the species (Hawkins *et al.* 2001).

Space and environmental stimulation

Ducks and geese should be able to exercise by walking and running, and should also have sufficient space to flap their wings without obstruction (Hawkins *et al.* 2001). Geese are adapted to walk while grazing and so will require a greater proportion of walking and grazing land than dabbling and diving ducks. Swimming exercise is more important for ducks and diving ducks need sufficient depth of water to dive in. Some diving ducks, *e.g.* stifftails such as the ruddy duck *Oxyura jamaicensis*, are very poorly adapted for walking and rarely leave the water so will need large ponds (Hawkins *et al.* 2001).

Although sufficient space to exercise is of primary importance for waterfowl, a stimulating environment is also necessary to encourage them to forage, play and use all three dimensions of their pond (Hawkins 1998). Features of their natural environment can be reproduced to an extent in the laboratory, and a variety of natural and synthetic objects should be used to provide environmental stimulation in pens and ponds (Hawkins *et al.* 2001).

Aggression may be associated with the provision of environmental stimulation if animals are competing for an insufficient number of objects or the space allowance is inadequate, but this can be reduced or prevented by allowing sufficient objects and space and observing the birds. Occasional competition for items accompanied by single pecks, rather than sustained attacks, should be regarded as normal social interaction and are no reason to stop providing environmental stimulation (Hawkins 1998).

Social housing and behaviour

Waterfowl are extremely gregarious and form strong attachments to one another. Geese in particular form long term, stable bonds, especially within family groups (Owen & Black 1990, Ely 1993). Some ducks such as the European teal *Anas crecca* and common eider *Somateria mollissima* do especially well in groups and display behaviour that may not be observed in single pairs, but others should be kept in single pairs only, *e.g.* shelducks *Tadorna tadorna* (Forbes & Richardson 1996). Group housing ducks and geese is vital for an acceptable standard of welfare in the majority of cases and the amount of time when any individual is left alone should be minimised (Hawkins *et al.* 2001). Ducks and geese generally become distressed if they cannot see conspecifics, so it may be necessary to provide a bird undergoing procedures with a companion who they can see (*e.g.* Stephenson 1994).

Many species of waterfowl become territorial during the breeding season. Male ducks and geese will defend females against other males until incubation has begun, and geese will defend their mate and her feeding resources throughout incubation. Some geese drive other families of geese away while rearing young. In most dabbling ducks, stifftails and some diving ducks, lone males will attempt to forcibly mate with other females. Many broods of mallards have mixed parentage as a result (Owen & Black 1990) but this extra pair forced copulation can also result in the death of the female. It may be necessary to reduce group sizes and ensure that there is sufficient space to reduce the risk of injury.

All geese and some ducks shed all the flight feathers simultaneously during moulting and are flightless until the new feathers have grown. This is often associated with ‘moult migrations’, where birds move to safer areas during the flightless period. This lasts for between 3 and 5 weeks and is often accompanied by a decrease in flight muscle mass (Owen & Black 1990, Saunders & Fedde 1994). The behaviour patterns of some species may also alter while their mobility is restricted, including changes in feeding times and stronger responses to stimuli interpreted as predators (Kahlert *et al.* 1996). Moulting birds may therefore require extra consideration, *i.e.* they should always have a refuge where they can feel secure and disturbance should be minimised.

Guidelines for housing the pigeon, *Columba livia*, in stock and during procedures

The various strains of domestic pigeon are believed to derive from the rock dove Columbia livia. Rock doves nest and roost on cliffs or within caves, and feral pigeons will utilise sheltered ledges on man-made structures in the same way. In their natural habitat pigeons usually occur in pairs to large flocks, feeding and roosting together, but will defend roosting spaces and nesting areas. Pigeons can be housed in mixed groups, and may lay eggs but will not incubate them if nest boxes are not provided.

Care must be taken when choosing a breed for laboratory use, as some strains may show abnormal or undesirable behaviours and should therefore be avoided. Pigeons are primarily seed eaters but are omnivorous, so food containing animal protein, such as commercially available turkey starter crumbs or chick rearing meal, should be offered regularly.

Pigeons should be allowed an area sufficient for flight wherever possible, with a separate perching area for each bird along at least one wall of the enclosure. Box perches approximately 30 cm square and 15 cm deep located in blocks should be provided. Branches hung from the roof and scaffolding can also be used for perching. Toys hung from chains should be provided, e.g. bird bells, mirrors and commercially available toys designed for companion animals. Each enclosure should have shallow water baths. Where pigeons must be handled frequently, 'nesting areas' or chambers can be provided so that birds can be trained to retreat to them for capture.

If enclosures large enough to permit flight are not feasible, access to 'flight rooms' with perches for exercise and social interaction (e.g. a modified animal room) should be considered, provided that birds are carefully monitored to ensure that subordinate birds are not bullied.

Larger, enriched enclosures with shelving, perches and toys should be used wherever possible rather than 'standard' pigeon enclosures. Pigeons benefit from being able to forage and should not be kept on grid floors without strong scientific justification.

The minimum enclosure size for group housed pigeons is 2 m^2, with a minimum height of 2 m. Enclosures should be long and narrow (e.g. 2 m by 1 m) rather than square so that birds are able to perform short flights.

Number of birds	Minimum area (m^2)	Minimum height (m)	Minimum length of food trough per bird (cm)	Minimum length of perch per bird (cm)
2 to 6	2	2	5	30
Up to 12	3	2	5	30
Each additional bird	0.15		5	30

Behaviour

The most commonly used Columbiform in the laboratory is the domestic pigeon, which is believed to derive from the rock dove *Columbia livia* (Hawes 1984). Rock doves nest and roost on cliffs or gorges or within potholes and caves, and so feral pigeons will utilise sheltered ledges on man-made structures (such as box perches) in the same way.

Wild and feral birds usually occur in large, mixed flocks and are usually housed in mixed groups in the laboratory, which helps to prevent aggression during the breeding season (Hutchison 1999). If breeding is not required, it can be prevented by withholding nesting places, as females may lay eggs but will not incubate them without a nesting site.

Pigeons are primarily seed eaters but will take a very wide range of grains, fruits, berries and vegetation and also small snails and other molluscs (Hutchison 1999). A variety of foods should be presented such as legumes and cereals with some smaller seeds (Harper 1996). Vegetable proteins alone do not provide an adequate diet for pigeons (Hutchinson 1999) and so foods containing animal protein should be offered regularly (Harper 1996, Hutchinson 1999). Pigeons fed *ad libitum* are liable to become obese, particularly females, and so regular monitoring of body weight and condition is essential (CCAC 1984, Hawkins *et al.* 2001). Obesity can be largely prevented by restricting feed to 28.5 g per bird per day and including low-palatability grains such as barley (FDW Harper, pers. comm.).

Domestic pigeons are kept in a variety of forms and over two hundred fancy breeds now exist, including strains that have been developed for appearance, endurance flying, racing and for meat production. Care must be taken when choosing a breed for laboratory use, as some strains may show abnormal or undesirable behaviours and should be avoided (Hutchison 1999).

Housing
If birds must be housed indoors, consideration must be given to providing sufficient quality and quantity space to allow a range of behaviours. This should include flight wherever possible (CCAC 1984, Hawkins *et al.* 2001), not least because pigeons have been demonstrated to express a strong preference for aviaries that are large enough for them to fly (Schmorrow & Ulrich 1991). Despite this, laboratory pigeons are often housed singly in small cages that do not permit them to extend their wings. This does not permit exercise or the provision of environmental stimulation. Furthermore, pigeons housed in ‘standard’ cages for long periods undergo a substantial loss in muscle tone and are not physiologically normal (Clarkson *et al.* 1963). Small cages are therefore not appropriate for long-term housing (CCAC 1984, Hawkins *et al.* 2001).

Modified cages
If pigeons must be housed in cages for scientific or veterinary reasons, large, modified cages with shelving, perches and toys should be used wherever possible (Nepote 1999, see also CCAC 1984) rather than ‘standard’ pigeon cages. Access to ‘flight rooms’ with perches for exercise and social interaction (*e.g.* a modified animal room) should also be seriously considered, provided that birds are carefully monitored to ensure that subordinate birds are not bullied (CCAC 1984, Nepote 1999).

Catching birds
If pigeons must be handled frequently, ‘nesting areas’ can be provided and birds trained to retreat to them for capture. It is possible to house pigeons in aviaries with outside access and with conspecifics even where they are required for training in small test chambers (*e.g.* Skinner boxes), by constructing connecting channels directly from the aviary and training the birds to enter the chambers for food. Following a habituation period of several days, a sliding door can be used to shut the pigeons in the experimental chamber, eliminating the need for stressful manual capture (see Huber 1994).

Perches
Flights and aviaries should allow a separate perching area for each bird, as sufficient box perches will allow birds to establish their own territories, reducing fighting and facilitating easy capture (CCAC 1984). Box perches approximately 30 cm square and 15 cm deep located in blocks on one wall simulate a ‘natural’ type of environment and also help to deposit faeces in one area. Each bird should have at least 30 cm of perching space (Hutchison 1999). Pens should have covered food, grit and water hoppers, with additional water baths (CCAC 1984, Hutchison 1999). It may be necessary to supply large waterproof

trays in which smaller baths can be placed, as pigeons splash considerably when they bathe and will soak the surrounding area (Hawkins *et al.* 2001).

Environmental stimulation

Pigeons are often housed in barren conditions in the laboratory, but they will benefit from and make good use of large pens or aviaries supplied with enrichment items (Hawkins *et al.* 2001). Nesting facilities, nesting material and perches should be provided when birds are paired or housed in aviaries (Hutchison 1999). Birds housed in the laboratory have been found to benefit from toys such as bird bells, mirrors and rubber toys designed for cats, hung from chains. Foliage can be attached to the sides of aviaries using thick gardening wire to provide additional perching and shelter. Branches hung from the roof and scaffolding can also be used for perching (J Archer, pers. comm.).

Flooring

Pigeons should not be housed on grid floors, as this prevents foraging. Birds housed on solid floors should be cleaned out regularly, the frequency depending on the degree of confinement. Smaller cages will require daily cleaning, but in larger flights it will only be necessary to clean heavily soiled areas beneath perching areas daily (Hawkins *et al.* 2001).

Guidelines for housing the zebra finch,* Taeniopygia guttata*, in stock and during procedures

Zebra finches occur across most of Australia and its neighbouring islands. They are highly mobile, ranging over wide areas in search of food, and live in flocks of up to several hundred individuals. The species is monogamous and sexually dimorphic, as the male's plumage is more ornate than that of the female. The breeding season is not fixed, but is triggered by the availability of ripening grass seeds. Zebra finches use nests for roosting as well as breeding; roosting nests may be old breeding nests or purpose-built. Nests can be provided for captive birds in the form of wicker or plastic baskets with dried grass for nesting material, but birds will defend these and it is important to monitor behaviour to ensure that sufficient nests are provided.

Zebra finches are social and non-breeding birds should be housed in groups. Mixed-sex groups are possible and breeding can be suppressed by feeding a diet of dry seeds supplemented with fresh greens, but never soaked or sprouted seeds. Sprays of Panicum millet should be continually available as dietary enrichment. As Zebra finches feed extensively on the ground, birds should be housed on solid floors to facilitate natural foraging behaviour.

Toys, perches and swings designed for companion birds will benefit zebra finches and these should be provided wherever possible. Perches are particularly important for wellbeing and should be provided at a range of heights to facilitate normal feeding and roosting behaviour. Water for bathing should be provided at least once a week in trays 0.5 to 1 cm deep.

Fitting zebra finches with coloured leg bands for identification can have significant effects on their social and reproductive behaviour (e.g. red can enhance dominance and green or blue reduce it). Colours and patterns that have minimum impact on social interactions should be researched before fitting leg bands.

Minimum enclosure sizes for zebra finches are set out below. Enclosures should be long and narrow (e.g. 2 m by 1 m) to enable birds to perform short flights. Zebra finches thrive in outdoor enclosures provided they have access to shelter and heating is provided in cold conditions.

Number of birds	***Minimum area (m^2)***	***Minimum height (m)***	***Minimum number of feeders***
Up to 6	*1*	*1*	*2*
Up to 12	*1.5*	*2*	*2*
12 to 20	*2*	*2*	*3*
Each additional bird	*0.05*	*2*	*1 per 6 birds*

Natural habitat and social behaviour

Zebra finches are found across most of Australia, as well as neighbouring islands (Zann 1996). They are a highly sociable species and usually live in flocks numbering a few dozen to a few hundred, often mixed with other species of small birds (Jones & Slater 1999, Hawkins *et al.* 2001). The zebra finch ranges over a wide area in search of food and local populations can be highly mobile, deserting an area if conditions are unfavourable. Perhaps as an adaptation for the need to be able to fly large distances at short notice, there is no synchronous moult and the feathers are shed gradually throughout the year (Zann 1996). The birds will use nests as roost sites even outside the breeding season, a habit which allows them to survive in areas where the temperature is low at night. Roosting nests are either old breeding nests or purpose-built (Zann 1996).

Housing and space requirements
As it is a sociable species, communal housing should always be provided for the zebra finch. The minimum enclosure dimensions presented in the table in Part A of this document are based on the experience of Expert Group members. It is best to optimise vertical height and length, to permit free flight. Steel or treated wood can be used to construct the framework of finch enclosures and plastic-coated wire mesh, of no greater than 1 cm width, is suitable for the walls of the enclosure and is also preferable to plastic aviary netting. The floor should be solid, as this encourages natural foraging behaviour when seeds are spilt or deliberately placed on the floor. Suitable substrates include bark chips, wood shavings or sand (Hawkins *et al.* 2001).

The range of the zebra finch is widespread within Australia, and it can sometimes be exposed to sub-zero temperatures over winter (Meijer *et al.* 1996). Zebra finches seem to thrive in outdoor accommodation, provided that some indoor shelter and/or heating is provided for particularly harsh conditions (Bates & Busenbark 1970).

Breeding and rearing
Zebra finches are monogamous and sexually dimorphic. The breeding season is not fixed, but is triggered by the arrival of rains. In a long-term population study, Zann *et al.* (1995) showed that there was a time-lag of one or 2 months between the start of the rains and breeding, such that hatching of the eggs coincided with the first availability of ripening grass seeds. Dry seed alone, however abundant, did not stimulate breeding.

Presumably so that they can take advantage of unpredictable breeding conditions without the delay imposed by courtship, zebra finches form long-term monogamous pair bonds in the wild. Once paired, much of the social behaviour of the male and female is directed towards maintaining this bond. The surest sign of pair-bonding is allopreening, where the male and female sit close to and preen each other. Young birds are capable of breeding at quite an early age, probably another adaptation for opportunistic breeding when conditions are unpredictable. The median age of first breeding is about 90 days in both sexes in wild birds (Zann 1996) and birds can breed even earlier in aviary conditions. The modal clutch size is five, both sexes incubating during the day and the female at night (Zann 1996). The average length of incubation is 14 days.

The zebra finch is amongst the easiest of birds to breed in captivity, due to its opportunistic breeding in response to the arrival of rains (Zann *et al.* 1995). If breeding is desired, it is important to stock birds at a lower density than for standard housing, provide an excess of nest sites, and maintain an equal sex ratio. This reduces sexual conflict and aggression between pairs. Zebra finches seem to breed best when a small number of pairs are housed together in a medium-sized aviary with sufficient breeding sites. Whenever pairs of birds are provided with a nesting site and with a supply of greens, live insects, and softened seeds, they are liable to attempt breeding. The ‘ideal’ conditions of captive housing can lead to over-production of young, or unhatched eggs (Bates & Busenbark 1970).

Dried grass and/or coconut fibre should be provided for nest-lining, although not in great excess as under the ‘cost free’ conditions of captivity some birds may overfill their nests with lining, constricting the space available to the nestlings. Suitable commercially produced nests are either a completely enclosed pear-drop-shaped wicker basket (or its more easily cleaned plastic equivalent), or a cubic wooden box of side about 12 cm and with either an entrance hole of about 4 cm or the top half of the front cut away. Dried grass for nest lining can be hung in a basket of wide-mesh chicken-wire within the aviary; this keeps it unsoiled by faeces and the aviary tidy. Some birds continue to add nesting material over clutches and then lay another clutch on top. Nesting material should therefore be removed after clutches have been laid. For further information on breeding and rearing, see Jones & Slater (1999) and Hawkins *et al.* (2001).

Diet

In the wild, zebra finches subsist on an almost exclusive diet of dry grass seeds. The basic diet for captive zebra finch consists of commercially available foreign finch seed mix (largely millet and canary seed). Finches also like both soaked and sprouted seeds and fresh greens, but these should not be provided to mixed flocks unless the birds are required to breed. Soaked seeds are produced by soaking the normal finch mix for a day, then rinsing the hydrated seeds before they are given to the birds. If soaked seeds are stored in a warm, dark place and kept moist, they will sprout; sprouted seeds should also be rinsed before they are given to the birds. Suitable greens for zebra finches include the darker outer leaves of lettuce or dandelion, water-cress, and spinach; pet shops also sell dehydrated greens which often have vitamin supplements added. It is best to give the birds a small amount of greens and protein-rich foods regularly, because an infrequent supply encourages them to overeat, which can result in diarrhoea (see Jones & Slater 1999, Hawkins *et al.* 2001).

Live insect prey, most conveniently mealworms or cricket nymphs, provide essential protein, but these should be limited to two or three a day, in order to prevent overeating. If birds are in a communal aviary where there is a risk of subordinate individuals having reduced access to these preferred foods, they are best supplied in several small, separated dishes, so that no one bird can monopolize the resource. Millet (*e.g. Panicum*) in spray form encourages natural foraging behaviour; fruit such as apple, and the yolk of hard- boiled eggs, are also recommended additions to the diet (Bates & Busenbark 1970).

Attempts should be made to carefully assess the nutrient balance of the diet and to correct any potential deficits with supplements (Kirkwood 1996). Supplements can be added to the birds' water in liquid form or to the food or grit as powder. Grit is best provided in a small dish separate from the seed mix, so that the bird can control its intake as necessary. Cuttlebone is the most convenient means of providing finches with calcium. Whole cuttlebone should be attached to the side of the bird's cage with the softer side outermost; alternatively, crushed cuttlebone can be added to the grit mix (Jones & Slater 1999).

Environmental stimulation

Toys: A range of toys designed for finches is commercially available. Birds are likely to benefit from these, provided that care is taken to ensure that all cage additions are well used, promote a range of desirable behaviours and are supplied in sufficient numbers to avoid competition (Hawkins *et al.* 2001).

Perches: Zebra finches are essentially perching birds, so are likely to feel most secure when they are able to rest above human head level. The most convenient perching materials are 0.5 cm doweling rods which should be easy to remove and clean regularly. Natural branches provide birds with extra interest and a range of different diameters and shapes to exercise the feet, and should be provided where possible. Swings may also help birds to exercise as their movement necessitates use of the wings for balance. Plentiful perching space, at several different levels with some high up (within 15 cm of the roof) for roosting, provides the birds with alternative perching heights. Like most birds, zebra finches feel safest on branches near the roof of the aviary, but prefer to approach feeders or the ground by moving to progressively closer perches. The aviary should not, however, be so crowded with perches as to make free flight or capture of the birds difficult. Perches should not be placed over food or water containers, so as to avoid fouling (Jones & Slater 1999, Hawkins *et al.* 2001).

Baths and water: As one might expect for a species that can persist in arid conditions, zebra finches can do without water for long periods, their needs being met by metabolic water from carbohydrate breakdown. However, in captivity it is essential to provide them with drinking water and also bathing water at least once a week (Jones & Slater 1999, Hawkins *et al.* 2001).

Coloured leg rings

An aspect of husbandry which may be unexpected and is of welfare significance, is that banding the birds with colour rings for identification can have profound effects on their social behaviour. The colour of rings which a bird wears can affect their attractiveness to mates, their breeding success, longevity and even the sex ratio of their broods (Burley 1985, 1986 a,b,c, 1988, Zann 1994). Females appear to prefer males with red leg rings, perhaps a redirected preference for the redness of the male's bill, and dislike green rings; females also find black-ringed males attractive and blue-ringed males unattractive (Burley *et al.* 1982). More recently, this pattern has been shown to be mirrored in intrasexual dominance interactions, with red-ringed males being dominant over green-ringed males (Cuthill *et al.* 1997). The important lesson for keeping zebra finches in captivity, is that colour-banding for identification is far from neutral in its effect on behaviour. Even banding a male with asymmetrical arrangements of multiple colours (*e.g.* orange over green on the left leg, green over orange on the right) will reduce his attractiveness to potential sexual partners (Swaddle & Cuthill 1994). If birds have to be banded for identification, it is best to use colours which have less impact on social interactions (see Burley *et al.* 1982, Burley 1986a) and/or to provide multiple food sources so that dominant birds cannot monopolize the resource (see Cuthill *et al.* 1997).

References

Andersson M, Nordin E, Jensen P (2001) Domestication effects on foraging strategies in fowl. *Applied Animal Behaviour Science* **72**, 51-62

Anonymous[1] (2001) Scientists' assessment of the impact of housing and management on animal welfare. *Journal of Applied Animal Welfare Science* **4**, 3-52

Ashton WLG, Pattison M, Barnett KC (1973) Light-induced eye abnormalities in turkeys and the turkey blindness syndrome. *Research in Veterinary Science* **14**, 42-46

Association of Avian Veterinarians (1999) *Basic pet bird care.* **www.aav.org/basic_care.html**

Bates HJ, Busenbark RL (1970) *Finches and Soft-Billed Birds.* Neptune City, New Jersey: TFH Publications Inc

Baxter MR (1994) The welfare problems of laying hens in battery cages. *Veterinary Record* **134**, 614-619

Bell DD, Adams CJ (1998) Environmental enrichment devices for caged laying hens. *Journal of Applied Poultry Research* **7**, 19-26

Bennett ATD, Cuthill IC (1994) Ultraviolet vision in birds: What is its function? *Vision Research* **34**, 1471-1478

Bennett ATD, Cuthill IC, Partridge JC, Lunau K (1997) Ultraviolet plumage colors predict mate preferences in starlings. *Proceedings of the National Academy of Science USA* **94**, 8618-8621

Bent AC (1963) *Life Histories of North American Gallinaceous Birds.* New York: Dover Publications, Inc

Berk J (1999) Influence of stocking density and environmental enrichment on behaviour and productivity by male, domestic turkeys. In: *33rd International Congress of the International Society for Animal Ethology* (Boe KE, M Bakken, BBO Bakken, eds). Ås, Norway: Agricultural University of Norway, p 197

Bilcik B, Keeling LJ (2000) Relationship between feather pecking and ground pecking in laying hens and the effect of group size. *Applied Animal Behaviour Science* **68**, 55-66

Blokhuis HJ (1986) Feather pecking in poultry: its relation to ground pecking. *Applied Animal Behaviour Science* **16**, 63-67

Blokhuis HJ (1989) The effect of a sudden change in floor type on pecking behaviour in chicks. *Applied Animal Behaviour Science* **22**, 65-76

Blokhuis HJ, Jones RB, de Jong IC, Keeling L, Preisinger R (2001) *Feather pecking: Solutions through understanding.* European Commission, RTD contract FAIR 5-CT97-3576

Bowmaker JK, Heath LA, Wilkie SE, Hunt DM (1997) Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research* **37**, 2183-2194

Buchwalder T, Wechsler B (1997) The effect of cover on the behaviour of Japanese quail (*Coturnix japonica*). *Applied Animal Behaviour Science* **54**, 335-343

Burley N (1985) Leg-band colour and mortality patters in captive breeding populations of zebra finches *Auk* **102**, 647-51

Burley N (1986a) Comparison of the band-colour preferences of two species of estrildid finches. *Animal Behaviour* **34**, 1732-41

Burley N (1986b) Sex-ration manipulation in colour-banded populations of zebra finches. *Evolution* **40**, 1191-206

Burley N (1986c) Sexual selection for aesthetic traits in species with biparental care. *American Naturalist* **127**, 415-45

Burley N (1988) The differential-allocation hypothesis and experimental test. *American Naturalist* **132,** 611-28

Burley N, Krantzberg G, Radman P (1982) Influence of colour-banding on the conspecific preferences of zebra finches. *Animal Behaviour* **30**, 444-55

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[1] This paper used the 'Delphi' method to define a framework for the assessment of farm animal welfare, making the assumption that welfare problems arise when animals are housed in environments to which they have difficulty adapting. A total of 22 welfare scientists from the Netherlands, Canada, Denmark, Italy, Sweden, the UK and France contributed to the finished article.

Butler PJ (1982) Respiratory and cardiovascular control during diving in birds and mammals. *Journal of Experimental Biology* **100**, 195-221

Butler PJ, Taylor EW (1983) Factors affecting the respiratory and cardiovascular responses to hypercapnic hypoxia, in mallard ducks. *Respiration Physiology* **53**, 109-127

CCAC (1984) *Guide to the care and use of laboratory animals. Volume 2.* Ottawa: Canadian Council on Animal Care

Channing CE, Hughes BO, Walker AW (2001) Spatial distribution and behaviour of laying hens housed in an alternative system. *Applied Animal Behaviour Science* **72**, 335-345

Clarkson TB, Prichard RW, Lofland HB, Goodman HO (1963) The pigeon as a laboratory animal. *Laboratory Animal Care* **16**, 767

Classen HL (1992) Management factors in leg disorders. In: *Poultry Science Symposium No. 23: Bone biology and skeletal disorders in poultry* (Whitehead CC ed.), Carfax Publishing Company, pp 195-211

Close B, Banister K, Baumans V, Bernoth E-M, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C (1997) Recommendations for euthanasia of experimental animals: Part 2. *Laboratory Animals* **31**, 1-32

Coles BH (1991) Cage and aviary birds. In: *Manual of Exotic Pets* (Beynon PH, Cooper JE, eds), Cheltenham, UK: British Small Animal Veterinary Association, pp 150-179

Cooper JJ, Appleby MC (1994) The use of aversive barriers to quantify nesting motivation in domestic hens. In: *Modified Cages for Laying Hens* (Sherwin CM ed.), Potters Bar: UFAW, pp 11-26

Cordiner LS, Savory CJ (2001) Use of perches and nestboxes by laying hens in relation to social status, based on examination of consistency of ranking orders and frequency of interaction. *Applied Animal Behaviour Science* **71**, 305-317

Cornetto T, Estevez I (1999) Utilizing artificial cover to improve use of pen center by domestic fowl. In: *33rd International Congress of the International Society for Animal Ethology* (Boe KE, M Bakken, BBO Bakken, eds). Ås, Norway: Agricultural University of Norway, p 57

Cornetto T, Estevez I, Douglass LW (2002) Using artificial cover to reduce aggression and disturbances in domestic fowl. *Applied Animal Behaviour Science* **75**, 325-336

Coulton LE, Waran NK, Young RJ (1997) Effects of foraging enrichment on the behaviour of parrots. *Animal Welfare* **6**, 357-363

Council of Europe (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. ETS123* Strasbourg: Council of Europe

Craig JV, Muir WM (1993) Selection for reduction of beak-inflicted injuries among caged hens. *Poultry Science* **72**, 411-420

Crowe R, Forbes JM (1999) Effects of four different environmental enrichment treatments on pecking behaviour in turkeys. *British Poultry Science* **40 (Suppl.)**, 11-12

Cuthill IC, Hunt S, Cleary, Clark C (1997) Colour bands, dominance, and body mass regulation in male zebra finches (*Taeniopygia guttata*). Proceedings of the Royal Society of London Series B, Biological Sciences **264**, 1093-9

Danbury TC, Weeks CA, Chambers JP, Waterman-Pearson AE, Kestin SC (2000) Self-selection of the analgesic drug Carprofen by lame broiler chickens. *Veterinary Record* **146**, 307-311

Davis GS, Siopes TD, Peiffer RL, Cook C (1986) Morphologic changes induced by photoperiod in eyes of turkey poults. *American Journal of Veterinary Research* **47**, 953-955

Dawkins MS, Hardie S (1989) Space needs of laying hens. *British Poultry Science* **30**, 418-416

de Jong I, Korte M, van Hierden Y, Ruesink W, Jones B, Blokhuis H (2001) Physiological and behavioural characteristics of birds showing high or low feather pecking. In: *Feather pecking: Solutions through understanding* (Blokhuis HJ, Jones RB, de Jong IC, Keeling L, Preisinger R, eds), European Commission, RTD contract FAIR 5-CT97-3576, pp 7-11

Dooling RJ (1992) Hearing in birds. In: *The Evolutionary Biology of Hearing*, Chapter 26 (Webster DB, Fay RR, Popper AN, eds), New York: Springer-Verlag

Duncan ET, Appleby MC, Hughes BO (1992) Effect of perches in laying cages on welfare and production of hens. *British Poultry Science* **33**, 25-35

Duncan IJH (1999) The domestic fowl. In: *The UFAW Handbook on the Care and Management of Laboratory Animals (7th Edition). Volume 1: Terrestrial vertebrates* (Poole T, ed*)*, London: Blackwell Science, pp 677-696

Duncan IJH, Hughes BO (1972) Free and operant feeding domestic fowls. *Animal Behaviour* **20**, 775-777

Duncan IJH, Slee GS, Seawright E, Breward J (1989) Behavioural consequences of partial beak amputation (beak trimming) in poultry. *British Poultry Science* **30**, 479-488

Duncan IJH, Wood-Gush DGM (1972) Thwarting of feeding behaviour in the fowl. *Animal Behaviour* **20**, 444-451

Ely CR (1993) Family stability in greater white-fronted geese. *Auk* **110**, 425-435

Elzanowski A (1991) Motivation and subjective experience in birds. *Acta XX Congressus Internationalis Ornithologici.* New Zealand Ornithological Congress Trust Board, pp 1921-1929

Ernst RA, Coleman TH (1966) The influence of floor space on growth, egg production, fertility and hatchability of *Coturnix japonica*. *Poultry Science* **45**, 437 - 440

Farm Animal Welfare Council (1993) *Second report on priorities for research and development in farm animal welfare.* Tolworth: Ministry of Agriculture, Fisheries and Food, PB1310, pp 3-4

Farm Animal Welfare Council (1995) *Report on the Welfare of Turkeys.* Tolworth: FAWC

Farm Animal Welfare Council (1997) *Report on the welfare of laying hens.* Tolworth: FAWC

Fisher J, Hinde RA (1949) The opening of milk bottles by birds. *British Birds* **42**, 347-357

Follett BK (1984) Birds. In: *Marshall's Physiology of Reproduction, Volume 1: Reproductive Cycles of Vertebrates.* London: Churchill Livingstone

Fölsch DW, Höfner M, Staack M, Trei G (2002) Comfortable quarters for chickens in research institutions. In: *Comfortable quarters for laboratory animals*, 9th edn (Reinhardt V, Reinhardt A, eds). Washington DC: Animal Welfare Institute, http://www.awionline.org/pubs/cq02/Cq-chick.html

Forbes NA, Richardson T (1996) Waterfowl: Husbandry and nutrition. In: *Manual of Raptors, Pigeons and Waterfowl* (Beynon PH, Forbes NA, Harcourt-Brown NH, eds), Cheltenham: British Small Animal Veterinary Association, pp 116-128

Fowler ME (1995) *Restraint and handling of wild and domestic animals (2nd Edition)*. Iowa: Iowa State University Press

Freire R, Appleby MC, Hughes BO (1997) Assessment of pre-laying motivation in the domestic hen using social interaction. *Animal Behaviour* **54**, 313-319

Freire R, Mendl M, Nicol CJ (1997) Object permanence in domestic hens: Visible displacements. *Proceedings of the 31st International Congress of the International Society for Applied Ethology* (Hemsworth PH, Spinka M, Kostal L, eds), p 146

Fritz J, Kotrschal K (1999) Social learning in common ravens, *Corvus corax*. *Animal Behaviour* **57**, 785-793

Gentle MJ (1991) Behavioural and physiological responses to pain in the chicken. *Acta XX Congressus Internationalis Ornithologici.* New Zealand Ornithological Congress Trust Board, pp 1915-1920

Gentle MJ (1971) Taste and its importance to the domestic chicken. *British Poultry Science* **12**, 77-86

Gentle MJ (1992) Pain in birds. *Animal Welfare* **1**, 235-247

Gentle MJ (2001) Attentional shifts alter pain perception in the chicken. *Animal Welfare* **10**, S187-94

Gentle MJ, Corr SA (1995) Endogenous analgesia in the chicken. *Neuroscience letters* **201**, 211-214

Gentle MJ, Tilston VL (1999) Reduction in peripheral inflammation by changes in attention. *Physiology and Behaviour* **66**, 289-292

Gentle MJ, Waddington D, Hunter LN, Jones RB (1990) Behavioural evidence for persistent pain following partial beak amputation in chickens. *Applied Animal Behaviour Science* **27**, 149-157

Gerken M (1983) *Untersuchungen zur gnetischen Fundierung und Beeinflubarkeit von Verhaltensmerkmalen des Geflügels, durchgeführt in einem Selektionexperiment auf Staubbadedeverhalten bei der japenischen Wachtel (*Coturnix coturnix japonica*).* Unpublished PhD thesis, Rheinsche Freidrich Wilhelms Universität, Bonn.

Gerken M, Mills AD (1993) Welfare of domestic quail. In: *Proceedings of the 4th European Symposium on Poultry Welfare* (Savory CJ, Hughes BO, eds), Potters Bar: UFAW

Goudie RI, Ankney CD (1986) Body size, activity budgets, and diets of sea ducks wintering in Newfoundland. *Ecology* **67**, 1475-1482

Green LE, Lewis K, Kimpton A, Nicol CJ (2000) Cross-sectional study of the prevalence of feather pecking in laying hens in alternative systems and its associations with management and disease. *Veterinary Record* **147**, 233-238

Grigor PN, Hughes BO, Appleby MC (1995) Effects of regular handling and exposure to an outside area on subsequent fearfulness and dispersal in domestic hens. *Applied Animal Behaviour Science* **44**, 47-55

Gunnarsson S, Matthews LR, Foster TM, Temple W (2000) The demand for straw and feathers as litter substrates by laying hens. *Applied Animal Behaviour Science* **65**, 321-330

Gunnarsson S, Yngvesson J, Keeling LJ, Forkman B (1999) Early access to perches enhances spatial awareness in the domestic hen. In: *33rd International Congress of the International Society for Animal Ethology* (Boe KE, M Bakken, BBO Bakken, eds). Ås, Norway: Agricultural University of Norway, p 62

Hansen I, Braastad BO, Storbråten J, Tofastrud M (1993) Differences in fearfulness indicated by tonic immobility between laying hens in aviaries and in cages. *Animal Welfare* **2**, 105-112

Harper FDW (1996) Pigeons: husbandry and nutrition. In: *Manual of Raptors, Pigeons and Waterfowl* (Beynon PH, Forbes NA, Harcourt-Brown NH, eds). Cheltenham: British Small Animal Veterinary Association, pp 233-237

Hatch KK, Lefebvre L (1997) Does father know best? Social learning from kin and non-kin in juvenile ringdoves. *Behavioural Processes* **41**, 1-10

Hawes RO (1984) Pigeons. In: *Evolution of domesticated animals* (Mason IL, ed), London: Longman

Hawkins P (1998) Environmental stimulation for waterfowl: the common eider duck. *Animal Technology* **49**, 91-99

Hawkins P, Morton DB, Cameron D, Cuthill I, Francis R, Freire R, Gosler A, Healy S,Hudson A, Inglis I, Jones A, Kirkwood J, Lawton M, Monaghan P, Sherwin C, Townsend P (2001) Laboratory birds: Refinements in husbandry and procedures. *Laboratory Animals* **35 Suppl. 1**, 1-163

Heffner HE (1998) Auditory awareness. *Applied Animal Behaviour Science* **57**, 259-268

Hodgetts B (1999) Quail production. In: *Management and Welfare of Farm Animals: The UFAW Farm Handbook (4th Edition)* (Ewbank R, Kim-Madslien F, Hart CB eds), Wheathampstead: UFAW, pp 269-271

Höfner M, Staack M, Fölsch DW (1997) Comfortable quarters for chickens. In: *Comfortable Quarters for Laboratory Animals (8th Edition)* (Reinhardt V ed.), Washington: Animal Welfare Institute

Home Office (1989) *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures.* London: HMSO

Huber L (1994) Amelioration of laboratory conditions for pigeons (*Columba livia*). *Animal Welfare* **3**, 321-324

Huber-Eicher B, Sebö F (2001) Reducing feather pecking when raising laying hen chicks in aviary systems. *Applied Animal Behaviour Science* **73**, 59-68

Hughes BO, Channing CE (1998) Effect of restricting access to litter trays on their use by caged laying hens. *Applied Animal Behaviour Science* **56**, 37-45

Hutchison RE (1999) Doves and pigeons. In: *The UFAW Handbook on the Care and Management of Laboratory Animals (7th Edition). Volume 1: Terrestrial vertebrates* (Poole T, ed), London: Blackwell Science, pp 714-721

Ito S, Tanaka T, Yoshimoto T (1999) Comparison of behaviour of commercial hens and Gifu native fowl. In: *33rd International Congress of the International Society for Animal Ethology* (Boe KE, M Bakken, BBO Bakken, eds). Ås, Norway: Agricultural University of Norway, p 164

Jacobs H, Smith N, Smith P, Smyth L, Yew P, Saibaba P, Hau J (1995) Zebra finch behaviour and effect of modest enrichment of standard cages. *Animal Welfare* **4**, 3-9

Jaksch W (1981) Euthanasia of day-old male chicks in the poultry industry. *International Journal for the Study of Animal Problems* **2**, 203-213

Johnson DB, Guthery FS (1988) Loafing coverts used by northern bobwhites in subtropical environments. *Journal of Wildlife Management* **52**, 464-469

Johnston ANB, Burne THJ, Rose SPR (1998) Observation learning in day-old chicks using a one-trial passive avoidance learning paradigm. *Animal Behaviour* **56**, 1347-1353

Jones AE, Slater PJB (1999) The zebra finch. In: *The UFAW Handbook on the Care and Management of Laboratory Animals Volume 1: Terrestrial Vertebrates,* 7th edn (Poole T, ed). London: Blackwell Science, pp 722-30

Jones DR (1976) The control of breathing in birds with particular reference to the initiation and maintenance of diving apnea. *Federation Proceedings* **35**, 1975-1982

Jones RB (1994) Regular handling and the domestic chick's fear of human beings – generalisation of response. *Applied Animal Behaviour Science* **42**, 129-143

Jones RB (2001) Developing environmental enrichment devices to reduce inter-bird pecking. In: *Feather pecking: Solutions through understanding* (Blokhuis HJ, Jones RB, de Jong IC, Keeling L, Preisinger R, eds), European Commission, RTD contract FAIR 5-CT97-3576, pp 13-16

Jones RB, Carmichael NL (1998) Pecking at string by individually caged, adult laying hens: colour preferences and their stability. *Applied Animal Behaviour Science* **60**, 11-23

Jones RB, Carmichael NL, Blokhuis HJ (1997) Domestic chicks' initial reactions to pecking devices made of feathers or string. *Poultry Science* **76 (S1)**, 127

Jones RB, Carmichael NL, Rayner E (2000) Pecking preferences and pre-dispositions in domestic chicks: implications for the development of environmental enrichment devices. *Applied Animal Behaviour Science* **69**, 291-312

Jones RB, Mills AD, Faure J-M (1991) Genetic and experimental manipulation of fear-related behavior in Japanese quail chicks (*Coturnix coturnix japonica*). *Journal of Comparative Psychology* **105**, 15-24

Kahlert H, Fox AD, Ettrup H (1996) Nocturnal feeding in moulting greylag geese *Anser anser* - an anti-predator response? *Ardea* **84**, 15-22

Kawahara T (1967) Wild *Coturnix* quail in Japan. *Quail Quarterly* **4**, 62-63

Keeling LJ (1997) A comparison of two basic characteristics of a perch for laying hens. In: *Proceedings of the 31st International Congress of the International Society of Applied Ethology, Prague, 13-16 August 1998*

Keeling LJ, Duncan IJH (1989) Interindividual distances and orientation in laying hens housed in groups of three in two differently sized enclosures. *Applied Animal Behaviour Science* **24**, 325-342

Keiper RR (1969) Causal factors of stereotypies in caged birds. *Animal Behaviour* **17**, 114-119

Keppler C, Fölsch DW (2000) Locomotive behaviour of hens and cocks (*Gallus gallus f. domesticus*): Implications for housing systems. *Archiv für Tierschutz* **43**, 184-88

Kestin SC, Su G, Sørensen P (1999) Different commercial broiler crosses have different susceptibilities to leg weakness. *Poultry Science* **78**, 1085-1090

King CE (1993) Environmental enrichment: Is it for the birds? *Zoo Biology* **12**, 509-512

Kirkwood JK (1996) Nutrition of captive and freeliving wild animals. In: *Manual of Companion Animal Feeding and Nutrition* (Kelly J, Wills J, eds). Cheltenham: British Small Animal Veterinary Association, pp 235-43

Kirkwood JK (1999a) Introduction to birds. In: *The UFAW Handbook on the Care and Management of Laboratory Animals (7th Edition). Volume 1: Terrestrial vertebrates* (Poole T, ed), London: Blackwell Science, pp 661-669

Kirkwood JK (1999b) Design of accommodation for wild animals: How do we know when we have got it right? *Fifth International Zoo Design Conference*, Paignton Zoological and Botanical Gardens, 18-22 May 1998 (Plowman A, Stevens P eds), Whitley Wildlife Protection Trust, Paignton, Devon, pp 51-61

Kirkwood JK (1996) Nutrition of captive and free-living wild animals. In: *Manual of Companion Animal Feeding and Nutrition* (Kelly J, Wills J eds), Cheltenham: British Small Animal Veterinary Association, pp 235-243

Kirkwood JK, Duignan P, Kember NF, Bennett PM, Price D (1989) The growth of the tarsometatarsus bone in birds. *Journal of Zoology, London* **217**, 403-416

Kjaer JB and Sorensen P (1997) Feather pecking behaviour in White Leghorns, a genetic study. *British Poultry Science* **38**, 333-341

Knowles TG, Broom DM (1990) Limb bone strength and movement in laying hens from different housing systems. *Veterinary Record* **126**, 354-356

Kreithen ML, Quine DB (1979) Infrasound detection by the homing pigeon: a behavioral audiogram. *Journal of Comparative Physiology* **129**, 1-4

Lambe NR, Scott GB (1998) Perching behaviour and preferences for different perch designs among laying hens. *Animal Welfare* **7**, 203-216

Lambooij E, Gerritzen MA, Engel B, Hillebrand SJW, Lankhaar J, Pieterse C (1999) Behavioural responses during exposure of broiler chickens to different gas mixtures. *Applied Animal Behavioural Science* **62**, 255-265

Laule G (1999) Training laboratory animals. In: *The UFAW Handbook on the Care and Management of Laboratory Animals (7th Edition) Volume 1: Terrestrial vertebrates* (Poole T, ed). Wheathampstead: UFAW, pp 21-27

Leach MC, Bowell VA, Allan TF, Morton DB (2002) Degrees of aversion shown by rats and mice to different concentrations on inhalational anaesthetics. *Veterinary Record* **150**, 808-815

Lewis PD, Perry GC, Sherwin CM, Moinard C (2000) Effect of ultraviolet radiation on the performance of intact male turkeys. *Poultry Science* **79**, 850-855

Lindberg AC (1999) Effects of vacuum and real dustbathing bouts on dustbathing motivation in domestic hens. In: *33rd International Congress of the International Society for Animal Ethology* (Boe KE, M Bakken, BBO Bakken, eds). Ås, Norway: Agricultural University of Norway, p 91

Lindberg AC, Nicol CJ (1996) Space and density effects on group size preferences in laying hens. *British Poultry Science* **37**, 709-721

Lint KC, Lint AM (1981) *Diets for Birds in Captivity.* Poole: Blandford Press

Manning A, Stamp Dawkins M (1998) *An Introduction to Animal Behaviour (5th Edition).* Cambridge: Cambridge University Press

Manser CE (1992) *The Assessment of Stress in Laboratory Animals.* Horsham: RSPCA

Manser CE (1996) Effects of lighting on the welfare of domestic poultry: a review. *Animal Welfare* **5**, 341-360

Marler P (1996) Social cognition: Are primates smarter than birds? In: *Current Ornithology Volume 13* (Nolan V, Ketterson ED eds), New York: Plenum Press

Mason GJ (1991) Stereotypy: a critical review. *Animal Behaviour* **41**, 1015-1-38

McAdie T, Keeling L (2002) Effects of manipulating feathers of laying hens on the incidence of feather pecking an cannibalism. *Applied Animal Behaviour Science* **68**, 215-229

McBride G, Parer IP, Foenander F (1969) The social organisation and behaviour of feral domestic fowl. *Animal Behaviour Monographs* **2**, 127-181

McFarland D (1993) *Animal Behaviour.* Harlow: Longman Scientific and Technical

McGeown D, Danbury TC, Waterman-Pearson AE, Kestin SC (1999) Effect of Carprofen on lameness in broiler chickens. *Veterinary Record* **144**, 668-671

Meijer T, Rozman J, Schulte M, Stach-Dreesmann C (1996) New findings in body mass regulation in zebra finches (*Taeniopygia guttata*) in response to photoperiod and temperature. *Journal of Zoology* **240,** 717-34

Mills AD, Crawford LL, Domjan M, Faure JM (1997) The behaviour of the Japanese or domestic quail. *Neuroscience and Biobehavioural Reviews* **21**, 261-281

Mills AD, Faure JM, Rault P (1999) The Japanese quail. In: *The UFAW Handbook on the*

Care and Management of Laboratory Animals (7th Edition). Volume 1: Terrestrial Vertebrates (Poole T ed.), London: Blackwell Science, pp 697-713

Moinard C, Sherwin CM (1999) Turkeys prefer fluorescent light with supplementary radiation. *Applied Animal Behaviour Science* **64**, 261-267

Murphy LB, Duncan IJH (1978) Attempts to modify the responses of domestic fowl towards human beings. II. The effect of early experience. *Applied Animal Ethology* **4**, 5-12

Neiworth JJ, Rilling ME (1987) A method for studying imagery in animals. *Journal of Experimental Psychology* **13**, 203-214

Nepote K (1999) Pigeon housing: Practical considerations and welfare implications. *Lab Animal* **28**, 34-37

Newberry RC (1999) Exploratory behaviour of young domestic fowl. *Applied Animal Behaviour Science* **63**, 311-321

Newberry RC, Estevez I, Keeling LJ (2001) Group size and perching behaviour in young domestic fowl. *Applied Animal Behaviour Science* **73**, 117-129

Newberry RC, Shackleton DM (1997) Use of visual cover by domestic fowl: a Venetian blind effect? *Animal Behaviour* **54**, 387-395

Nicol CJ (1995) Environmental enrichment for birds. In: *Environmental Enrichment Information Resources for Laboratory Animals 1965-1995*. Maryland: AWIC, pp 1-3

Nicol CJ, Lindberg AC, Phillips AJ, Pope SJ, Wilkins LJ, Green LE (2001) Influence of prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. *Applied Animal Behaviour Science* **73**, 141-155

Nicol CJ, Pope SJ (1996) The maternal feeding display of domestic hens is sensitive to perceived chick error. *Animal Behaviour* **52**, 767-774

Nicol CJ, Pope SJ (1999) The effects of demonstrator social status and prior foraging success on social learning in laying hens. *Animal Behaviour* **57**, 163-171

Olsson IAS, Keeling LJ (2000) Night-time roosting in laying hens and the effect of thwarting access to perches. *Applied Animal Behaviour Science* **68**, 243-256

Olsson IAS, Keeling LJ (2001) The push-door for measuring motivation in hens: laying hens are motivated to perch at night. Ch. II in: *Motivation in laying hens: studies of perching and dustbathing behaviour*. Uppsala: Swedish University of Agricultural Sciences, Doctoral Thesis by Anna Olsson

Ottinger MA, Rattner BA (1999) Husbandry and care of quail. *Poultry and Avian Biology Reviews* **10**, 117-120

Owen M, Black J (1990) *Waterfowl Ecology.* New York: Chapman & Hall

Paulus, SL (1988) Time-activity budgets of nonbreeding Anatidae: A review. In: *Waterfowl in Winter* (Weller MW, ed), Minneapolis: University of Minnesota Press

Pepperberg IM (1994) Evidence for numerical competence in an African Grey parrot. *Journal of Comparative Psychology* **108**, 36-44

Pepperberg IM, Funk MS (1990) Object permanence in four species of psittacine birds. *Animal Learning and Behaviour* **18**, 97-108

Pettit-Riley R, Estevez I (2001) Effects of density on perching behaviour of broiler chickens. *Applied Animal Behaviour Science* **71**, 127-140

Poole T, Stamp Dawkins MS (1999) Environmental enrichment for vertebrates. In: *The UFAW Handbook on the Care and Management of Laboratory Animals (7th Edition) Volume 1: Terrestrial Vertebrates* (Poole T, ed), London: Blackwell Science, pp 13-20

Preisinger R (2001) Recommendations for the future from a breeder's perspective. In: *Feather pecking: Solutions through understanding* (Blokhuis HJ, Jones RB, de Jong IC, Keeling L, Preisinger R, eds), European Commission, RTD contract FAIR 5-CT97-3576, pp 21-25

Raj ABM (1996) Aversive reactions of turkeys to argon, carbon dioxide and a mixture of carbon dioxide and oxygen. *Veterinary Record* **138**, 592–593

Redfern CPF, Clark JA (eds) (2001) *Ringers' Manual (4th Edition).* Thetford: British Trust for Ornithology

Redig PT (1996) Nursing avian patients. In *Manual of Raptors, Pigeons and Waterfowl* (Beynon PH, Forbes NA, Harcourt-Brown NH eds), Cheltenham: British Small Animal Veterinary Association, pp 42-46

Rietveld-Piepers B, Blokhuis HJ, Wiepkema PR (1985) Egg-laying behaviour and nest-site selection of domestic hens kept in small floor-pens. *Applied Animal Behaviour Science* **14**, 75-88

Ristau CA (1991) Aspects of the cognitive ethology of an injury-feigning bird, the piping plover. In: *Cognitive Ethology, Chapter 5* (Ristau CA, ed). Mahwah, New Jersey: Lawrence Erlbaum Associates, pp 91-126

Robinson I (1996) Waterfowl: feathers and skin. In: *Manual of Raptors, Pigeons and Waterfowl* (Beynon PH, Forbes NA, Harcourt-Brown NH, eds). Cheltenham: British Small Animal Veterinary Association, pp 305-10

Royal Society for the Prevention of Cruelty to Animals (1997) *Welfare Standards for Turkeys.* Horsham: RSPCA

Royal Society for the Prevention of Cruelty to Animals (1999a) *Welfare Standards for Ducks.* Horsham: RSPCA

Royal Society for the Prevention of Cruelty to Animals (1999b) *Welfare Standards for Laying Hens and Pullets.* Horsham: RSPCA

Sanotra GS (2000) Leg problems in broilers: A survey of conventional production systems in Denmark. Dyrenes Beskyttelse, Alhambravej 15, 1826 Frederiksberg C, Denmark

Saunders DK, Fedde MR (1994) Exercise performance of birds. *Advances in Veterinary Medicine and Comparative Medicine* **38B**, 139-190

Schmid I, Wechsler B (1997) Behaviour of Japanese quail kept in semi-natural aviary conditions. *Applied Animal Behaviour Science* **55**, 103-112

Schmorrow DD, Ulrich RE (1991) Improving the housing and care of laboratory pigeons and rats. *Humane Innovations and Alternatives* **5**, 299-304

Schorger AW (1966) *The Wild Turkey.* Norman: University of Oklahoma Press

Schütz KE, Forkman B, Jensen P (2001) Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (*Gallus gallus*) and a modern layer strain. *Applied Animal Behaviour Science* **74**, 1-14

Seastedt TR, Maclean SF Jr (1977) Calcium supplements in the diet of nestling Lapland longspurs (*Calcarius lapponicus*) near Barrow, Alaska. *Ibis* **119**, 531-533

Sedinger JS (1992) Ecology of prefledging waterfowl. In: *Ecology and Management of Breeding Waterfowl* (Batt BDJ, Afton AJ, Anderson MG, Ankney CD, Johnson DH, Kadlec JA, Krapu GL, eds), Minneapolis: University of Minnesota Press

Sherry DF (1977) Parental food-calling and the role of the young in the Burmese red junglefowl (*Gallus gallus spadiceus*). *Animal Behaviour* **25**, 594-601

Sherry DF, Galef BG (1990) Social learning without imitation: More about milk bottle opening by birds. *Animal Behaviour* **40**, 987-989

Sherwin CM (1993) Pecking behaviour of laying hens provided with a simple motorised environmental enrichment device. *British Poultry Science* **34**, 235-240

Sherwin CM (ed) (1994) *Modified Cages for Laying Hens.* Potters Bar: UFAW

Sherwin CM (1995) Environmental enrichment for laying hens – spherical enrichment objects in the feed trough. *Animal Welfare* **4**, 41-51

Sherwin CM (1998) Light intensity preferences of male domestic turkeys. *Applied Animal Behaviour Science* **58**, 121-130

Sherwin CM (1999) Domestic turkeys are not averse to compact fluorescent lighting. *Applied Animal Behaviour Science* **64**, 47-55

Sherwin CM, Devereux CL (1999) Preliminary investigations of ultraviolet-induced markings on domestic turkey chicks and a possible role in injurious pecking. *British Poultry Science* **40**, 429-433

Sherwin CM, Kelland A (1998) Time-budgets, comfort behaviours and injurious pecking of turkeys housed in pairs. *British Poultry Science* **39**, 325-332

Sherwin CM, Lewis PD, Perry GC (1999a) Effects of environmental enrichment, fluorescent and intermittent lighting on injurious pecking among turkey poults. *British Poultry Science* **40**, 592-598

Sherwin CM, Lewis PD, Perry GC (1999b) The effects of environmental enrichment and intermittent lighting on the behaviour and welfare of male domestic turkeys. *Applied Animal Behaviour Science* **62**, 319-333

Sherwin CM, Nicol CJ (1993) Factors influencing floor-laying by hens in modified cages. *Applied Animal Behaviour Science* **36**, 211-222.

Siopes TD, Timmons MB, Baughman GR, Parkhurst CR (1984) The effects of light intensity on turkey poult performance eye morphology and adrenal weight. *Poultry Science* **63**, 904-909

Skutch AF (1996) *The Minds of Birds.* Texas: Texas A&M University Press

Stokes AW (1971) Parental and courtship feeding in Red jungle fowl. *Auk* **88**, 21-29

Stephenson R (1994) Diving energetics in lesser scaup (*Aythya affinis*, Eyton). *Journal of experimental Biology* **190**, 155-178

Su G, Sørensen P, Kestin SC (1999) Meal feeding is more effective than early feed restriction at reducing the prevalence of leg weakness in broiler chickens. *Poultry Science* **78**, 949-955

Swaddle JP, Cuthill IC (1994) Preference for symmetrical males by female zebra finches. *Nature* **367**, 165-6

Tolman CW (1964) Social facilitation of feeding behaviour in the domestic chick. *Animal Behaviour* **12**, 245-251

Tolman CW (1967) The feeding behaviour of domestic chicks as a function of rate of pecking by a surrogate companion. *Behaviour* **29**, 57-62

Tolman CW, Wilson GF (1965) Social feeding in domestic chicks. *Animal Behaviour* **13**, 134-142

Turner ERA (1964) Social feeding in birds. *Behaviour* **24**, 1-46

Vallortigara G, Regolin L, Rigoni M, Zanforlin M (1998) Delayed search for a concealed imprinted object in the domestic chick. *Animal Cognition* **1**, 17-24

Van Hoek CS, King CE (1997) Causation and influence of environmental enrichment on feather pecking of the crimson-bellied conure (*Pyrrhura perlata perlata*). *Zoo Biology* **16**, 161-172

Watts CR, Stokes AW (1971) The social order of turkeys. *Scientific American* **224**, 112-118

Webster AB, Fletcher DL (2001) Reactions of laying hens and broilers to different gases used for stunning poultry. *Poultry Science* **80**, 1371-7

Wechsler B, Schmid I (1998) Aggressive pecking by males in breeding groups of Japanese quail (*Coturnix japonica*). *Applied Animal Behaviour Science* **39**, 333-339

Weeks CA, Danbury TC, Davies HC, Hunt P, Kestin SC (2000) The behaviour of broiler chickens and its modification by lameness. *Applied Animal Behaviour Science* **67**, 111-125

Welty JC, Baptista L (1988) *The Life of Birds (4th Edition).* New York: Saunders College Publishing

Widowski TM, Duncan IJH (2000) Working for a dustbath: are hens increasing pleasure rather than reducing suffering? *Applied Animal Behaviour Science* **68**, 39-53

Widowski TM, Duncan IJH (1992) Prefrences of hens for compact fluorescent light. *Canadian Journal of Animal Science* **72**, 203-211

Williams LE (1981) *The Book of the Wild Turkey.* Tulsa, Oklahoma: Winchester Press

Zann R (1994) Effects of band colour on survivorship, body condition and reproductive effort of free-living Australian zebra finches. *Auk* **111**, 131-42

Zann R (1996) *The Zebra finch: A Synthesis of Field and Laboratory Studies.* Oxford: Oxford University Press

Zann RA, Morton SR, Jones KR, Burley NT (1995) The timing of breeding by zebra finches in relation to rainfall in central Australia. *Emu* **95**, 208-22

Zeigler HP (1975) Trigeminal deafferentiation and hunger in the pigeon. *Journal of Comparative Physiology & Psychology* **89**, 827-844

Zimmerman PH, Koene P, van Hooff JARAM (2000) Thwarting of behaviour in different contexts and the gakel-call in the laying hen. *Applied Animal Behaviour Science* **69**, 255-264

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Cats

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)

6th meeting
Strasbourg, 25-27 March 2003

Species specific provisions for cats

Background information for the proposals presented by the Group of Experts on dogs and cats

PART B

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Species-specific sections – Cats

Preamble

In 1997, the Council of Europe (CoE) established four Expert Groups in order to advise the CoE Working Party on revisions to Appendix A of the Convention ETS123 (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 1986). One of these Expert Groups was established to consider proposals for dogs and cats; its remit was later extended to include ferrets when the CoE Working Party decided that other species covered by ETS123 should be included in the revision. Organisations represented both on the Group and at its meetings were:

Federation of European Laboratory Animal Science Associations (FELASA) – Coordinator of Group
Eurogroup for Animal Welfare
European Federation of Pharmaceutical Industries and Associations (EFPIA)
Federation of Veterinarians in Europe (FVE)
Federation of European Laboratory Animal Breeders Associations (FELABA)
International Society for Applied Ethology (ISAE)

The members of the Expert Group on dogs, cats and ferrets met on several occasions and also exchanged information by e-mail. The Coordinator of the Expert Group, accompanied by one or more other members, attended all meetings of the Working Party in Strasbourg, in order to present the Group's proposals, discuss their content and answer questions, and refer matters back to the Group where appropriate.

The CoE Expert Group on Dogs, Cats and Ferrets has provided three separate reports, covering each of these species. Each report comes in two parts. Part A details the proposals for amendments to Appendix A, as agreed by the Working Party and including amendments to the Expert Group's original proposals as required by the Working Party. Part B provides background information for these proposals. Where possible, recommendations have been based upon scientific evidence; where this is not available, they take account of established good practice, based both on the experience of the members of the Expert Group and also on further consultations with other experts. Additional comments have been received from members of the Working Party and from a range of non-governmental organisations and individuals. These have been considered by the Expert Group and incorporated in the proposals where appropriate.

The proposals and their rationale are therefore the outcome of extensive and detailed discussions within the Group and should be regarded as expert recommendations, reflecting the scientific evidence and information on good practice available at the time. Their intention is to increase the welfare of animals used in research, taking into account the purposes for which such animals are used, which may place some constraints upon their housing and husbandry.

The Expert Group considered that, although the provisions of Appendix A formally are guidelines, its finalised proposals should be regarded as minimum requirements. Knowledge gained by further research and scientific evidence, as well as changing views on what is current 'good practice', may mean that the accommodation and care provided for animals in research in the future may vary from these proposals such that future revision of Appendix A is necessary.

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1. Introduction

The domestic cat is derived from the solitary African wild cat (*Felis silvestris libyca*), but has a strong tendency to learn social behaviour. With appropriate socialisation provided at an early age, such behaviour can be expressed both to conspecifics and man.
Good social interactions with humans encourage suitable temperament for subsequent studies. However, as cats lack dominance hierarchies and appear to lack mechanisms for reconciliation post-conflict, forming social relationships may be stressful. Visible signs that cats are stressed are not as straightforward to interpret as are those in dogs.
As cats are territorial and will become attached to particular locations they are likely to be stressed by relocation. Cats are excellent climbers and utilise raised structures (e.g. shelves) extensively, both as vantage points and, when group housed, to maintain distance from other cats.

2. The environment and its control

2.1. Ventilation (*See item 2.1. of the General section of Appendix A*)

Background: The number of air changes per hour in the European (15-20), UK (10-12) and US (10-15) guidelines differ to some extent, although no evidence is offered to support the specific ranges. All guidelines allow for lower ventilation rates if stocking density is low. These factors were taken into account in drafting the General Section of Appendix A.

2.2. Temperature

A temperature range of 15 to 21° should be maintained when precise control is required for cats under procedure**.** In all other circumstances, cats may be maintained within a wider temperature range provided that their welfare is not compromised.
As kittens have limited thermoregulatory control for around the first ten days of life, additional local heating should be provided during this period.

Background: The range of temperatures given in the European (15-21°C), UK (15-24) and US (18-29) guidelines differ. Narrow ranges aim to avoid wide fluctuations of the ambient temperature so as to reduce experimental variation, although there is no evidence that a temperature outside of the least stringent range would impact on the welfare of the cats.

It is more important that there are no abrupt changes, which would affect e.g. the respiratory system, or too wide a fluctuation, which could affect physiological parameters and interfere with certain procedures.

In view of the above, there would seem no reason to recommend a specific temperature range, except for cats undergoing procedures. Young kittens will need higher temperatures to take account of their limited thermoregulatory control (Olmstead et al, 1979).

In some cases, cats will have access to an outside run at ambient temperatures. As this could mean extremes of temperature being encountered with potential impact on welfare, the cats should also have access to an indoor area that allow them to exercise some control over their living environment.

Consideration of the above factors led to the Working Party agreeing the above proposal.

2.3. Humidity

It is considered unnecessary to control relative humidity, as cats can be exposed to wide fluctuations of ambient relative humidity without adverse effects.

Background: The ranges in European and UK (both 45-65% with extremes 30-70) and US (30-70%) guidelines are near identical. However, there is no evidence that a relative humidity exceeding these ranges would impact the welfare of the cats. The Working Party therefore agreed the above proposal.

N.B. The Expert Group considered there could be benefits in recording and logging Relative Humidity on a regular basis, in order to help identify any potential problems at an early stage.

2.4. <u>Lighting</u>

Holding of cats under the natural 24 hours light-dark cycle is acceptable. Where the light part of the photoperiod is provided by artificial lighting, this should be within a range of 10 to 12 hours daily.

If natural light is totally excluded, low level night lighting (5-10 lux) should be provided to allow cats to retain some vision and to take account of their startle reflex.

Background: Prolonged periods in excess of the natural dark period can stress the cats. A minimum light period should therefore be provided daily, not less than the extreme within the natural light-dark cycle throughout the seasons. In addition, sufficient lighting is essential to examine the clinical condition of the animals and allow for routine husbandry practices.

During the dark period, total darkness should be avoided. In nature total darkness does not exist and, contrary to folklore, cats cannot 'see in the dark' if there is a complete absence of any light. Furthermore, minimal night lighting is necessary to avoid a startle reflex.

Some lighting systems have been shown to be aversive to some animals, possibly because the cat's Critical Fusion Frequency is higher than humans' (Berkley, 1976), and so they may perceive light sources as flickering whereas we do not.

2.5. <u>Noise</u> (*See item 2.5. of the General section of Appendix A)*

Background: The hearing range of cats is 0.07-91 kHz, with peak sensitivity 1-40 kHz.

All existing guidelines mention that noise can be a disturbing factor or even be damaging. Unpredictable noises cause stress (Carlstead et al, 1993). The hearing range of cats is included in these recommendations to give an indication of the audio-frequencies involved. Sound-absorbing materials will help reduce the disturbance caused by husbandry-generated noise.

2.6. <u>Alarm systems</u> (*See item 2.6. of the General section of Appendix A)*

3. Health *(See items 4.1 and 4.4 of the General Section of Appendix A)*

Background: The FELASA recommendations are used as a common reference to determine the health status of animals. A minimum quarantine period of two weeks is proposed as the incubation periods of most diseases are less than two weeks and this duration also allows for additional sampling and analysis if required.

In relation to acclimatisation, a period of two weeks is common practice.

This recommendation from the Expert Group should be considered in conjunction with the information provided in the General Section.

4. Housing and enrichment

4.1. Housing

Female cats and neutered cats of both sexes are generally sociable and are commonly held in groups of up to 12. However, the establishment of groups of two or more such cats requires careful monitoring of the compatibility of all individuals in the group. Special care is needed when regrouping cats, introducing an unfamiliar cat to a group, housing unneutered males in group or maintaining cats in larger groups.

Where cats are normally group-housed, single housing may be a significant stress factor. Therefore, cats should not be single housed for more than 24 hours without justification on veterinary or welfare grounds. Single housing for more than 24 hours on experimental grounds should be determined in consultation with the animal technician and with the competent person charged with advisory duties in relation to the well-being of the animals.

Cats which are repeatedly aggressive towards other cats should be housed singly only if a compatible companion cannot be found. Social stress in all pair- or group-housed individuals should be monitored at least weekly using an established behavioural and/or physiological stress scoring system. This is especially important for unneutered males.

Females with kittens under four weeks of age or in the last two weeks of pregnancy may be housed singly. During this time, consideration should also be given to allowing females which are normally group-housed to have access to their group e.g. by connecting nesting enclosures to the group housing animal enclosures.

Early Socialisation with conspecifics and humans

The development of social behaviour in cats is profoundly affected by social experience between two and eight weeks of age. During this period it is particularly important that the cat has social contacts with other cats (e.g. litter mates) and with humans and is familiarised with environmental conditions likely to be encountered during subsequent use. Daily handling during this sensitive stage of development is a prerequisite for the social behaviour of the adult cat and it has been shown that a short period of handling even on the first day after birth is of importance as the young animals are already able to respond to scent and tactile stimulation.

All cats should have a period of play and general social interactions with humans on a daily basis, plus additional time for regular grooming. Particular attention should be paid to the social enrichment for single-housed cats by providing additional human contact.

Background: The domestic cat has evolved from an essentially solitary species, the African wildcat Felis sylvestris libyca and its capacity to live in groups is consequently limited compared to, for example, that of the domestic dog. For example, groups of cats lack distinct dominance hierarchies, and mechanisms for reconciliation post-conflict appear to be absent (van den Bos & de Cock Buning, 1994a; van den Bos, 1998). Naturally-occurring social groups generally consist of related females, and males of breeding age are essentially asocial (Kerby & Macdonald, 1988). Castrated males may form permanent amicable associations with groups of females (Bradshaw & Brown, 1996), and neutered pet cats of both sexes can co-exist amicably at densities up to at least 1 per 10 square metres (Bernstein & Strack, 1996). The criteria which determine whether one cat is likely to behave sociably towards another are not fully understood, but cats which have lived together since both were kittens (e.g. littermates) are more likely to be friendly towards one another than cats which first meet when one or both are adult (Bradshaw & Hall, 1999). Enforced proximity to unfamiliar conspecifics is a significant source of stress for many cats (Smith et al, 1994; Kessler & Turner, 1999). This will be obvious if it leads to fighting, but long-term it is more likely to lead to the most stressed individuals reducing their activity and retreating to locations which provide security, e.g. high shelves (van den Bos & de Cock Buning, 1994b). This may not be noticed by caretakers unless formal assessment of stress is

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conducted. In the absence of clear guidelines for predicting which of a candidate group of cats are likely to be socially incompatible, the welfare of group-housed cats can be protected most effectively by careful monitoring of social behaviour using a standard method (Kessler & Turner, 1997) and removing the most chronically-stressed individuals. This should ensure social harmony in groups consisting of females, young cats and/or neutered males; groups containing more than one reproductively entire male of breeding age are more difficult to monitor in this way, and are not recommended at the current state of knowledge.

Cats that are not socialised to people become highly stressed when approached by caretakers and other personnel, and this is likely to be most acute during handling (McCune, 1992; McCune, 1995a). This stress may be minimised, and possibly replaced, by positive feelings on the part of the cat, if kittens are correctly socialised to people during the primary socialisation period, which is between two and approximately eight weeks of age (McCune et al, 1995). Optimum socialisation is achieved if handling is increased from a few minutes each day to a minimum of 40 minutes per day by six weeks old, and it is most effective if performed in the presence of the mother and littermates (McCune et al, 1995).

It is generally believed that the same period is critical for socialisation to other cats, but since the litter is normally not divided or separated from the mother until towards or after the end of this time, few kittens fail to achieve minimal experience of members of their own species. However, it is known that separation from the mother at two weeks of age is detrimental to the development of social behaviour , and that hand-raised kittens are very likely to react fearfully to other cats, and to develop attachment-related behavioural disorders (Baerends-van Roon & Baerends, 1979).

The time of onset of the socialisation period is not known precisely, but may begin as soon as the appropriate senses have developed. Thus kittens begin to learn about the visual and auditory characteristics of their surroundings from about their third week of life, as their senses of vision and hearing mature (Bradshaw, 1992). Since their senses of touch and smell are already functional at birth, and are used in associative learning from the second or third day of life (Rosenblatt, 1972), it is likely that gentle handling from this age onwards is beneficial in priming socialisation, although the reaction of the mother should also be taken into account.

4.2. Enrichment

Raised, part-enclosed structures should be provided (e.g. a bed with three walls and a roof on a shelf approximately 1 metre off the floor) to give the cats a view of their surroundings and, if pair- or group-housed, the opportunity to maintain a comfortable distance from other cat(s). There should be a sufficient number of these structures to minimise competition. Structures should be distributed within the enclosure so that animals can fully use the space available.
There should also be provision for the cats to seek refuge and privacy within their own enclosure and, in particular, away from the sight of cats in other enclosures. Vertical wooden surfaces should be provided to allow claw-sharpening and scent-marking.

Pseudo-predatory and play behaviour should be encouraged. A selection of toys should be available and these should be changed on a regular basis in order to ensure ongoing stimulation and avoid familiarity, which decreases the motivation to play.

Background: The ancestral species of the domestic cat is a solitary predator, and modern cats retain both the species-typical repertoire of hunting behaviour patterns, and the motivational system which controls their expression. This is not closely controlled by appetite (Adamec, 1976), and therefore even well-fed cats, which spend much of their time sleeping, have a need to express predatory behaviour, in the form of play with prey-like objects (Hall, 1998). In the wild, cats forage for small food items, such as insects, in addition to larger prey (Fitzgerald, 1988), and it is beneficial to provide opportunities for

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simulating this type of predatory behaviour also. Pseudo-predatory behaviour should be encouraged by the provision of toys simulating mouse-sized prey (which can be included in social interactions with humans) and by feeders which require manipulation by the cat to dispense food (e.g. a clean plastic bottle containing 20-30 pieces of dry cat food, with 2-3 holes of sufficient diameter to allow the food to fall out a piece at a time when the bottle is rolled.

A further enrichment for some cats may be provided in the form of trays of growing grass, which are replaced as the grass is eaten. However, certain cats may be observed not to eat grass.

Healthy free-roaming cats never defecate in locations where food is available (Bateson & Turner, 1988), and have to overcome this natural aversion when confined. Cats which are given no option but to feed close to a litter box (e.g. when boarded in catteries) may continue to show this disinhibition after returning home, expressed as a loss of house-training (Overall, 1997). Areas for feeding and for litter trays should therefore not be less 0.5 metres apart and should not be interchanged.

Cats have a much more sensitive sense of smell than humans (Bradshaw, 1992). The olfactory environment therefore has a significance to them which people cannot directly experience. Scent-marking behaviour (e.g. object-rubbing, claw-sharpening) is an important element of social behaviour (Bradshaw & Cameron-Beaumont, in press). Sites where scent signals can be deposited and remain undisturbed, such as by cleaning procedures, for extended periods may therefore make an important contribution to social harmony in group-housed cats, although knowledge of this is currently incomplete.

A cat which is group-housed reduces its opportunities for conflict by maintaining distance from the other cats (Leyhausen, 1979), and monitoring both their location and behaviour (Smith et al, 1994). This is made easier if elevated structures are available, since these enable both spacing in three dimensions, and vantage points: such structures are used more than the floor of the enclosure (Podberscek et al, 1991;Rochlitz et al, 1998).

Well-socialised cats actively seek to engage in social interaction with people. During this they perform species-typical behaviour (e.g. tail up, rubbing, purring) indicating both that the interactions are amicable (Bradshaw & Cameron-Beaumont, in press) and that this may also be an adequate substitute for interactions with other cats.

4.3. Animal enclosures – dimensions and flooring

Enclosures, including the divisions between enclosures, should provide a robust and easily cleaned environment for the cats. Their design and construction they should seek to provide an open and light facility giving the cats comprehensive sight outside of their animal enclosure.

Background: Freedom to express a range of normal behaviours will place some demands on the structure of enclosures. Those which allow extensive visibility of the immediate surroundings will promote normal behaviours and encourage animals to take an interest in their surroundings. The ability to watch other animals and staff may also provide a useful function in the cat's time utilisation. Careful consideration must therefore be given to providing an appropriate balance between solid walls and metal bars or mesh. Glass can be used beneficially in cat enclosures so as to provide visual contact but at the same time maintain separation. The design of enclosures should also provide an enclosed area out of view of other cats, while at the same time allowing for easy inspection of animals by staff.

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Dimensions
Table 1. Minimum space allowances

	Floor* m²	Shelves m²	Height m
Minimum for one adult cat	1.5	0. 5	2
For each additional cat add	0.75	0.25	-

Note: * Floor area excluding shelves.

The minimum space in which a queen and litter (to weaning) may be held is the space for a single cat, which should be gradually increased so that by 4 months of age litters have been re-housed to conform with the above space requirements for adults. The normal age for weaning is 7-9 weeks.

Areas for feeding and for litter trays should be not less than 0.5 metres apart and should not be interchanged.

Constraint in a space below the minimum requirement detailed above, such as in a metabolism cage or any similar type of housing for scientific purposes, may severely compromise the welfare of the animals. Such constraint should be for the minimum time and within a space that is as close as possible to that defined above and no less than that required for the animal to stretch fully horizontally and vertically, to lie down and turn around.

Background: Cats are descended from a territorial species and, while they show considerable flexibility in how much space they require in the wild, there is evidence that the smaller the space a cat is confined in, the more likely it is to develop behavioural abnormalities indicative of chronic stress (McCune, 1995b). Further research is required to establish the precise minimum space needed, but it is likely that increasing the quality of space may provide more benefit than floor area per se (McCune, 1995b). The recommendation for single-housed cats is therefore based on providing sufficient space for the enrichments in (4) to be implemented effectively and safely by animal caretakers. Unlike dogs, there is no evidence that cats benefit from periods of 'exercise' outside their primary enclosure. Enriched single housing for cats is illustrated in Loveridge et al (1995).

For pair- and group-housed cats, behavioural indicators of stress increase at densities greater than one cat per two square metres (Kessler & Turner, 1999; Rochlitz, 2000); the recommendations are based on the assumption that this density can be increased slightly if the cats can make use of the full height of the enclosure, and that the other enrichment in (4) is also implemented. Enriched group-housing for cats is illustrated in Loveridge (1994).

Outside runs
Outside runs provide an environmental enrichment opportunity for cats in both breeding and user establishments and should be provided where possible.

Cats should never be forced to spend their entire lives outside and should always have access to an internal enclosure that meets all standards including minimum dimensions detailed in these guidelines.

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The quality and finish of the floor of an outside run need not be to the standard of the inside enclosure, providing it is easily cleanable and not physically injurious to the cats.

Flooring

The preferred flooring for cat enclosures is a solid continuous floor with a smooth non-slip finish. Additional enclosure furniture should provide all cats with a comfortable resting place.

Open flooring systems such as grids or mesh should not be used for cats**.** Where there is justification for open flooring, the highest level of attention should be given to their design and construction in order to avoid pain, injury and diseases and allow the animals to express normal behaviours. Practical experience shows that metabolism cages are not always necessary as cat's urine and faeces can be collected direct from litter trays.

Background: The question of whether cat accommodation should have solid or open flooring was considered at the Berlin Workshop (1993). Cats are considered by the Expert Group to require a solid floor and should not be kept on open flooring at any time, except for specific scientific purposes, when such use should be authorised by the Responsible Authority. The provision of a comfortable resting place is considered particularly important for this species.

4. 4. Feeding (*See item 4.6.of the General section of Appendix A)*

4.5. Watering (*See item 4.7. of the General section of Appendix A)*

4.6. Substrate, litter, bedding and nesting material

At least one litter tray of minimum dimension 300 x 400 mm should be provided for every 2 cats and should contain a suitable absorbent and non-toxic litter or substrate material that is acceptable to and used by the cats. If urine and faeces are regularly deposited outside the trays, additional trays containing alternative substrates should be provided. If this is ineffective in pair- or group-housed cats, social incompatibility is indicated and cats should be removed from the group one at a time until the problem is resolved.

Sufficient beds should be provided for all cats and should be made of a suitable easily cleanable material. These beds should contain bedding material such as polyester fleece or similar bedding material.

Background: Cats can be naturally trained to use litter trays and so absorbent substrate on the floor of the pen is not necessary. The litter material should be non-toxic, absorbent, dust-free and produce little ammonia; dust-free wood chips have been shown to be acceptable. The dust content of older 'chalky granules' has been considered to be too high (Saigeman, 1998). Cats require sleeping areas that are quiet and warm and, if kept in groups, should have the choice to sleep on their own. Cats also, when allowed to exhibit natural instincts (Rochlitz, 2000) will often sleep at a height, so sleeping boxes may be hung on walls (Hurni & Rossbach, 1987) or at different heights especially in the corners of rooms where they cannot be approached from behind (Roy, 1992). Generally non-toxic, durable and easily washable bedding material is used with cats. Man-made material is popular as cats prefer those materials that maintain a constant temperature, though shredded paper or wood shavings have been shown to be acceptable (Roy, 1992). As cats are more fastidious eaters than dogs, the possibility of them poisoning themselves by ingesting bedding material or causing intestinal foreign bodies is much reduced.

4.7. Cleaning

Each occupied enclosure should be cleaned at least daily. Litter trays should be emptied daily and litter material replaced.

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Cleaning of enclosures should not result in cats becoming wet. When enclosures are hosed down, the cats should be removed from the enclosure to a dry place and returned only when it is reasonably dry.

Background: Maintenance of good cleaning regimes is an obvious requirement to meet within cat facilities. It is particularly important with this species that animals do not become wet, as this is generally intensively disliked and is also undesirable in relation to health and welfare. It should be noted that cleaning is a task which should bring staff into close contact with animals. Consideration should be given to exploiting this opportunity to develop 'social contacts' between carers and cats.

4.8. Handling (*See item 4.10. of the General section of Appendix A)*
For cats, close contact with the persons caring for the animals is crucial, especially for single housed cats.

4.9. Humane killing (*See item 4.11. of the General section of Appendix A)*

4.10. Records *(See item 4.12. of the General section of Appendix A)*

4.11. Identification (*See item 4.13. of the General section of Appendix A)*

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PART B - CATS

References

Adamec R E (1976) The interaction of hunger and preying in the domestic cat (*Felis catus*): an adaptive hierarchy? Behavioral Biology 18, 263-272.

Baerends-van Roon J M & Baerends G (1979) The Morphogenesis of the Behaviour of the Domestic Cat: with a special emphasis on the development of prey-catching. Amsterdam: North-Holland.

Bateson P & Turner D C (1988) Questions about cats. In "The Domestic Cat: the biology of its behaviour", ed. D C Turner & P Bateson, pp. 193-201. Cambridge: Cambridge University Press.

Berkley MA (1976) Cat visual psychophysics: neural correlates and comparisons with man. Progress in Psychobiology and Physiological Psychology 6, 63-119.

Berlin Workshop (1993) The accommodation of laboratory animals in accordance with animal welfare requirements: proceedings of an International Workshop held at the Bundesgesundheitsamt, Berlin; 17-19 May 1993.

Bernstein P L & Strack (1996) A game of cat and house: spatial patterns and behaviour of 14 cats (*Felis catus*) in the home**.** Anthrozoös 9, 25-39.

Bradshaw J W S (1992) The Behaviour of the Domestic Cat. Wallingford, UK: CAB International.

Bradshaw J W S & Brown S L (1996) Social behaviour in a small colony of neutered feral cats. Journal of the Feline Advisory Bureau 34, 35-37.

Bradshaw J W S and Cameron-Beaumont C (in press). The signalling repertoire of the domestic cat and its undomesticated relatives. In: "The Domestic Cat: the biology of its behaviour", 2nd edition, ed. D C Turner and P Bateson, pp. 67-93. Cambridge: Cambridge University Press.

Bradshaw J W S & Hall S L (1999) Affiliative behaviour of related and unrelated pairs of cats in catteries: a preliminary report. Applied Animal Behaviour Science 63, 251-255.

Carlstead K, Brown JL & Strawn W (1993) Behavioral and physiological correlates of stress in laboratory cats. Applied Animal Behaviour Science 38, 143-158.

Fitzgerald B M (1988) Diet of domestic cats and their impact on prey populations. In "The Domestic Cat: the biology of its behaviour", ed. D C Turner & P Bateson, pp. 123-147. Cambridge: Cambridge University Press.

Hall S L (1998) Object play by adult animals. In "Animal Play: Evolutionary, Comparative and Ecological Perspectives", ed. M Bekoff & J A Byers, pp. 45-60. Cambridge: Cambridge University Press.

Hurni H & Rossbach W (1987) The Laboratory Cat. In The UFAW Handbook on the care and management of laboratory animals 6th edition. Ed Poole T.

Kerby G and Macdonald D W (1988) Cat society and the consequences of colony size. In "The Domestic Cat: the biology of its behaviour", ed. D C Turner & P Bateson, pp. 67-81. Cambridge: Cambridge University Press.

Kessler M R & Turner D C (1997) Stress and adaptation of cats (*Felis silvestris catus*) housed singly, in pairs and in groups in boarding catteries.

Kessler M R & Turner D C (1999) Effects of density and cage size on stress in domestic cats (*Felis silvestris catus*) housed in animal shelters and boarding catteries. Animal Welfare 8, 259-267.

Leyhausen P (1979) Cat behaviour: the predatory and social behaviour of domestic and wild cats. New York: Garland STPM Press.

Loveridge GG (1994) Provision of environmentally enriched housing for cats. Animal Technology 45: 69-87.

Loveridge GG, Horrocks LJ & Hawthorne AJ (1995) Environmentally enriched housing for cats when housed singly. Animal Welfare 4: 135-141.

McCune S (1992) Temperament and the welfare of caged cats. D. Phil thesis, University of Cambridge.

McCune S (1995a) The impact of paternity and early socialisation on the development of cats' behaviour to people and novel objects. Applied Animal Behaviour Science, 45, 109-124.

McCune S (1995b) Enriching the environment of the laboratory cat. In Environmental Enrichment Information Resources for Laboratory Animals 1965-1995. AWIC Resource Series No 2, pp 27-33.

McCune S, McPherson J A & Bradshaw J W S (1995) Avoiding problems; the importance of socialisation. In "The Waltham Book of Human-Animal Interactions", ed. I H Robinson, pp. 71-86. Oxford: Pergamon Press.

Overall K L (1997) Clinical Behavioral Medicine for Small Animals, Chapter 8. New York: Mosby.

Olmstead CE, Villablanca JR, Torbiner M & Rhodes D (1979) Development of thermoregulation in the kitten. Physiology & Behaviour 23, 489-495.

Podberscek A L, Blackshaw J K & Beattie A W (1991) The behaviour of laboratory colony cats and their reactions to a familiar and unfamiliar person. Applied Animal Behaviour Science 31, 119-130.

Rochlitz I (2000) Recommendations for the housing and care of domestic cats in laboratories. Laboratory Animals 34: 1-9.

Rochlitz I, Podberscek A L & Broom D M (1998) The welfare of cats in a quarantine cattery. Veterinary Record 143, 35-39.

Rosenblatt J S (1972) Learning in newborn kittens. Scientific American 227, 18-25.
Roy D (1992) Environmental enrichment for cats in rescue centres. BSc thesis University of Southampton.
Saigeman S (1998) Environmental enhancement of cats – what? Why? How? Animal Technology 49, 3: 145-154.
Smith D F E, Durman K J, Roy D B & Bradshaw J W S (1994) Behavioural aspects of the welfare of rescued cats. Journal of the Feline Advisory Bureau 31, 25-28.
van den Bos R & de Cock Buning T (1994a) Social and non-social behaviour of domestic cats (*Felis catus* L.): a review of the literature and experimental findings. In "Welfare and Science", Proceedings of the 5th FELASA Symposium, ed. J Bunyan, pp. 53-57. London: Royal Society of Medicine Press.
van den Bos R & de Cock Buning T (1994b) Social behaviour of domestic cats *(Felis lybica catus* L.): a study of dominance in a group of female laboratory cats. Ethology 98, 14-37.
van den Bos R (1998) Post-conflict stress response in confined group-living cats (*Felis silvestris catus*). Applied Animal Behaviour Science 59, 323-330.

General references

86/609/EEC: Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.
Home Office (1989) Code of Practice for the Housing and Care of Animals used in Scientific Procedures. HMSO: London.
Home Office (1995) Code of Practice for the Housing and Care of Animals in Designated Breeding and Supply Establishments. HMSO: London.
ILAR Commission on Life Sciences (1996) Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington DC.
White WJ, Balk MW & Slaughter LJ (1974) Housing requirements – dogs and cats. In Handbook of Laboratory Animal Science Vol 1. Eds Melby EC & Altman NH.

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Dogs

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)

6th meeting
Strasbourg, 25-27 March 2003

Species specific provisions for dogs

Background information for the proposals presented by the Group of Experts on dogs and cats

PART B

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Species-specific sections – Dogs

Preamble

In 1997, the Council of Europe (CoE) established four Expert Groups in order to advise the CoE Working Party on revisions to Appendix A of the Convention ETS123 (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 1986). One of these Expert Groups was established to consider proposals for dogs and cats; its remit was later extended to include ferrets when the CoE Working Party decided that other species covered by ETS123 should be included in the revision. Organisations represented both on the Group and at its meetings were:

Federation of European Laboratory Animal Science Associations (FELASA) – Coordinator of Group
Eurogroup for Animal Welfare
European Federation of Pharmaceutical Industries and Associations (EFPIA)
Federation of Veterinarians in Europe (FVE)
Federation of European Laboratory Animal Breeders Associations (FELABA)
International Society for Applied Ethology (ISAE)

The members of the Expert Group on dogs, cats and ferrets met on several occasions and also exchanged information by e-mail. The Coordinator of the Expert Group, accompanied by one or more other members, attended all meetings of the Working Party in Strasbourg, in order to present the Group's proposals, discuss their content and answer questions, and refer matters back to the Group where appropriate.

The CoE Expert Group on Dogs, Cats and Ferrets has provided three separate reports, covering each of these species. Each report comes in two parts. Part A details the proposals for amendments to Appendix A, as agreed by the Working Party and including amendments to the Expert Group's original proposals as required by the Working Party. Part B provides background information for these proposals. Where possible, recommendations have been based upon scientific evidence; where this is not available, they take account of established good practice, based both on the experience of the members of the Expert Group and also on further consultations with other experts. Additional comments have been received from members of the Working Party and from a range of non-governmental organisations and individuals. These have been considered by the Expert Group and incorporated in the proposals where appropriate.

The proposals and their rationale are therefore the outcome of extensive and detailed discussions within the Group and should be regarded as expert recommendations, reflecting the scientific evidence and information on good practice available at the time. Their intention is to increase the welfare of animals used in research, taking into account the purposes for which such animals are used, which may place some constraints upon their housing and husbandry.

The Expert Group considered that, although the provisions of Appendix A formally are guidelines, its finalised proposals should be regarded as minimum requirements. Knowledge gained by further research and scientific evidence, as well as changing views on what is current 'good practice', may mean that the accommodation and care provided for animals in research in the future may vary from these proposals such that future revision of Appendix A is necessary.

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1. Introduction

The domestic dog *(Canis familiaris)* is an inquisitive and highly social animal which actively seeks information about its surroundings, reflecting the behaviour of its ancestors in the wolf family.
Although much of the day is spent resting, the dog requires a complex physical and social environment during the active phase.
Bitches seek solitude in a quiet area for parturition and rearing of young.
As aggression is a significant risk, care is needed to maintain dogs in socially harmonious groups.
The recommendations are provided for the beagle, the most commonly used breed. Account should be taken of individual breed characteristics if other breeds are used.

2. The environment and its control

2.1. Ventilation (See *item 2.1 of the General section of Appendix A)*

Background: The number of air changes per hour in the European (15-20), UK (10-12) and US (10-15) guidelines differ to some extent, although no evidence is offered to support the specific ranges. All guidelines allow for lower ventilation rates if stocking density is low. These factors were taken into account in drafting the General Section of Appendix A.

2.2. Temperature

A temperature range of 15 to 21°C should be maintained when precise control is required for dogs under procedure. In all other circumstances, dogs may be maintained within a wider temperature range provided that welfare is not compromised.

Puppies have limited thermoregulatory control in the first 10 days or so of life and additional local heating should be provided within the whelping enclosure.

Background: The range of temperatures given in the European (15-21°C), UK (15-24) and US (18-29) differ. Narrow ranges aim to avoid wide fluctuations of the ambient temperature so as to reduce experimental variation, although there is no evidence that a temperature outside the most stringent range but within the largest range would impact on the welfare of the dogs.

It is more important that there are no abrupt changes, which would affect e.g. the respiratory system, or too wide a fluctuation, which could affect physiological parameters and interfere with certain procedures.

In view of the above, there would seem no reason to recommend a specific temperature range except for dogs undergoing procedures. In addition, young puppies will need higher temperatures to take account of their limited thermoregulatory control.

In some cases, dogs will have access to an outside run at ambient temperatures. As this could mean extremes of temperature being encountered with potential impact on welfare, the dogs should also have access to an area that enables them to exercise some control over their living environment.

Consideration of the above factors resulted in the Working Party agreeing the above proposal.

2.3. Humidity

It is considered unnecessary to control relative humidity, as dogs can be exposed to wide fluctuations of ambient relative humidity without adverse effects.

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Background: The ranges in European and UK guidelines (both 45-65% with extremes 30-70) and US (30-70%) are near identical. However, there is no evidence that a relative humidity exceeding these ranges would impact the welfare of dogs. The Working Party therefore agreed the above proposal.

N.B. The Expert Group considered there could be benefits in recording and logging Relative Humidity on a regular basis, in order to help identify any potential problems at an early stage.

2.4. Lighting

Holding of dogs under the natural 24 hours light-dark cycle is acceptable. Where the light part of the photoperiod is provided by artificial lighting, this should be within a range of 10 to 12 hours daily.

If natural light is totally excluded, low level night lighting (5-10 lux) should be provided to allow dogs to retain some vision and to take account of their startle reflex.

Background: Prolonged periods in excess of the natural dark period can stress the dogs. A minimum light period should therefore be provided daily, not less than the extreme within the natural light-dark cycle throughout the seasons. In addition, sufficient lighting is essential to examine the clinical condition of the animals on a regular basis and allow for routine husbandry practices.

During the dark period, total darkness should be avoided. In nature, total darkness does not exist. Furthermore, minimal night lighting is necessary to avoid a startle reflex.

Some lighting systems have been shown to be aversive to some animals.

2.5. Noise

Noise in dog kennels can reach high levels which are known to cause damage to humans, and which could have effects on dogs' health or physiology. For these reasons it is important to consider methods of reducing noise in dog facilities. Much of the noise is generated by the dogs' own vocalisations, but may also be generated by husbandry operations within the facility and also ingress from outside sources. Any source of noise that may stimulate further dog barking should therefore be limited as far as possible. Ingress of noise can be controlled by appropriate siting of the facility and by appropriate architectural design. Noise generated within the facility can be controlled by noise absorbent materials or structures. By addressing the dogs' behavioural needs in the facility design, the level of vocalisation may be decreased. Expert advice should be taken when designing or modifying dog accommodation.

Background: The hearing range of dogs is 0.04-46 kHz, with peak sensitivity of 0.5-16 kHz.

All existing guidelines mention that noise can be a disturbing factor or even be damaging. The hearing range above indicates the frequencies involved. In dog kennels, it is known that most noise is created by the animals themselves and that the noise levels can be high for a substantial proportion of the day (Hubrecht et al. 1997, Sales et al. 1997). Sound-absorbing materials should therefore be used. Socialisation and habituation of the dogs to the presence of humans will reduce the barking behaviour.

2.6. Alarm systems (*See item 2.6. of the General section of Appendix A*)

3. Health (See items 4.1 and 4.4 of the General section of Appendix A

Background: The FELASA recommendations have been used as a common reference to determine the health status of animals. A minimum quarantine period of two weeks is

suggested as the incubation periods of most diseases are less than two weeks and this duration also allows for additional sampling and analysis if required.

In relation to acclimatisation, a period of two weeks is common practice.

This recommendation from the Expert Group should be considered in conjunction with the information provided in the General Section.

4. Housing and enrichment

Background: As with other sections, proposals are based on research data where available, and otherwise on good practice. The over-riding principle with dogs is the need to encourage and motivate social housing. Dogs should be held in socially-harmonious groups with a minimum of two, i.e. a pair. Long-term single housing and social isolation are associated with a range of behavioural disturbances (Hetts et al, 1992).

4. 1. Housing

Dogs should be housed in socially harmonious groups within the animal enclosure, unless the scientific procedures or welfare requirements make this impossible. Special care is needed when regrouping dogs or introducing an unfamiliar dog to a group. In all cases, groups should be monitored for social compatibility on an ongoing basis.

Single housing of dogs for even short periods can be a significant stress factor. Therefore, dogs should not be single housed for more than 4 hours without justification on welfare or veterinary grounds. Single housing for more than 4 hours on experimental grounds should be determined in consultation with the animal technician and with the competent person charged with advisory duties in relation to the well-being of the animals.

In such circumstances, additional resources should be targeted to the welfare and care of these dogs. Additional human socialisation time, and visual, auditory and, where possible, tactile contact with other dogs should be provided for all single-housed animals on a daily basis.

Unless contra-indicated on scientific grounds, single-housed dogs should be allowed to exercise in a separate area with, if possible, other dogs, and with staff supervision and interaction, on a daily basis.

Stud dogs should, wherever possible, be housed in socially harmonious pairs or groups or with bitches.

Peri-parturient bitches should only be moved to the whelping enclosure between one and two weeks of expected parturition. While in the whelping enclosure they should have additional daily human contact.

Early Socialisation with Conspecifics and Humans

Social behaviour in dogs develops between 4 and 20 weeks of age. During this period it is particularly important that the dog has social contacts with both littermates and adult dogs (e.g. the bitch) and with humans and is familiarised with conditions likely to be encountered during subsequent use. Daily handling during this sensitive stage of development is a prerequisite for the social behaviour of the adult dog and it has been shown that a short period of handling even from the first day after birth on is of importance as the young animals are already able to respond to scent and tactile stimulation.

Background: Dogs are social animals and it is therefore not surprising that single housing is associated with an increased incidence of behavioural abnormalities (Hetts et al. 1992). Hetts argues that social isolation is even more damaging than spatial restriction. There is abundant literature on dogs kept as companion animals to show that they frequently suffer

from separation anxiety when left on their own (e.g. review in Serpell & Jagoe, 1995). From 3 weeks of age, puppies become increasingly distressed if placed alone in a strange environment (Elliott & Scott, 1961). There are no clear data to indicate what an optimum group size might be, but pair housing would seem to be a reasonable minimum as dogs in pairs spend a similar proportion of their time socialising to those in larger groups (Hubrecht, 1993). Aggression in group housing can be a serious problem, in some cases leading to the death of animals, so it is important to ensure that there is an adequate husbandry routine to monitor the dogs and forestall potential problems.

The Expert Group considered that, with the exception of the provision for temporary separation of up to 4 hours for pair- or group-housed dogs (see Section 4.3), special justification and approval from the Responsible Authority should be required if dogs are to be single-housed for scientific purposes. The Expert Group also considered that, as single housing for even short periods can be a stress factor, any period in excess of 24 hours should be considered as severely compromising the welfare of the animals and therefore taken fully into account when considering the justification for the procedures.

With regard to early socialisation with conspecifics and humans, socially restricted rearing conditions lead to the development of behavioural abnormalities and crippled behavioural repertoires (Thompson et al, 1956; Fuller, 1967). The early development of the dog can be divided into four stages: (1) the neonatal stage when the puppy is comparatively helpless; (2) the transition period at about 13 days when the eyes first open, and lasting for about 1 week; (3) the socialisation period between 4 and 20 weeks; and (4) the juvenile period. It has long been accepted that the socialisation period is critical in determining the dog's social behaviour (Scott & Fuller, 1965; Scott et al, 1974; Wright, 1983). All dogs should therefore have experienced adequate socialisation during the 'socialisation period', and should receive gentle and sympathetic handling thereafter. The earlier periods are also important. Short periods of daily handling or other stimuli can have a marked influence on development. For example, early handling can result in a more rapid maturation of the brain (Fox & Stelzner, 1966), more rapid weight gain and earlier opening of the eyes. Puppies can learn associations between smell and suckling at one day of age (Fox, 1971), and Fox (1978) showed that puppies exposed to varied stimulation between birth and 5 weeks of age were later more confident and exploratory in a strange environment. The easiest way to minimise stress in the dog is to ensure that he/she reacts well to handling.

4. 2. Enrichment

The design of indoor and outdoor enclosures should allow some privacy for the dogs and enable them to exercise some control over their social interactions.

Separate areas for different activities should be provided. This can be achieved by, for example, inclusion of raised platforms and pen sub-divisions.

Dog treats and toys afford welfare benefits to the animals, providing these are used sensibly and adequately monitored. As chewing is an important behaviour, items should be provided which meet this need.

The primary advantages of exercise are to allow additional opportunities for dogs to experience a complex and varied environment and to increase interactions with other dogs and humans. These will be particularly important where these needs cannot be fully met within the space provided by the animal enclosure. Therefore, unless contra-indicated on scientific or veterinary grounds, dogs should be removed to a separate area and allowed to exercise, with other dogs where possible, and with staff supervision and interaction, ideally on a daily basis.

Background: There are two basic methods of enriching a caged environment: (1) by social enrichment, and (2) by physical enrichment. These can be sub-divided as follows:

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- ***By providing more, or more varied, opportunities for social interactions with (a) humans or (b) dogs***
- ***By allowing dogs a greater choice of activity through the provision of physical items within the pen***
- ***By allowing dogs a greater choice of location of micro-environment within the pen***

As discussed under (4.1) above, housing dogs in pairs is considered an effective strategy for conspecific socialisation. It is also common practice in many dog facilities to allow dogs to run together while the pens are being cleaned, for example by making use of corridor areas. The dogs appear to enjoy this activity although there have been no specific studies on its effectiveness for enrichment. Given that puppies have been properly socialised with humans during the socialisation period, they will be motivated to socialise with humans (Scott & Fuller, 1965).

Studies on the provision of physical enrichment items indicate that dogs will make extensive use of items, particularly if they are food-flavoured (DeLuca & Kranda, 1992; Hubrecht, 1993; Hubrecht, 1995). Proper presentation, for example by suspending chews a few centimetres from the floor of the pen on a spring, can help to minimise cleaning and possessive aggression problems whilst also allowing the animals to chew in a species-specific manner (Hubrecht, 1993).

Social and environmental enrichment can be combined into an integrated programme (Loveridge, 1994).

Kennels or open pen designs should not overtly restrict the dogs' ability to obtain information about the surroundings. High walls or partitions between pens result in the dogs being unable to see to the end of their rooms. This can lead to them spending a relatively high proportion of time on their hind-legs (Hubrecht et al, 1992) or in apparently repetitive, possibly stereotypical, jumping behaviour. Obvious ways around this problem include reducing solid partition height between pens for at least a portion of their length, or providing platforms within pens. Hubrecht (1993) has shown that platforms are extensively used by dogs to play and rest on. These structures have the benefit that they increase the complexity of pens, thus allowing the dogs more choices within their environment, and that, if they are properly designed so they have sufficient height, they do not block the existing floor area. They also provide a greater floor area within a given space, as they make use of the otherwise inaccessible third dimension within a pen.

Dog housing should be designed so that the dogs can retreat to an area that provides them with a sense of security. This need not cause a problem of visibility for the animal carer as it can simply be an area with a few barriers shielding the animal from view on some sides. It is particularly important to provide such structures when dogs are housed in large social groups, to allow the dogs more control over their social interactions.

Exercise

One obvious effect of confinement is to restrict locomotory behaviour. Small enclosures not only discourage exercise, but also restrict the type of locomotion that is possible. Clark et al (1991) have shown that increasing pen dimensions beyond the minimum acceptable in the US (USDA Code of Federal Regulations) does not seem to make much difference to the dog in terms of physical fitness, nor does this seem to make much difference to the frequency of aggression or play (Bebak & Beck, 1993). However, a further study (Hubrecht et al, 1992) of the behaviour of mixed breeds housed in pens with spacious outdoor runs (744 square metres) showed that both the activity of the dogs and the range of locomotory behaviours were greater than that of dogs in pens of less than 7 square metres.

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4. 3. Animal enclosures – dimensions and flooring

Animal enclosures, including the divisions between enclosures, should provide a robust and easily cleaned environment for the dogs. In their design and construction they should seek to provide an open and light facility giving the dogs comprehensive sight of other dogs and staff, outside of their immediate animal enclosure.

Background: Freedom to express a range of normal behaviours will place some demands on the structure of enclosures. Those which allow extensive visibility of the immediate surroundings will promote normal behaviours and encourage animals to take an interest in their surroundings. The ability to watch other animals and staff may also provide a useful function in the dog's time utilisation, thereby playing a role in minimising the risk of developing stereotypies. Careful consideration must therefore be given to providing an appropriate balance between solid walls and metal bars or mesh. Reinforced plate glass has also been used for pen dividers in order to allow visual contact but at the same time maintaining separation, e.g. of dosing groups in toxicology studies. The design of enclosures should provide an enclosed area out of view of other dogs while at the same time allowing for visual, auditory and, where possible, tactile contact between dogs. This design can at the same time allow easy inspection by staff.

Outside runs

Outside runs provide an environmental enrichment opportunity for dogs in both breeding and user establishments and should be provided where possible.

Dogs should never be forced to spend their entire lives outside and should at all times have access to an internal enclosure that meets the standards for construction and environmental control detailed in these guidelines. The internal enclosure should represent not less than 50% of the minimum space to be made available to the dogs, as detailed in table 1 below.

The quality and finish of the floor of an outside run need not be to the standard of the inside enclosure, providing it is easily cleanable and not physically injurious to the dogs.

Dimensions

These guidelines are intended to encourage the social housing of dogs and to permit adequate enrichment of the environment. It should be noted that within this concept and strategy every encouragement is given to holding dogs in larger and socially-harmonious groups both to extend the available floor space even further and to enhance the socialisation opportunities.

The space allowances detailed below are based on the requirements of beagles, but it should be noted that allowances significantly in excess may be required for giant breeds such as the St Bernard or Irish Wolfhounds. In the cases of breeds other than the laboratory beagle, space allowances should be decided in consultation with veterinary staff and the responsible authority.

Table 1. Minimum space allowances

Weight of dog kg	Minimum floor area For one or two dogs m^2	For each additional dog add a minimum of m^2	Minimum height m
= 20	4	2	2
> 20	8	4	2

Dogs that are pair or group housed may each be constrained to half the total space provided (2 m^2 for a dog under 20 kg, 4 m^2 for a dog over 20 kg) while they are undergoing procedures as defined in the Convention, if this separation is essential for scientific purposes. The period for which a dog should be so constrained should be minimised and should not in any event exceed 4 hours. This

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provision is made to encourage pair housing (particularly in toxicology studies) while at the same time allowing for the need to monitor feed intake and post-dosing observations.

Any further social or physical constraint, such as in a metabolism cage or physical restraint in a sling, may severely compromise the welfare of the animals. Constraint in a metabolism cage or any similar type of housing for scientific purposes should be within a space that is as close as possible to that defined above and no less than that required for the animal to stretch fully, lie down and turn around.

Nursing bitches and litters, and puppies up to 7.5 kg

The normal weaning age for puppies is 6-9 weeks.

The following recommendations are based on good practice and address the situation in dog breeding establishments.

A nursing bitch and litter should have the same space allowance as a single bitch of equivalent weight. The whelping pen should be designed such that the bitch can move to an additional compartment or raised area away from the puppies.

Table 2. Minimum post weaned stock space allowances

Weight of dog kg	Minimum floor area/animal m^2	Minimum height m
= 2	0.5	2
= 5	1.0	2
= 10	1.5	2
= 15	2	2
= 20	4	2

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The table above indicates the minimum floor space which should be continuously available to each dog of the weight indicated.

The minimum pen size for any animal is $4m^2$.

***Background:** Confining dogs in small pens prevents them from retreating when alarmed and restricts their ability to perform species-specific behaviour. For example, in a pen too small a dog will not be able to run in a 'dog-specific' manner (Hubrecht, 1997). Small enclosures are also associated with a higher incidence of circling and other stereotypies, compared to larger enclosures (Hubrecht et al, 1992). Dogs given larger enclosures choose to keep significantly more distance between themselves (Bebak & Beck, 1993). Adequate space is required for social interactions (but also to enable an individual to control its social interactions), and to permit play with enrichment devices such as toys. Regarding good practice, the UK Home Office recommends that no dog is kept in less than 4.5 square metres (Home Office, 1995). The Home Office has also identified appropriate space allowances for post-weaned stock.*

Therefore, in order to encourage housing in pairs (at least), and taking account of many existing pen sizes, the minimum floor area recommended for one dog is the same as that for two dogs, i.e. 4 square metres for dogs weighing under 20 kg. Each additional dog above two and weighing up to 20 kg will required an additional 2 square metres.

Dogs weighing 20 kg or more should be given a minimum floor space of 8 square metres, i.e. two standard pens interlinked. Again, two dogs may be kept in this space, but each additional dog above two and weighing more than 20 kg will require additional floor space of 4 square metres. These requirements may be met by linking two or more adjacent pens.

Dogs which are normally pair- or group-housed may each be constrained to half the space provided (e.g. 2 square metres for a dog under 20 kg) while they are undergoing scientific procedures if this separation is essential to the purpose of the study, but with a maximum of 4 hours separation. This space provision is to encourage pair- or group-housing particularly in toxicology studies. The Expert Group considered that any period of isolation may cause some stress and so compromise welfare; however a permissible period of separation of 4 hours was established in discussion with the Working Party as a reasonable period to allow adequate time for dosing, feeding and making individual observations of animals. The dogs should therefore be returned to pair- or group-housing as soon as possible, and certainly by the end of the working day. The Expert Group also considered that any period of separation exceeding 4 hours should require the authorisation of the Responsible Authority. This above is consistent with good practice in toxicology. For example, a survey of husbandry practices in toxicology studies in the UK found that 7 out of 8 respondents housed their dogs two or more to a pen, separating them for feeding; and six respondents provided pen floor areas of 4.5 square metres or more (Hubrecht, 1994). One establishment had pens of 6.8 square metres floor space. It was also noted that separation for dosing was not always considered necessary and depends on the compound under study.

Any further constraint such as in a metabolism cage or physical restraint in a sling may severely compromise the welfare of dogs. The Expert Group considered this should be specifically authorised by the Responsible Authority. The optimum size of metabolism cage which balances welfare and scientific objectives is yet to be determined, but these or any other type of similar confinement should provide a dog with a space as close as possible to the minimum standards for normal housing and at the very minimum should allow the dog to stretch fully, lie down and turn around.

Flooring

The preferred flooring for dog accommodation is a solid continuous floor with a smooth non-slip finish. All dogs should be provided with a comfortable solid resting area, for example by the use of enclosure furniture such as raised beds or platforms.

Open flooring systems such as grids or mesh should not be used for dogs. Where there is a justification for open flooring, the highest level of attention should be given to their design and construction in order to avoid pain, injury or disease and allow the animals to express normal behaviours. If any welfare problems do arise related to the flooring, veterinary advice should be sought and, if necessary, dogs relocated onto solid flooring.

Pre-weaned puppies and peri-parturient and suckling bitches should not be held on an open floor system.

Background: The question of whether dog accommodation should have solid or grid floors was considered at length at the Berlin Workshop (1993). Most experts recommended solid or at least only partly gridded floors and agreed that dogs preferred solid flooring. In considering this issue, the Expert Group aimed to satisfy the concept of the 5 Freedoms (FAWC, 1993), comprising freedom: from thirst, hunger and malnutrition; from discomfort; from pain, injury and disease; to express normal behaviour; and from fear or distress. In relation to the relevant Freedoms, the floor of a dog enclosure needs to be comfortable (physical and thermal), non-injurious, easily cleanable and of a design suited to the anatomy of the canine limb. There are arguments to support both strategies of flooring (open vs solid), but no firm evidence that open flooring systems do actually equal poor welfare for the dogs. However, the positive benefits of an open flooring system tend to accrue primarily to the provider in respect of comparative capital cost and efficient husbandry practices with respect to cleaning, rather than in positive benefits to animal welfare. Indeed, there can be significant negative effects if open flooring systems are poorly designed, although this comment can be applied to any type of floor to some extent. The above recommendations therefore recognise the desirability of providing the animal with an element of choice, whatever the flooring system, and, specifically, with a comfortable resting place. A solid area, of sufficient size for an animal to lie down in comfort, should be provided in any system, whether solid or open. In certain situations, open flooring should never be used, such as for heavily pregnant or suckling bitches, and very young animals.

4. 4. Feeding (*See item 4.6. of the General section of Appendix A*)

4. 5. Watering (*See item 4.7. of the General section of Appendix A*)

4. 6. Substrate, litter, bedding and nesting material

When dogs are held on solid floors, some litter or substrate material facilitates cleaning and minimises the necessity to wash/hose down regularly.

Peri-parturient and suckling bitches should be provided with a bed and bedding material to support whelping and the nursing of puppies. Puppies also benefit from the provision of bedding materials, as may certain breeds such as the greyhound.

Background: In general dogs are housed on sawdust or wood chips, perhaps more for historical reasons than to meet dogs' needs. It is common for concrete or similar flooring to be used for dogs, but provision of a warm dry place to sleep is paramount. Under-floor heating may be used but should not be instead of bedding materials as dogs could then be lying in direct contact with urine and faeces. Raised bed boards are popular and also allow visualisation of the environment. The beds may be wood-based but must then be metal-edged to reduce chewing (MacArthur, 1987). Nesting boxes for whelping bitches that are insulated and raise pups from the floor have been in use for some time.

The idea of having a bedding material is to soak up urine and faecal water content, reducing the need for cleaning and also the amount of free water in the enclosure. In puppies there is also the need for an insulating layer on the floor as young animals regulate their body temperatures less effectively.

Any bedding material used must clearly be non-toxic, produce little ammonia, be absorbent, non-allergenic and atraumatic (Voipio et al, 1986; Potgieter & Wilke, 1996). Soft wood bedding has been found to induce liver enzymes in mice (Vessell, 1973) and so hard wood is the material of choice, also being more absorbent (Voipio et al, 1986). A study between pine wood, eucalyptus pulp and vermiculite showed that the dust content of vermiculite and pine was higher than eucalyptus, and both had lower water absorbency. However, the vermiculite produced the least ammonia and pine the highest (Potgieter & Wilke, 1996). There have however been reports of vermiculite inducing neoplastic changes in the lungs of mice (Jager et al, 1997) and cedar has been implicated in inducing tumours in rats (Sabine, 1975), whereas other workers have found that sawdust gave a similar incidence of neoplasia as control groups (Tennekes et al, 1981). It therefore appears that, although vermiculite may be best avoided, compelling evidence not to use a particular type of wood bedding, due to carcinogenicity, is lacking.

Dogs have been bedded on straw or hay. However, they may not be a good choice due to the lack of quality control of these products and their ability to induce atopic skin disease. There are many cases of atopy, but pollens, fungi and moulds are frequently incriminated.

Paper has been used in domestic situations but newspaper print will make animals dirty and there is insufficient evidence concerning the toxicity of shredded paper when ingested. Recycled, non-toxic and sterile paper products may be acceptable.

Fabric type bedding materials are popular in whelping units. There are several types that are hypoallergenic, non-toxic, durable and easily washed, but they can be expensive and care must be taken to clean them regularly.

4.7. Cleaning

Each occupied enclosure should be cleaned at least daily. All excreta and soiled materials should be removed from all areas used by dogs at least daily and more frequently if necessary.

Wet cleaning by hosing down of enclosures should be carried out as necessary but should not result in dogs becoming wet. When enclosures are hosed down, the dogs should be removed from the enclosure to a dry place and returned only when it is reasonably dry.

Background: Maintenance of good cleaning regimes is an obvious requirement to meet within dog facilities. It is emphasised by the Expert Group that persistent daily hosing down of enclosures with the dogs present can lead to them being almost constantly wet and this was also addressed during the Berlin Workshop discussions (1993). This situation is undesirable in relation to health and welfare. It should also be noted that cleaning is a task which should bring staff into close contact with animals. Consideration should be given to exploiting this opportunity to develop 'social contacts' between carers and dogs.

4.8. Handling (*See item 4.1 above and item 4.10 of the General section of Appendix A*)

4.9. Humane killing (*See item 4.11 of the General section of Appendix A)*

4.10. Records (*See item 4.12 of the General section of Appendix A*)

4. 11. Identification (*See item 4.13 of the General section of Appendix A)*

PART B - DOGS

References

Bebak J & Beck AM (1993) The effect of cage size on play and aggression between dogs in purpose-bred beagles. Laboratory Animal Science 43: 457-459.

Berlin Workshop (1993) The accommodation of laboratory animals in accordance with animal welfare requirements: proceedings of an International Workshop held at the Bundesgesundheitsamt, Berlin; 17-19 May 1993.

Clark, J.D., Calpin. J.P., & Armstrong, R.B. (1991) Influence of type of enclosure on exercise fitness of dogs. American Journal of Veterinary Research, 52: 1024-1028.

DeLuca AM & Kranda KC (1992) Environmental enrichment in a large animal facility. Lab Animal 21: 38-44.

Elliot O & Scott JP (1961) The development of emotional distress reactions to separation in puppies. Journal of Genetic Psychology 99: 3-22.

FAWC (1993) Second Report on Priorities for Research and Development in Farm Animal Welfare. Tolworth: Ministry of Agriculture, Fisheries and Food.

Fox MW (1965) Environmental factors influencing stereotyped and allelomimetic behaviour in animals. Lab Animal Care 15(5): 363-370.

Fox MW (1971) Integrative development of Brain and Behaviour in the Dog. University of Chicago Press, Chicago.

Fox MW (1978) The dog its domestication and behaviour. New York & London: Garland STPM Press.

Fox MW and Stelzner D (1966) Behavioural effects of differential early experience in the dog. Animal Behaviour 14 : 273-281

Fuller JL (1967) Experiential deprivation and later behaviour. Science 158: 16-45.

Grant D Skin Disease in the Dog and Cat 2nd Edition. Blackwell Scientific Publications.

Hetts S, Clark JD, Calpin JP, Arnold CE & Mateo JM (1992) Influence of housing conditions on beagle behaviour. Applied Animal Behaviour Science 34: 137-155.

Home Office (1995) Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments. London: HMSO.

Hubrecht RC (1993) A comparison of social and environmental enrichment methods for laboratory housed dogs. Applied Animal Behaviour Science 37: 4 345-361.

Hubrecht RC (1995) Enrichment in puppyhood and its effects on later behavior of dogs. Laboratory Animal Science 45: 1 70-75.

Hubrecht R (1994) Housing husbandry and welfare provision for animals used in toxicology studies: results of a UK questionnaire on current practice.

Hubrecht RC (1997) Comfortable quarters for laboratory dogs, in Comfortable Quarters for Laboratory Animals, pp 63-74, ed. Reinhardt V, Washington DC: Animal Welfare Institute.

Hubrecht RC, Serpell JA & Poole TB (1992) Correlates of pen size and housing conditions on the behaviour of kennelled dogs. Applied Animal Behaviour Science 34: 365-383.

Hubrecht R, Sales G, Peyvandi A, Milligan S & Shield B (1997) Noise in dog kennels, effects of design and husbandry. Animal Alternatives, Welfare and Ethics (Series: Developments in Animal and Veterinary Sciences) pp 215-220.

Jager L, Bruyan L & Potgieter F (1997) Effect of vermiculite bedding material on the incidence of lung malignancies: implications for case-control studies. Animal Technology Vol 48, No 1.

Loveridge G (1994) Provision of environmentally enriched housing for dogs. Animal Technology 45: 1-19.

MacArthur J (1987) The Dog. In The UFAW Handbook on the Care and Management of Laboratory Animals 6th edition, Poole T (ed).

Potgieter F & Wilke P (1996) The dust content, dust generation, ammonia production and absorption properties of three different rodent bedding types. Laboratory Animals 30, 79-87.

Sabine J (1975) Exposure to an environment containing the aromatic red cedar, Juniperus virginiana: procarcinogenic, enzyme-inducing and insecticidal effects. Toxicol 5:221-235.

Sales G, Hubrecht R, Peyvandi A, Milligan S & Shield B (1997) Noise in dog kennelling: Is barking a welfare problem for dogs? Applied Animal Behaviour Science 52: 321-329.

Scott, J.P. & Fuller, J.L. (1965). Genetics and the Social Behaviour of the Dog. Chicago: University of Chicago Press.
Scott J P, Stewart, J M & De Ghett, VJ (1974) Critical periods in the organisation of systems. Developmental Psychobiology, 7: 489-513.
Serpell, J., & Jagoe, J.A. (1995). Early experience and the development of behaviour. In J. Serpell (Ed.), The domestic dog: its evolution, behaviour and interactions with people (pp 79-102). Cambridge: Cambridge University Press.
Tennekes H, Wright A & Dix K (1981) Effects of dieldrin, diet and bedding on enzyme function and tumour incidence in livers of CF-1 mice. Cancer Research 41:3615-3620.
Thompson WR, Melzack R, Scott TH (1956) "Whirling behaviour" in dogs as related to early exposure. Science 123: 393.
Vessell E (1973) Induction of drug-metabolising enzymes in liver microsomes of mice and rats by soft wood bedding. Science 179: 896-897.
Voipio H, Haataja H & Nevalainen T GLP requirements of bedding materials (1986) In Safety Evaluation of Chemicals on Laboratory Animals pp171-182, Nevalainen et al (eds).
Wright JC (1983) The effects of differential rearing on exploratory behaviour in puppies. Applied Animal Ethology 10: 27-34.

General References

86/609/EEC: Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.
Andersen AC & Good LS (1970) The Beagle as an experimental animal. Ames, Iowa State University Press.
Home Office (1989) Code of Practice for the Housing and Care of Animals used in Scientific Procedures. London: HMSO.
ILAR Commission on Life Sciences (1996) Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington DC.
White WJ, Balk MW, Slaughter LJ (1974) Housing requirements – dogs and cats. In Handbook of Laboratory Animal Science Vol 1, Melby EC & Altman NH (eds).

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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)

6th meeting
Strasbourg, 25-27 March 2003

Species-specific provisions for ferrets

Background information for the proposals presented by the Group of Experts on Dogs, Cats and Ferrets

PART B

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Species-specific section - Ferrets

Preamble

In 1997, the Council of Europe (CoE) established four Expert Groups in order to advise the CoE Working Party on revisions to Appendix A of the Convention ETS123 (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 1986). One of these Expert Groups was established to consider proposals for dogs and cats; its remit was later extended to include ferrets when the CoE Working Party decided that other species covered by ETS123 should be included in the revision. Organisations represented both on the Group and at its meetings were:

Federation of European Laboratory Animal Science Associations (FELASA) – Coordinator of Group
Eurogroup for Animal Welfare
European Federation of Pharmaceutical Industries and Associations (EFPIA)
Federation of Veterinarians in Europe (FVE)
Federation of European Laboratory Animal Breeders Associations (FELABA)
International Society for Applied Ethology (ISAE)

The members of the Expert Group on dogs, cats and ferrets met on several occasions and also exchanged information by e-mail. The Coordinator of the Expert Group, accompanied by one or more other members, attended all meetings of the Working Party in Strasbourg, in order to present the Group's proposals, discuss their content and answer questions, and refer matters back to the Group where appropriate.

The CoE Expert Group on Dogs, Cats and Ferrets has provided three separate reports, covering each of these species. Each report comes in two parts. Part A details the proposals for amendments to Appendix A, as agreed by the Working Party and including amendments to the Expert Group's original proposals as required by the Working Party. Part B provides background information for these proposals. Where possible, recommendations have been based upon scientific evidence; where this is not available, they take account of established good practice, based both on the experience of the members of the Expert Group and also on further consultations with other experts. Additional comments have been received from members of the Working Party and from a range of non-governmental organisations and individuals. These have been considered by the Expert Group and incorporated in the proposals where appropriate.

The proposals and their rationale are therefore the outcome of extensive and detailed discussions within the Group and should be regarded as expert recommendations, reflecting the scientific evidence and information on good practice available at the time. Their intention is to increase the welfare of animals used in research, taking into account the purposes for which such animals are used, which may place some constraints upon their housing and husbandry.

The Expert Group considered that, although the provisions of Appendix A formally are guidelines, its finalised proposals should be regarded as minimum requirements. Knowledge gained by further research and scientific evidence, as well as changing views on what is current 'good practice', may mean that the accommodation and care provided for animals in research in the future may vary from these proposals such that future revision of Appendix A is necessary.

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1. Introduction

Ferrets are small carnivores, weighing up to 2.5kg in the case of adult males, and are thought to be a domesticated form of the wild polecat. There is a pigmented and an albino form of the ferret, the former being genetically dominant, having a brown or ginger and black coat and being known as the polecat ferret. The fur in albino males may become very yellow owing to the secretions of the sebaceous glands.

Under natural conditions they feed on small mammals, birds, fish and invertebrates. They do not digest vegetable material well, hence the need for a high animal protein level in the diet. They tend to hoard food, but will not eat decayed matter.

They have a complex hunting behaviour and have therefore been used for hunting small animals such as rabbits. This is linked to their living habits, normally in burrows, and it is therefore important to provide in their living environment enrichments such as tubes and boxes, in which they can crawl, sleep and play games.

Studies of feral ferrets indicate that they are normally solitary animals, with a single male occupying a large territory with several females. However, in captivity they seem to benefit greatly from social housing in groups and spend a considerable amount of time in play behaviour.

Ferrets usually breed once a year, mating in the spring. During the breeding season, male animals are hostile to and will fight vigorously with unfamiliar males, which can result in serious injuries and even to death. As a consequence, at this time single housing of males is advisable.

The ferret is an intelligent, inquisitive, playful and agile animal and is often kept as a pet. It can be taught to come in response to a voice command and also to work on leads or lines. It will investigate all areas of its enclosure and will exploit any opportunity for escape. This should be taken into account in the design of the accommodation and a complex, escape-proof enclosure is required which also provides opportunities to the ferret to exhibit a wide behavioural repertoire.

Research uses of the ferret include studies associated with emesis, as it has a strong vomiting reflex, and viral diseases such as human influenza, owing to its susceptibility. It is highly susceptible to canine distemper virus, with high mortality, and vaccination of stock is advised. The vaccine must be indicated by the manufacturers for use in ferrets, as some distemper vaccines contain live virus produced in ferrets, which could produce clinical infection post-vaccination.

2. The environment and its control

2.1. Ventilation (*See item 2.1. of the General section of Appendix A*)

Background: The air in ferret enclosures should be renewed 10 to 15 times per hour but without draughts. It is important both to attenuate the musk odour while minimising the risk of viral respiratory diseases, to which the ferret is very sensitive. Tiered racks of cages are likely to require higher rates than floor pens (Home Office, 2002). Lower stocking densities will require fewer air changes.

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2.2. Temperature
Ferrets should be maintained in the temperature range 15 to 24°C.
Ferrets do not have well-developed sweat glands and heat exhaustion may occur if they are exposed to high temperatures

Background: The ferret does not have well-developed sweat glands, and is prone to heat exhaustion when exposed to high temperatures (Plant, 1999). Adult ferrets can tolerate well temperatures as low as 4oC, however young unweaned ferrets should not be maintained below 15oC. Heating or cooling may therefore be required in cold winters or hot summers to ensure a comfortable environment is maintained. Particular care must be taken during transport to avoid the animals being exposed to high temperatures (Home Office, 2002).

2.3. Humidity
It is considered unnecessary to control or record relative humidity as ferrets can be exposed to wide fluctuations of ambient relative humidity without adverse effects.

Background: There are no specific adverse effects reported as a result of ferrets being exposed to wide fluctuations of ambient relative humidity. However, high humidity levels should be avoided, especially if the temperature is low, to minimise the occurrence of viral respiratory disease.

2.4. Lighting
The light source and type should not be aversive to the animals and particular care should be taken with ferrets, especially if albino, in the top tier of tiered racking systems.

Holding of ferrets under the natural 24 hours light-dark cycle is acceptable.

Where the light part of the photoperiod is provided by artificial lighting, this should be for a minimum of 8 hours daily and should generally not exceed 16 hours daily.

However, it should be noted that the duration of light-dark cycles is important for the manipulation of the reproductive cycle in the ferret and the light period may be reduced to 6 hours under these circumstances. This will need to be increased to 14-15 hours when it is required to stimulate oestrus in the female.

If natural light is totally excluded, low level night lighting should be provided to allow animals to retain some vision and to take account of their startle reflex.

Background: Lighting levels and durations must not only allow for easy and regular inspection of the ferrets but also allow for necessary manipulation of the reproductive cycle. The natural light cycle is normally acceptable; where artificial lighting is provided, a minimum light period of 8 hours is considered adequate. This can be reduced to 6 hours and then increased to a longer day period (up to 15 hours of light) to stimulate the occurrence of oestrus (Plant, 1999). It should be noted that the male is light negative and requires the opposite light cycles to the female to stimulate their season; this should commence several months before required so as to ensure sperm maturity. In tiered racking systems, and particularly with albino ferrets, it is important to avoid the exposure of animals to high intensity lighting, although no specific levels are referenced.

2.5. Noise
Lack of sound or auditory stimulation can be detrimental and make ferrets nervous. However, loud unfamiliar noise and vibration have been reported to cause stress-

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related disorders in ferrets and should be avoided. It is important to consider methods of reducing sudden or unfamiliar noise in ferret facilities, including that generated by husbandry operations within the facility and also by ingress from outside sources. Ingress of noise can be controlled by appropriate sitting of the facility and by appropriate architectural design. Noise generated within the facility can be controlled by noise absorbent materials or structures. Expert advice should be taken when designing or modifying accommodation.

Background: No data are available on the hearing range of the ferret, however the evoked auditory response range is 1-6 kHz up to 32 days of age, 4-15 kHz for adults, but up to 100 kHz for pregnant females (Plant, personal communication). It is necessary to avoid sharp, loud or otherwise unfamiliar noises and vibration, as these are considered to cause stress-related disorders in ferrets. The most important factor is familiar noise, and the lack of sound or auditory stimulation can be detrimental, making animals jumpy and nervous. A soft and varied background noise may stimulate the sensory and social development of the young ferret.

<u>2.6. Alarm systems</u> *(See item 2.6 of the General section of Appendix A)*

3. Health *(See items 4.1 and 4.4 of the General Section of Appendix A)*

Background: The ferret is particularly sensitive to certain virus conditions, such as Aleutian disease and canine distemper. Human influenza virus may cause clinical disease in ferrets and appropriate preventive measures should therefore be taken in consultation with the attending veterinary surgeon. It should be noted that humans may also be infected by ferrets suffering from influenza.

Pregnancy toxaemia and Oestrus-induced anaemia are not uncommonly encountered in jills. Pregnancy toxaemia results from feeding an inadequate diet during pregnancy, particularly in jills carrying large litters. Oestrus-induced anaemia occurs in jills kept in the absence of a male during the breeding season. As the ferret is an induced ovulator, such jills may remain in oestrus for several months. The vulva may become very swollen and susceptible to trauma, also haematopoiesis is suppressed and a severe anaemia may develop. This may be managed by mating with a vasectomised male or by use of hormonal preparations, on the advice of the attending veterinary surgeon.

Breeding males should be housed singly during the breeding season as they are highly aggressive during this time and severe injuries can result from fighting. During mating, females may suffer neck injuries.

4. Housing and enrichment

These guidelines have been formulated to encourage the social housing of ferrets and to permit adequate enrichment of the environment. It should be noted that within this concept and strategy every encouragement is given to holding ferrets in larger and socially-harmonious groups both to extend the available floor space even further and to enhance the socialisation opportunities.

Background: The domestic ferret is thought to descend from the European polecat. Porter & Brown (1993) describe the natural behaviour of the polecat as follows. The wild polecat uses underground burrows, often with more than one entrance. Side chambers are used for sleeping areas and to store food. The sleeping chamber is lined with dry nesting material by the polecat, which keeps its nest clean and may even air its bedding. The polecat defaecates and urinates away from its burrow and

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uses vertical surfaces to scent mark. Nesting jills dig out a chamber usually near the end of the burrow, far away from the entrance. Feral ferrets when established behave in a similar way to the polecat. The ferret in captivity therefore requires a dry warm sleeping chamber, discrete eating and food storage areas, and a vertical surface for scent-marking well away from the sleeping and eating areas (Porter & Brown, 1993; Plant, 1999). Enclosures can be made from various materials such as wood, plastic, fibreglass or metal but care should be taken when using galvanised metal as zinc toxicity has been noted (Fox, 1998). Soiled litter should be removed daily and it may be useful to provide a soil tray in a corner of the enclosure.

4. 1. Housing

Although in the wild the ferret is a generally a solitary animal, there would seem to be welfare benefits by providing more opportunities for enrichment by housing them in socially harmonious groups within the animal enclosure, unless there are scientific or welfare justifications for single housing.

During the breeding season, adult males may need to be maintained singly to avoid fighting and injury. However, males can be maintained successfully in groups at other times.

Pregnant females should be housed singly only during late pregnancy, no more than 2 weeks prior to parturition.

Separation of animals that are normally group housed can be a significant stress factor. Where this is for a period of more than 24 hours, it should be regarded as severely compromising the welfare of the animals. If for scientific purposes, this should be taken fully into account when considering the justification for the procedures.

Where animals are single-housed, whether for scientific or welfare reasons, additional resources should be targeted to the welfare and care of these animals. Additional human socialisation time, and visual, auditory and, where possible, tactile contact with other ferrets should be provided for all single-housed animals on a daily basis.

Background: Male polecats occupy large territories which include those of females but are thought to be relatively solitary (Plant, 1999). Experience shows, however, that ferrets are sociable animals and should normally be housed socially as social interactions are a major environmental enrichment for them (Wolfensohn & Lloyd, 1998; Plant, 1999). Care should always be taken when grouping animals, or introducing new animals to a group, to ensure that they are compatible. It has been found that gradual introduction, first by vision and smell, may be beneficial. However, group housing is not always advisable for males in the breeding season and females with litters, mated or in oestrus (Plant, 1999). Where animals are normally group-housed, the Expert Group considered that any isolation could be a stress factor and should therefore be avoided. Where this was for a period in excess of one day, it was considered this potentially could have a significant impact on the animal's welfare and should therefore be specifically justified to, and approved by, the Responsible Authority. Practical experience has shown that singly-caged ferrets show reduced exploratory movements during routine maintenance of litter material and generally are less active. They put on weight more quickly and are in poorer bodily condition than group-housed animals, which may be associated with their lower activity levels as well as the prompting of behaviours by other members of the social group in group-housing situations.

Socialisation

The ferret is an inquisitive, intelligent, playful and agile animal and this should be taken into account by providing regular interactions with other ferrets by group housing and by regular handling. In general, ferrets seem to benefit from such regular and confident handling and this should be encouraged. It will result in a better quality and more sociable animal.

Social behaviour in ferrets develops at an early age and it is important that the young ferret has social contacts with other ferrets (e.g. litter mates) and with humans (e.g. animal caretakers). Daily handling during this sensitive stage of development is a prerequisite for the social behaviour of the adult ferret. It is reported that the more frequent the interactions, the more placid the animal will become, and these interactions should be continued through into adult life.

A major form of environmental enrichment for ferrets is the provision of group housing. Where this is not possible, additional resources should be targeted.

4.2. Enrichment

The design of animal enclosures should meet the animals' species- and breed-specific needs. It should be adaptable so that innovations based on new understanding may be incorporated.

The design of animal enclosures should allow some privacy for the ferrets and enable them to exercise some control over their social interactions.

Separate areas for different activities, such as by raised platforms and pen sub-divisions, should be provided in addition to the minimum floor space detailed below. Where nesting boxes are provided, these should be designed to contain the young ferrets within the nest.

Provision of containers and tubes, of cardboard or rigid plastic, and paper bags stimulates both investigative and play behaviour. Water baths/bowls have been reported to have been used extensively by ferrets.

Background: It is generally accepted that the ferret is an inquisitive and active animal and requires an interesting and complex environment (Porter & Brown, 1993; Wolfensohn & Lloyd, 1998; Plant, 1999). Ferrets in all situations may show stereotypies, such as running movements repeated over a short distance, and enrichment should be aimed at reducing their incidence. Social enrichment should be provided by housing socially as described. Physical enrichment of enclosures to stimulate investigation and play can be provided through the use of tubes, either plastic or cardboard, containers of various materials, and paper bags. Boxes are a form of enrichment that will not only help meet the species-specific needs but will also provide privacy for the ferrets. Deep litter for group-housed animals is also beneficial as an enrichment (Plant, 1999). An example of the type of enrichment device that can be provided in a ferret enclosure is shown in the photograph:

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Photograph courtesy of GlaxoSmithKline R&D

4.3. Animal enclosures – dimensions and flooring

Animal enclosures, including the divisions between enclosures, should provide an easily cleaned and robust environment for the ferrets. In their design and construction they should seek to provide an open and light facility giving the ferrets comprehensive sight of other ferrets and staff, outside of their immediate animal enclosure. There should also be provision for the ferrets to seek refuge and privacy within their own enclosure and, in particular, away from the sight of ferrets in other enclosures.

It should be noted that ferrets are inquisitive and agile animals with a remarkable ability to escape. The design of the enclosure should be such that the animal is unable to escape and also unable to injure itself should any attempt be made.

The recommended minimum height of the enclosure should be 50 cm. The ferret is an inquisitive animal that enjoys climbing and this height allows for some vertical enrichment. The floor space should provide an adequate area for movement and to allow the animal the opportunity to select sleeping, eating and urination/defecation areas. In order to provide enough space for environmental complexity, no animal enclosure should be less than 4500 cm². Minimum space requirements for each ferret are therefore as follows:

Single housing	4500 cm²
Group housing	
up to 600g	1500 cm² per animal
over 600g	3000 cm² per animal
Adult male	6000 cm² per animal
Jill + litter	5400 cm²

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It is recommended that animal enclosures should be of a rectangular shape rather than square, to facilitate locomotor activities.

Constraint in less than the above space requirements for scientific purposes, such as in a metabolism cage, may severely compromise the welfare of the animals

Background: Housing should provide adequate area for movement and ample height to allow the animal to stand on its hind legs. It should also provide sufficient space to meet the requirements described under (4) above. Most recommended dimensions refer to the breeding and keeping of ferrets as working animals or pets (e.g. Porter & Brown, 1993). Recommended enclosure dimensions for ferrets have been developed by a UK expert group and have been adopted by the Home Office as a supplement to their code of practice for breeders and suppliers. This expert group finalised its recommendations after examining excellent ferret accommodation created by modifying disused dog pens. These housed ferrets in an area of 4.5 square metres with ample room for enrichment. Some breeders and users have provided complex wooden sleeping boxes complete with tunnels within these pens. A further set of recommendations produced by the UK Animal Procedures Committee advocated more spacious accommodation. In preparing the above space dimensions, the Council of Europe Expert Group considered both of these sets of recommendations, but formulated its proposals after further discussions with those with considerable practical experience in the housing of ferrets both for breeding and research purposes. The basic criterion used was the need to accommodate the minimum environmental enrichment that it was considered should be provided, in the form of tubes, tunnels, boxes, ladders etc, while also meeting the physiological needs of the ferrets for separate areas of their accommodation for feeding, sleeping and urination/defaecation. The above figures are therefore those that it is considered are the minimum required to allow animals to express a basic behavioural repertoire.

Flooring

The flooring for ferret accommodation should be a solid continuous floor with a smooth non-slip finish. Additional enclosure furnishment such as beds or platforms should provide all ferrets with a warm and comfortable resting place.

Open flooring systems such as grids or mesh should not be used for ferrets.

4.4. Feeding (See item 4.6 of the General section of Appendix A)

Background: The ferret is a carnivore. The diet should provide high levels of animal protein and fat. High carbohydrate levels can lead to protein deficiency because the animals will eat to meet their calorie requirements. Dietary fibre is not a particular requirement. These requirements are met by ferret diets available from commercial manufacturers. The nutritional requirements of the pregnant or lactating jill require particular attention, as poor or inappropriate diets can lead to poor reproductive performance, including poor conception rates, small litter numbers and pregnancy toxaemia. Plant (1999) notes that feeding of raw fish and eggs should be avoided as they contain an excess of thiaminase and may predispose to thiamine deficiency. Also, raw fish may have high levels of nitrates.

4.5. Watering *(See item 4.7 of the General section of Appendix A)*

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<u>4.6. Substrate, litter, bedding and nesting material</u>
Bedding material is required for all ferrets. In addition, nesting materials such as hay, straw or paper should be provided. Deep litter systems are considered to provide additional enrichment.

It is good practice to use some litter or substrate material at least to facilitate cleaning and minimise the necessity to wash/hose down regularly.

<u>4.7. Cleaning</u>
Wet cleaning by hosing down of animal enclosures should not result in ferrets becoming wet. When animal enclosures are hosed down, the ferrets should be removed from the enclosure to a dry place and returned only when it is reasonably dry.

Ferrets tend to defecate in one area of the enclosure against a vertical surface. Provision of a litter tray may be beneficial and reduce the frequency of cleaning required for the remainder of the enclosure.
Each occupied enclosure should be cleaned at least daily. All excreta and soiled materials should be emptied from litter trays and/or removed from all other areas used by ferrets at least daily and more frequently if necessary.

Background: An appropriate cleaning regime is a pre-requisite of good husbandry and health for ferrets. This regime needs to take account of the normal behaviours of the ferret, in particular the use of a vertical surface for urination, defaecation and scent-marking. However, ferrets may use litter trays and this will reduce the frequency of cleaning for the rest of the enclosure. The zoning of the enclosure into eating, sleeping and urination/defaecation areas should be respected when replacing litter trays. Ferrets do not normally like becoming wet and should be removed when the enclosure is being hosed down.

<u>4.8 Handling</u> (*See item 4.11. of the General section of Appendix A)*

Background: Handling of ferrets from an early age is particularly important and will lead to a more placid animal that is less stressed by handling when adult. Frequency of handling is more important than the duration of the interaction. Particular care should be taken when handling nursing mothers and sick animals, as these can be aggressive. It is essential that handlers are experienced and confident.

<u>4.9. Humane killing</u> (*See item 4.12. of the General section of Appendix A)*

<u>4.10. Records</u> (*See item 4.13. of the General section of Appendix A)*

<u>4. 11. Identification</u> (*See item 4.14. of the General section of Appendix A)*

Background: Use of collars, as for cats, may be a suitable method of identification for temporary purposes, as may the use of coat dyes for albino animals. However, the preferred method of permanent identification is by microchipping. Ear tattooing and ear tags are not suitable as the ferret ear is small and comparatively delicate.

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References

Clingerman KJ & Fox JG, 1991: Ferrets as Laboratory Animals: a Bibliography USDA, NAL

Fox JG, 1998: Biology and Diseases of the Ferret. Lea & Febiger, Philadelphia

Home Office Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments – Supplement, 2002. HMSO, London

Plant M, 1999: The Ferret. In: The UFAW Handbook on the Care and Management of Laboratory Animals, 7th edition (Ed T Poole). Blackwell Science, Oxford

Porter V & Brown N: The complete book of ferrets (revised edition). D & M Publications, Bedford

Wolfensohn S & Lloyd M, 1998: Handbook of laboratory animal management and welfare, 2nd edition. Blackwell Science, Oxford

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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)

6th Meeting
Strasbourg, 25-27 March 2003

Species-specific provisions for Non-Human Primates

Background information for the proposals presented by the Group of Experts on Non-Human Primates

PART B

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NON-HUMAN PRIMATES

SECTION B : SCIENTIFIC JUSTIFICATION

1 Introduction

Normal behavioural repertoire

The aim for captive primates should be for them to have the facility to carry out a wide range of normal behaviour providing it does not result in injury or distress to itself or another animal (Schapiro et. al. 1996). It is particularly important that the enclosure should enable the animals to adopt normal postures and a range of locomotor behaviour (Buchanan-Smith 1997, Home Office 1989, International Primatological Society 1993, Marriott *et al.*1993, Olfert *et al.*1993, Poole 1991, Poole *et al.*1994, Reinhardt 1997a, Snowdon and Savage 1989, Whitney and Wickings 1987). Test conditions lacking adequate environmental enrichment should be regarded as testing under abnormal conditions (Kessel and Brent, 1995a; Reinhardt *et al.*1996), which in certain cases may lead to false conclusions from the results.

However, in captivity, non-human primates may perform abnormal behaviours, such as locomotor stereotypies, self directed behaviour and even self mutilation. Such behaviours are indicative of unsatisfactory environmental conditions according to Bayne *et al.* (1992), Broom and Johnson (1993). However, the presence of stereotypies may reflect past, not present, environments (Brent and Hughes 1997, Broom and Johnson 1993, Mason 1991a, b, Mason and Mendl 1993). The causes may be either a restricted social environment during development or early infant separation from the mother and the behaviour may be untreatable (Capitanio 1986, Mason and Berkson 1975, Novak and Drewson 1989, O'Neill 1989) or only eliminated with considerable effort (Kessel and Brent 1997). Cage height may also be a factor, as long-tailed macaques were found to exhibit less self-directed stereotypy in taller cages as compared with those in shorter ones (Watson and Shively, 1996). In a study of the influence of cage size and behaviour, pairs of common marmosets were found to show stereotypies and higher levels of aggression and startle responses in smaller cages (Kitchen and Martin, 1996). From this literature, it is apparent that three factors can induce abnormal behaviour in mammals, but that these act in combination, for primates they are single housing, small cages and lack of environmental complexity. Thus, minimum or even optimum cage size alone cannot be quantified scientifically.

From this literature, it can be concluded that the captive environment should provide non-human primates with an adequately complex social and physical environment. Cage dimensions should allow for sufficient structures to enable them to carry out a wide range of normal behaviour and exhibit a minimum of abnormal ones.

2 Health

Health:

Non-human primates are known to sometimes harbour viruses, bacteria, protozoa and other endoparasites that can be harmful or even fatal when transmitted to humans. Herpes B virus, Marburg virus and *Mycobacterium tuberculosis* are the three pathogens which have received the widest concern. (Hazards of handling simians, Laboratory Animal Handbooks 4, 1969). Additional agents, e.g. of the retrovirus group pose a health risk to other primates in a colony. Some diseases are endemic in certain areas in the wild population but not in others and may lead to severe outbreaks when transmitted to primates from areas that are free of the agent. Unfortunately the reservoir species for certain diseases are not always known and not all species show clinical symptoms after infection.

The use of purpose-bred animals has helped to minimise such health problems, and reputable breeders will supply a health certificate based on their health monitoring scheme. This may help shorten the compulsory quarantine period. The fact that infections may go unobserved for a long time necessitates a regular screening of the animals. Eradication of the disease is not always easy through treatment, but knowing the microbiological status of the animals will allow the necessary precautions to be taken to prevent transmission to other animals and the staff. Guidelines for health monitoring have been proposed by FELASA (1999).

Some diseases may be transmitted from humans to primates. Typical is tuberculosis for which most non-human primates are highly susceptible and cannot effectively be treated. Therefore, screening of the staff, and precautions with personnel with health problems is essential.

Rodents, birds and insects, and for animals in the source country, wild conspecifics may be a threat to the captive bred groups. Especially for groups in outdoor enclosures preventive measures against intrusion of possible disease vectors are essential.

The reason for scrupulous separation of animals from different geographical areas during transport and until the health status of the animals has been clarified is that transmission from some silent pathogens in reservoir species causing severe outbreaks of disease in animals from other geographical areas cannot be excluded.(FELASA, 1997).
The following references cover the subject of health monitoring: FELASA (1997, 1999), OIE (1999a,b).

3 Housing and enrichment

3.1 Social housing

It has already been pointed out that being part of a compatible group provides a sense of security for the vast majority of non-human primates. It also provides opportunities for a whole range of species-specific social activities such as grooming, embracing, huddling, patting and kissing (Cheney *et al.*1987, Jolly 1985).In most species, social bonds are forged and maintained by grooming which is therefore of paramount importance in maintaining social cohesion (Williams and Bernstein 1995). In addition it has been demonstrated that grooming has a relaxing effect on the animal being groomed, lowering the heart rate (Boccia et al. 1989). Singly housed primates are particularly prone to show abnormal behaviour, whereas keeping them in groups reduces the incidence of this behaviour (in long tailed macaques Line *et al.*1990, rhesus monkeys, Schapiro *et al.*1996, squirrel monkeys, Spring *et al.*1997, macaques and baboons, Woolley 1997). Isolation and prevention of contact with companions may also lead to altered physiological parameters e.g. elevated blood pressure in baboons (Coelho *et al.*1991), altered cell mediated immune responses in rhesus monkeys (Schapiro *et al.*1997).

Reinhardt *et al.*(1995a) pointed out that many of the reasons commonly given to justify single housing, such as difficulties in creating compatible pairs and aggression between cage mates are not supported by the evidence. Most Old World primates are put into groups as juveniles aged up to twelve months as this avoids most of the problems of incompatibility, but it is possible to form compatible groups of macaques well into adolescence. In some species such as vervets and rhesus monkeys there may be disruption in such groups as they approach maturity. However, Reinhardt *et al.*(1995a) and Lynch and Baker (1998) were successful in establishing adult macaques as long term compatible pairs. For this procedure to succeed, it is necessary to assess the attitudes of the animals to the prospective partners and to carefully monitor their behaviours after introduction. Reinhardt *et al.* (1995a) outlined a nine step partner evaluation and introduction technique. It is important, however, to bear in mind that placing two animals in a bare small cage can lead to conflict; an appropriately complex cage environment should also be provided (APHIS 1999).

For groups of maturing same sex monkeys, the proximity of members of the opposite sex in nearby cages can lead to intra-group aggression among previously amicable animals, especially males. Same sex grouped monkeys should therefore be well separated from enclosures containing members of the opposite sex.

To conclude, virtually all primates commonly used in laboratories are highly social and need to be kept with one or more compatible conspecifics, this provides them with a sense of security, companionship and opportunities for a wider range of normal behaviour. Their physiological condition also benefits.

Separation and weaning
Young monkeys have a long period of dependency on their mothers and natal group. During this period they learn about their environment under the mother's protective vigilance, they also learn social and parenting skills from other group members.

Early separation results in extreme distress to the infant at the time, but it is now well established that it damages normal development and results in animals which are physiologically and immunologically abnormal, as adults. Nursery rearing, in the absence of adults, also commonly results in behavioural abnormalities, such as locomotor stereotypies and auto-aggression (Capitanio 1986, Marriner and Drickamer 1994). Even separation for as little as two weeks during the animal's first year of life can have permanent adverse effects on the immune system of pig tailed macaques (Laudenslager *et al.* 1990).

For most species, the best way to produce behaviourally and physiologically normal monkeys, suitable for breeding and long term study, is to ensure, wherever practicable, that they remain in the natal group for as long as possible, ideally for the first 18 months of their lives. Juveniles separated from their mothers for whatever reason should be reared in social, preferably well organised groups.

3.2 Environmental complexity
Primates should be housed in enriched environments which allow them to carry out a normal behavioural repertoire, showing species-typical behaviour which is complete and well balanced (Brent and Long 1995, Chamove and Anderson 1989, Fragaszy 1991, Olfert et. al. 1993, Poole 1991, 1992, Rose 1994, Toates 1995, Line 1987).

Animals cannot be expected to carry out every natural behaviour in captivity but, excluding the extremes, behaviour seen in nature provides a useful guide (Veasey et. al. (1996), Rosenblum and Andrews 1995, McGrew 1981). Captive conditions should fit within the adaptive range of the species (Kaumanns 1997).

However the animals should be able, as in the wild to carry out a complex daily programme of activity. While it is seldom possible to provide the majority of features of the wild habitat in a laboratory, major attributes can be provided. In the wild, primates require a secure environment (provided by a familiar home range and their social group), a sufficient amount of appropriate complexity (to enable them to carry out a wide behavioural repertoire) and facilities to enable them to achieve objectives. Finally, as natural environments are not invariant, a level of novelty to which they can respond adaptively is necessary. If these features are incorporated into the captive environment, primates can lead a full and interesting life (Poole 1998).

A sense of security
Non-human primates show adaptive flight responses. They respond to ground predators by fleeing upwards into trees or cliffs and downwards to mid-level branches or to the ground to avoid aerial predators (Seyfarth and Cheney 1980). In addition, while social groups are cohesive, a certain amount of dispersion of individuals has been observed in both field and captive studies. There is general agreement among primatologists that enclosures for primates should enable them to fully utilise the vertical dimension (Abee 1985, Buchanan-Smith 1997, Dukelow and Asakawa 1987, International Primatological Society 1993, Maple and Perkins 1996, Olfert *et al.*1993, Poole *et al.*1994, Reinhardt *et al.*1996, Queyras et al.1997).

Their sense of security both from predators and also from rivals, depends on being able to reach a high point in their environment; dominant squirrel monkeys used the highest available perches (Williams *et al.*1988). Furthermore, long tailed macaques (Woodbeck and Reinhardt 1991), rhesus monkeys (Watson and Shively 1996) marmosets and tamarins (Caine et. al. 1992; Prescott and Buchanan-Smith 2002; Ely et al. 1998; Kerl and Rothe 1996; Buchanan-Smith et al. 2002) all show preferences for the upper part of their cages.

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Hediger (1964) pointed out that a captive mammal's liability to panic and hence its sense of security depends on the enclosure allowing for an adequate flight response. In the case of primates, this relates to the enclosure being of adequate height to allow adequate vertical movement ; primates tend to move to a position where they can look down on the perceived threat.

A second, important, factor which provides the primate with a sense of security, is the presence of one or more social companions. Apart from orang utans and some prosimians, primates live in social groups. This enables them to detect predators more efficiently and escape from them or defend themselves against their smaller enemies. The most vulnerable time in the life of an adult wild primate is when it emigrates from one troop to another. Even when unwelcome, the isolated animal will attempt to attach itself to a group, for example, living on the periphery of an established social group, as in forest dwelling guenons, or joining bachelor troops, as in langurs and desert baboons. For the vast majority of primates, therefore, a social group is essential to ensure their security.

Complexity

A complex environment, which includes swings, perches and branches allows the animals to display a wide locomotor repertoire. Captive rhesus macaques were observed by Dunbar (1989) to walk, gallop, leap, climb, swim and hang from climbing structures. Long tailed macaques, rhesus monkeys and vervets are good swimmers. Leaping is a common mode of locomotion for arboreal species, such as callitrichids, squirrel monkeys and long tailed macaques. Observations in the wild show that squirrel monkeys can leap considerable distances when travelling from branch to branch (Fleagle *et al.*1981) and long tailed macaques commonly make leaps of 2.2 m (Cant 1988). Enclosures should allow for leaping in these species. Space allowances for juveniles should be the same as adults of the species as the former require plenty of space for play (Goosen *et al.*1984). Play is a good indicator of welfare in the young as it is only carried out in a relaxed situation (Fagen 1993, O'Neill et. al. 1990, Thompson, 1996, Pereira et. al. 1989).

Tactile stimuli are also valuable (such as a soft substrate for foraging). Where primates need to be singly housed, fleece pads can be sprinkled with food items. Lam et. al (1991) provided such fleece pads and found that stereotyped behaviour declined by 73%; after the food had been depleted, rhesus monkeys continued to use the fleece for grooming.

Opportunities to achieve objectives.

Primates need to be able to exert some control over their environment, both physical and social; this is essentially a need to be able to achieve objectives. As Sambrook and Buchanan-Smith (1997) have pointed out, this is an adaptive aspect of behaviour. It enables them to adjust to change, alter confronting stimuli, organise response strategies and apply their cognitive capacities (Rosenblum 1991, Neveu & Deputte 1996). It also reduces stress, as indicated by plasma cortisol levels, in a captive environment (Hanson *et al.*1976). A primate's ability to produce predictable environmental changes through its own actions enables it to be comfortable in a captive environment (Fragaszy and Adams-Curtis 1991).

Although puzzle feeders and foraging boxes (Meunier *et al.*1989, Florence & Riondet 2000) are more effective in reducing stereotypic behaviour and increasing activity in rhesus monkeys, watching videos and manipulating video game joysticks, can also be beneficial (Platt and Novak, 1997).

Novelty

Some novelty should also be introduced at intervals (for example objects, which can include destructible materials) or minor changes in the conformation or arrangement of cage furniture (Sambrook and Buchanan-Smith 1997).

It can be concluded that primates require an environment which encourages them to carry out a daily complex programme of activity.

3.3 Enclosures - dimensions and flooring

As previously mentioned, primates' ability to utilise three dimensions and the expression of complex social behaviour necessitates the use of cages and enclosures which have a vertical dimension sufficiently high for them to feel secure and control their social environment. Primates should be able to perch higher than a perceived threat and IPS (1993) recommends that this should be above human eye level. If the cages are to be of adequate height, two tier housing will be impractical as lower-row animals are forced to remain below human eye level (NRC/ILAR 1998). Two tier housing is also unsuitable because the animals in the lower cages are not only subject to poorer lighting conditions, which is unsatisfactory for a diurnal animal, but the stressful situation also increases unnecessary experimental variables (CCAC 1993, IPS 1993). Primates in lower tier cages are also less easily observed by the staff (Reinhardt 1997c, 1999). Visual barriers will help to prevent monkeys in social housing from being stressed by agonistic behaviour of other group members.

For various reasons, it may be necessary to separate an animal from a partner or group. In this situation, it is advantageous for housing to be provided within visual contact of the original cagemate(s) (Lynch 1998, Reinhardt et. al. 1995a).

The animals will spend much time foraging if a substrate is provided in which food can be scattered. The substrate, which must be clean and free from toxic residues, can take the form of straw, wood wool, wood chips, shredded paper, vegetation or soil (Westergaard and Munkenbeck-Fragaszy 1985, McKenzie *et al.* 1986). Chamove *et al.* (1982) found that the provision of a wood chip substrate with scattered food significantly reduced aggression in the majority of socially housed primates. Foraging can also be encouraged by the provision of browse (Shumaker 1995).

Cage inclusions should be sufficient to encourage a natural range of locomotor behaviour (walk, jump, climb, run) by providing, platforms, perches and climbing frames. Young primates also like to use mobile furniture, such as swings and ropes. A complex environment reduces both inactivity and aggression in social groups (Chamove *et al.* 1982, McKenzie *et al.* 1986, Chamove and Anderson 1989).

To conclude, the size of the enclosure/cage will depend not only on the size and number of animals, but also on the inclusion of adequate space for foraging facilities, high platforms or perches and opportunities to carry out a typical repertoire of locomotor and cognitive behaviour. Cages for experimental animals should also be in a single tier to allow for them to be of adequate height, to enable them to retreat from fear-inducing stimuli as they will be stressed if unable to reach an appropriate elevation (Burt and Plant 1990, King and Norwood 1989, Whitney and Wickings 1987)

To avoid the stresses associated with social isolation, unless absolutely essential, experimental animals should not be singly housed. Separation of an animal for experimental purposes can be achieved either by training or enticing the animal into a small subdivision of the cage, with or without further restraint.

3.4 Feeding

Scattered food will encourage foraging (Chamove *et al.* 1982), or where this is difficult puzzle feeders can be provided. Variations in dietary components can provide interest and environmental enrichment

A varied diet, however, should not be provided if it is likely to influence experimental results (Coates 1999). However, many standard diets are available in different flavours and these can be used to provide variation.

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3.5 Substrate

Except for disease eradication programmes, deep litter systems have been shown to be both hygienic and labour saving as Chamove *et al.* (1982) found that bacterial growth was inhibited in wood chip substrate and that it need only be swept out and removed once every 1-4 weeks. In outdoor enclosures the base may be natural vegetation, however, larger primates can root up grass and low herbs, leaving an unsatisfactory muddy substrate. This can be prevented by planting under wire mesh or, as is common practice in many zoos, covering the soil with a non-toxic bark chip substrate with rapidly growing shrubs planted at intervals.

3.6 Handling and training the animals

Staff/animal relations

From the standpoint of non-human primate welfare, the caregiver's role is of prime importance. Good, friendly relations between familiar carers and monkeys reduce stress and also act as enjoyable stimulation for both staff and animals (Bayne et. al. 1993).

In contrast, where keepers make no effort to socialise with the animals, because the animals cannot avoid them, any contact will be stressful (Heath 1989, Olfert et. al. 1993, Van Vlissingen 1997,)

Training

Handling or anaesthetising primates is stressful and an increasing number of facilities are training animals to co-operate in many routine procedures, such as injections, blood sampling, urine collection, vaginal smears, oral drug administration and moving to another cage (UFAW 1992, Biological Council 1992, Reinhardt 1997d, Laule 1999). Training animals is not difficult and does not require specialist knowledge (see Pryor 1984) and most caregivers will have trained their monkeys to take treats from the hand. Positive reinforcement must always be used. It is but a short step to familiarise an animal with, say being touched by a needle, to getting it to accept an injection followed by a suitable reward (Reinhardt *et al.* 1995a, Laule 1999). It is even possible to train a macaque to willingly enter a restraint box and accept dosing through a stomach tube, a relatively unpleasant procedure (Jaeckel 1989). As primates are highly intelligent, training need not be time consuming and can save much time and stress to the animals and their caregivers in the long run. There will, of course, also be some individuals who may be difficult to train and some procedures which may be too aversive so that, for these situations, traditional methods will be unavoidable.

3.7 Training staff

As non-human primates require specialist care, it is essential that staff should receive special training covering the biology, husbandry,health, behavioural needs and psychological wellbeing and safety aspects of primatology. Special courses are usually available run by veterinary, laboratory animal science or technicians' organisations or in university laboratory animal science or primatology departments. A useful document outlining the levels of expertise required to manage non-human primates was published by the International Primatological Society (1993) in a section entitled « IPS Code of Practice : 2 Levels of Training for Care Giving Staff ».

GENERAL READING

A continuously updated database of relevant publications, established can be found at the following web site http://www.animalwelfare.com/Lab_animals/biblio/enrich.htm. There is an excellent, well documented review in the American APHIS' Final Report on Environmental Enhancement to Promote the Psychological Well-Being of NonHuman Primates : http://www.aphis.usda.gov/ac/eejuly15.html. Also the document from the Council of Europe – GT 123 (99) 9 « Report on workshop of the European Federation for Primatology » and « The Psychological well-being of nonhuman primates » published by the National Research Council in 1998 provide useful information.

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The following are valuable for reference and general reading and information on species not considered specifically in the above guidelines (such as *Cebus* and *Aotus*):

Poole, T (ed.) (1999) *The UFAW Handbook on the Care and Management of Laboratory Animals (7th edition): Volume 1 Terrestrial Vertebrates* Blackwell Science Ltd., Oxford

Taylor Bennett B, Abee CR, Henrickson R. (1995) *Nonhuman Primates in Biomedical Research, Biology and Management*. San Diego, Academic Press,

Taylor Bennett B, Abee CR, Henrickson R, 1998 *Nonhuman Primates in Biomedical Research, Diseases*. San Diego, Academic Press.

FURTHER RESEARCH WHICH WOULD BE OF VALUE

The value of various forms of dietary variation and content (for example, access to medicinal herbs, and dietary effects (if any) on excess multiple births in marmosets, and on psychological well-being).

Further studies on the effects of weaning age and post-weaning rearing conditions on social skills, immune competence, physiology and breeding success.

Assessment of possible adverse auditory stimuli, in the typical laboratory environment.

The value of visual stimulation, including light quality, using choice experiments.

The correlation between group size, cage size enrichment, and group compatibility using behavioural and non-invasive physiological measures.

The influence of staff working in the animal unit during nocturnal animals' rest periods.

REFERENCES

Bayne, K. A. L., S. L. Dexter, and G. M. Strange (1993). Effects of food treats and human interaction. *Contemporary Topics in Laboratory Animal Science* 32(2): 6-9.

Bayne, K. A. L., J. K. Hurst, and S. L. Dexter (1992). Evaluation of the preference to and behavioral effects of an enriched environment on male rhesus monkeys. *Laboratory Animal Science* 42(1): 38-45.

Biological Council (1992) *Guidelines on the Handling and Training of Laboratory Animals.* Universities Federation for Animal Welfare, Potters Bar, UK

Boccia, M.L., Reite, M. & Laudenslager, M. 1989. On the physiology of grooming in a pigtail macaque. Physiology and Behavior 45: 667-670.

Brent, L. and A. Hughes (1997). The occurrence of abnormal behavior in group housed baboons. *American Journal of Primatology* 42(2): 96-97.

Brent, L. and K. E. Long (1995). The behavioral response of individually caged baboons to feeding enrichment and the standard diet: A preliminary report.
Contemporary Topics in Laboratory Animal Science 34(2): 65-69.

Broom, D. M. and K. G. Johnson (1993). *Stress and animal welfare*. Chapman and Hall: London, England.

Buchanan-Smith, H. M. (1997). Considerations for the housing and handling of New World primates in the laboratory. In *Comfortable Quarters for Laboratory Animals*, Eighth Edition, 1997, V. Reinhardt, ed., Animal Welfare Institute: Washington, D.C., pp. 75-84.

Buchanan-Smith HM, Shand C and Morris K (2002). Cage use and feeding height preferences of captive common marmosets (*Callithrix jacchus*) in two-tier cages. Journal of Applied Animal Wekfare Science 5: 139-149.

Burt, D.A. and M. Plant (1990). Observations on a caging system for housing stump-tailed macaques. *Animal Technology* 41(3): 175-179.

Canadian Council on Animal Care (CCAC) (1993). *Guide to the Care and Use of Experimental Animals, Volume 1* (2nd Edition). E. D. Olfert, B. M. Cross, and A.
A. McWilliam, eds., Canadian Council on Animal Care: Ottawa, Canada, 212 p.

Cant, J. (1988). Positional behavior of long-tailed (Macaca fascicularis) macaques in Northern Sumatra (Indonesia). *American Journal of Physical Anthropology* 76(1): 29-38.

DRAFT

Capitanio, J. P. (1986). *Behavioral pathology. Comparative Primate Biology, Vol. 24: Behavior, Conservation, and Ecology* 24: 411-454.

Chamove, A. S. and J. R. Anderson (1989). Examining environmental enrichment. In *Housing, Care, and Psychological Well Being of Captive and Laboratory Primates,* E. F. Segal, ed., Noyes Publications: Park Ridge, New Jersey, pp. 183-202.

Chamove, A. S., J. R. Anderson, S. C. Morgan-Jones, and S. P. Jones (1982). Deep wood chip litter: hygiene, feeding and behavioral enhancement in eight primate species. *International Journal for the Study of Animal Problems* 3(4): 308-318.

Cheney, D. L., R. M. Seyfarth, B. B. Smuts, and R. W. Wrangham (1987). The Study of Primate Societies, In *Primate Societies*, B. B. Smuts, D. L. Cheney, R. M.

Seyfarth, R. W. Wrangham, and T. T. Struhsaker, eds., University of Chicago Press: Chicago, Illinois, pp.1-8.

Coates, M. E. (1999) Chapter 6 Nutrition and feeding. In *The UFAW Handbook on the Care and Management of Laboratory Animals* Volume 1 7th Edition, T. Poole ed. , Blackwell Science, Oxford pp.

Coe, C.L. & Scheffler, J. 1989. Utility of immune measures for evaluating psychological well-being in nonhuman primates. Zoo Biology Supplement 1: 89-99.

Coe, C.L., Rosenberg, L.T. Fischer, M. & Levine, S. 1987. Psychological factors capable of preventing the inhibition of anibody responses in separated infant monkeys. Child Development, 58: 1420-1430

Coelho, A. M., K. D. Carey, and R. E. Shade (1991). Assessing the effects of social environment on blood pressure and heart rates of baboons. *American Journal of Primatology* 23: 257-267.

Dahl, J. F. (1989). An inexpensive climate-controlled enclosure for gibbons utilizing appropriate technology, In *Housing, Care and Psychological Well Being of Captive and Laboratory Primates*, E. F. Segal, ed., Noyes Publications: Park Ridge, New Jersey, pp.323-335.

Deputte, B.L. 2000. Primate socialization revisited: theoretical and practical issues in social ontogeny. Advances in the Study of Behavior, 29: 99-157.

Dunbar, D. (1989). Locomotor behavior of rhesus macaques on Cayo Santiago.
Puerto Rican Health Sciences Journal 8(1): 79-85.

Ely A, Freer A, Windle C and Ridley RM (1998). Assessment of cage use by laboratory-bred common marmosets (*Callithrix jacchus*). Laboratory Animals 32: 427-433.

Fagen, R. (1993). Primate juveniles and primate play. In *Juvenile primates: life history, development, and behavior*, Pereira, M. E. and L. F. Fairbanks, eds.
Oxford University Press: New York. Chapter 13, pp. 182-196.

FELASA Working Group on Non-Human Primate Health (1997) Sanitary aspects of handling primates during transport. *Laboratory Animals* 31, 298 - 392

FELASA Working Group on Non-Human Primate Health (1999) Recommendation of Non-Human Primate Colonies. *Laboratory Animals*, 33, (Suppl.1) S1:3 - S1:18

Fleagle, J. G., R. A. Mittermeier, and A. L. Skopec (1981). Differential habitat use by Cebus apella and Saimiri sciureus in central Surinam. *Primates*
22(3):361--367.

Florence, G. Riondet, L. 2000 Influence of a puzzle feeder on rhesus macaque behaviour: learning phase. Folia Primatologica, 71: 249-267.

Fragaszy, D. M. (1991). The expression of natural behavioural repertoires in captivity. Primatology Today: *Proceedings of the XIIIth Congress of the International Primatological Society*, Elsevier Science Publishers BV: Amsterdam, pp.726-727.

Fragaszy, D. M. and L. Adams-Curtis (1991). Environmental challenges in groups of capuchins. In *Primate Responses to Environmental Change*, H. O. Box, ed., Chapman and Hall: New York, pp.237-264.

Goosen, C., W. Van der Gulden, H. Rozemond, H. Balner, A. Bertens, R. Boot, J. Brinkert, H. Dienske, G. Janssen, A. Lammers, and P. Timmermans (1984).
Recommendations for the housing of macaque monkeys. *Laboratory Animals* 18: 99-102.

Gust, D. A., T. P. Gordon, A. R. Brodie, and H. M. McClure (1994). Effect of a preferred companion in modulating stress in adult female rhesus monkeys.
Physiology and Behavior 55: 681-684.

Hanson, J. P., M. E. Larson, and C. T. Snowdon (1976). The effects of control over high intensity noise on plasma cortisol levels in rhesus monkeys.*Behavioral Biology* 16: 333-340.

Heath, M. (1989). The training of cynomologus monkeys and how the human/animal relationship improves with environmental and mental enrichment. *Animal Technology* 40: 11-22.

Hediger, H. (1964). *Wild animals in captivity.* Dover Publications Inc.: New York, p 207.

Home Office, Animals (Scientific Procedures) Act (1989). *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures*. Her Majesty's Stationery Office, London. Available on line at http://www.homeoffice.gov.uk/hcasp.htm on 12/21/98.

International Primatological Society (IPS) (1993). International guidelines for the acquisition, care, and breeding of nonhuman primates. Codes of practice 1-3

Primate Report 35: 3-29. (Also *Primate Report* Special Issue (1993) (eds) T. B. Poole and M. Schwibbe)

Jaeckel, J. (1989) The benefits of training rhesus monkeys living under laboratory conditions. In: *Laboratory Animal Welfare Research – Primates*. pp. 23-25 Universities Federation for Animal Welfare, Potters Bar, England (also see video produced by Ciba-Geigy, Switzerland)

Jolly, A. (1985). *Evolution of Primate Behavior*, 2nd Edition, Macmillan: New York, p 526 .

Kaumanns, W. (1997). General aspects of primate keeping and colony management.

In Abstracts of the Second EUPREN/EMRG Winter Workshop : *The housing of non-human primates used for experimental and other scientific purposes: Issues for consideration*, Rome 27.09.1996. [Monograph online available from: http://www.dpz.gwdg.de:80/eupren/eupren.htm (March 23, 1998)]. European Primate Resources Network (EUPREN).

Kerl J and Rothe H (1996). Influences of cage size and cage equipment on physiology and behaviour of common marmosets (*Callithrix jacchus*). Laboaratory Primate Newsletter 3: 10-13.

Kessel, A.L. and L. Brent (1995). An activity cage for baboons, Part I.

Contemporary Topics in Laboratory Animal Science 34(1): 74-79.

Kessel, A.L. and L. Brent (1997). Rehabilitating a baboon (*Papio hamadryas hamadryas* X *Papio hamadryas cynocephalus*), from single housing to social housing: a case study. *American Journal of Primatology* 42(2): 121.

King, J.E. and V.R. Norwood (1989). Free-environment rooms as alternative housing for squirrel monkeys. In *Housing, Care and Psychological Well-being of Captive and Laboratory Primates*, E. F. Segal, ed. Noyes Publications: Park Ridge, New Jersey, pp. 102-114.
King, J.E. and V.R. Norwood (1989). Free-environment rooms as alternative housing for squirrel monkeys. In *Housing, Care and Psychological Well-being of Captive and Laboratory Primates*, E. F. Segal, ed. Noyes Publications: Park Ridge, New Jersey, pp. 102-114.
Kitchen, A. M. and A. A. Martin (1996). The effects of cage size and complexity on the behaviour of captive common marmosets, Callithrix jacchus jacchus. *Laboratory Animals* 30: 317-326.

Lam, K., N. M. J. Rupniak, and S. D. Iversen (1991). Use of a grooming and foraging substrate to reduce cage stereotypies in macaques. *Journal of Medical Primatology* 20: 104-109.

Laudenslager, M. L., D.E. Held, M. L. Boccia, M. L. Reite, and J. J. Cohen (1990). Behavioral and immunological consequences of brief mother-infant separation: a species comparison. *Developmental Psychobiology* 23: 247-64.

Laule, G. E. (1999) Chapter 4 Training laboratory animals. In *The UFAW Handbook on the Care and Management of Laboratory Animals* Volume 1 7th Edition, T. Poole ed. , Blackwell Science, Oxford pp. 21-27

Laule, G. E. and T. Desmond (1998). Chapter 17: Positive reinforcement training as an enrichment strategy. In *Second nature: environmental enrichment for captive animals*, Shepherdson, D. J., J. D. Mellen, and M. Hutchins, eds., Smithsonian Institution Press: Washington, D.C.

Line, S. W. (1987). Environmental enrichment for laboratory primates. *Journal of the American Veterinary Medical Association* 190(7): 854-859.

Line S.W, K. N Morgan, H Markowitz, JA Roberts and M Ridell 1990, Behavioural reponses of female long-tailed macaques (M. fascicuaris) to pair formation. *Laboratory Primate Newsletter*. 29 1-5

Lynch, R. (1998). Successful pair housing of male macaques. *Laboratory Primate Newsletter* 37(1): 4-6.

Lynch, R. and D. C. Baker (1998). Enrichment and exercise room for free roaming.

Laboratory Primate Newsletter 37(1): 6.

OIE (1999) Zoonoses transmissible from non-human primates In: *International Animal Health Code*. World Organisation for Animal Health, Paris pp 48-53.

OIE (1999) Quarantine Measures applicable to non-human primates. In: *International Animal Health Code*. World Organisation for Animal Health, Paris pp 364-367

Marriner, L. M. and L. C. Drickamer (1994). Factors influencing stereotyped behavior of primates in a zoo. *Zoo Biology* 13(3): 267-275.

Marriott, B. M., R. W. Marriott, J. Norris, and D. Lee (1993). A semi-natural habitat for housing small nonhuman primates. *Journal of Medical Primatology* 22(6): 348-354.

Mason, G. J. (1991a). Stereotypies: a critical review. *Animal Behaviour* 41: 1015-1037.

Mason, G. J. (1991b) . Stereotypies and suffering. *Behavioral Processes* 25:103-115.

Mason, G. J. and M. Mendl (1993). Why is there no simple way of measuring animal welfare? *Animal Welfare* 2(4): 301-319.

Mason, W. A. and G. Berkson (1975). Effects of maternal mobility on the development of rocking and other behaviors in rhesus monkeys: A study with artificial mothers. *Developmental Psychobiology* 8: 197-211.

McGrew, W. C. (1981). Social and cognitive capabilities of nonhuman primates: lessons from the wild to captivity. *International Journal for the Study of Animal Problems* 2(3): 138-149.

McKenzie, S. M., A. S. Chamove, and A. T. C. Feistner (1986). Floor-coverings and hanging screens alter arboreal monkey behavior. *Zoo Biology* 5(4): 339-348.

Mendl, M. and R. C. Newberry (1997). Social conditions. In *Animal Welfare*, M.C. Appleby and B. O. Hughes, eds. CAB International: New York, New York, pp. 171-203.

Meunier, L. D., J. T. Duktig, and M. S. Landi (1989). Modifications of stereotypic behavior in rhesus monkeys using videotapes, puzzlefeeders, and foraging boxes. *Laboratory Animal Science* 39(5): 479.

National Research Council, Institute of Laboratory Animal Resources (NRC/ILAR) (1968). *Nonhuman primates: standards and guidelines for the breeding, care, and management of laboratory animals, a report*. National Academy Press: Washington, D.C., 35 p.

Neveu, H. & Deputte, B.L. 1996. Influence of the availability of perches on the behavioral well-being of captive, group-living mangabeys. American Journal of Primatology, 38: 175-185

Novak, M. A. and K. H. Drewson (1989). Enriching the lives of captive primates: Issues and problems. *In Housing, Care and Psychological Well-being of Captive and Laboratory Primates*, E. F. Segal, ed. Noyes Publications: Park Ridge, New Jersey, pp. 161-182.

Olfert, E. D., B. M. Cross, and A. A. McWilliam, (eds.), (1993). *Guide to the Care and Use of Experimental Animals*, Volume 1 (2nd Edition). Canadian Council on Animal Care, Ottawa, Canada, 211 p.

O'Neill, P. (1989). A room with a view for captive primates: Issues, goals, related research and strategies. In *Housing, Care and Psychological Well-being of Captive and Laboratory Primates*. E. F. Segal, ed., Noyes Publications: Park Ridge, New Jersey, pp. 135-160.

O'Neill, P., Price, C., and Suomi. S. Designing captive primate environments sensitive to age and gender: Related activity profiles for rhesus monkeys (Macaca mulatta). *American Association of Zoological Parks and Aquariums (AAZPA) Regional Proceedings 1990*. pp. 546-551. AAZPA, Wheeling, WV.

Pereira, M. E., J. M. Macedonia, D. M. Haring, and E. L. Simons (1989). Maintenance of primates in captivity for research: the need for naturalistic environments. In *Housing, Care and Psychological Well-being of Captive and Laboratory Primates*, E. F. Segal, ed., Noyes Publications: Park Ridge, New Jersey, pp. 40-60.

Platt, D. M. and M. A. Novak (1997). Videostimulation as enrichment for captive rhesus monkeys (Macaca mulatta). *Applied Animal Behaviour Science* 52: 139-155.

Poole, T. (1991). Criteria for the provision of captive environments. In *Primate responses to environmental change*, H. O. Box, ed., Chapman and Hall: London, England, pp. 357-374.

Poole, T. (1992). Nature and evolution of behavioral needs in mammals. *Animal Welfare* 1(3): 203-220.

Poole, T., P. Costa, W. J. Netto, K. Schwarz, B. Wechsler, and D. Whittaker (1994). *Non-human primates. In The Accommodation of Laboratory Animals in Accordance with Animal Welfare Requirements*: Proceedings of an International Workshop Held at the Bundesgesundheitsamt, Berlin, 17-19 May 1993, O'Donoghue, P. N., editor, Bundesministerium für Ernährung, Landwirtschaft und Forsten: Bonn, Germany, pp. 81-86.

DRAFT

Poole, T, (1998) A systematic approach to environmental enrichment using the “scan” system. *Animal Technology* 49: 7-17.
Prescott MJ and Buchanan-Smith HM (2002). Predation sensitive foraging in captive tamarins. In Miller L (Ed). Eat or be eaten: Predation sensitive foraging among primates, pp. 44-57. Cambridge: Cambridge University Press.
Pryor, K. (1984) *Don’t Shoot the Dog* Simon and Schuster, New York
Queyras, A., M. Scolavino, and A. Vitale (1997). Research and animal welfare needs when studying social learning: the case of a colony of captive common marmosets. In Abstracts of the Second EUPREN/EMRG Winter Workshop : *The housing of non-human primates used for experimental and other scientific purposes:*
Issues for consideration, Rome 27.-27.09.1996. [Monograph online available from:
http://www.dpz.gwdg.de:80/eupren/eupren.htm (March 23, 1998)]. European Primate
Reinhardt, V. (1997a). Species-adequate housing and handling conditions for old world nonhuman primates kept in research instutions. In *Comfortable Quarters for Laboratory Animals*, V. Reinhardt, ed., Animal Welfare Institute: Washington, DC., pp. 85-93.
Reinhardt, V. (1997b). Training nonhuman primates to cooperate during handling procedures: a review. *Animal Technology* 48(2): 55-73.
Reinhardt, V. (1997c) Lighting conditions for laboratory monkeys: are they adequate? *AWIC Newsletter* 8 (2): 3-6
Reinhardt, V. (1999) The monkey cave: the dark lower row. *Laboratory Primate Newsletter* 38 (3): 8-9
Reinhardt, V., C. Liss, and C. Stevens (1995a). Restraint methods of laboratory non-human primate: a critical review. *Animal Welfare* 4(3): 221-238.
Reinhardt, V., C. Liss, and C. Stevens (1995b). Social housing of previously single-caged macaques: What are the options and the risks? *Animal Welfare* 4(4): 307-328.
Reinhardt, V., C. Liss, and C. Stevens (1996). Space requirement stipulations for caged non-human primates in the United States: A critical review. *Animal Welfare* 5(4): 361-372.
Rose, M. A. (1994). Environmental factors likely to impact an animal's well-being. In *Improving the well-being of animals in the research environment--Proceedings of the conference held at the Marriott Hotel, Sydney,October, 1993*, Baker, R.M., G. Jenkin, and D. J. Mellor (eds.) Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART),
Glen Osmond, Australia, pp. 99-116. ISBN 0 646 181165.
Rosenblum, L. A. (1991). Subjective and objective factors in assessing psychological well-being in nonhuman primates. In *Through the Looking Glass: Issues of Psychological Well-Being in Captive Nonhuman Primates*, M. A. Novak and A. J. Petto, eds., American Psychological Association: Washington, D.C., pp. 43-49.
Rosenblum, L. A. and M. W. Andrews (1995). Environmental enrichment and psychological well-being of nonhuman primates. In *Nonhuman Primates in Biomedical Research, Biology, and Management*, B. T. Bennett, C. R. Abee, and R. Henrickson, eds., Academic Press: New York., pp. 101-112.
Sambrook, T. D. and H. M. Buchanan-Smith (1996). What makes novel objects enriching? A comparison of the qualities of control and complexity. Laboratory *Primate Newsletter* 35(4): 1-4.
Schapiro, S. J., M. A. Bloomsmith, S. A. Suarez, and L. A. Porter (1996).
Effects of social and inanimate enrichment on the behavior of yearling rhesus monkeys. *American Journal of Primatology* 40(3): 247-260.
Schapiro, S. J., P. N. Nehete, J. E. Perlman, and K. J. Sastry (1997). Social housing condition affects cell-mediated immune responses in adult rhesus macaques. *American Journal of Primatology 42(2): 147*.
Seyfarth, R.M. & Cheney, D.L. 1980. The ontogeny of vervet monkey alarm-calling behavior: a preliminary report. Zeitschrift für Tierpsychologie. 54:37-56.
Shumaker, R. (1995). List of browse species used by the National Zoo in Washington, D.C. In *Primate-Talk* (internet listserv), Wisconsin Regional Primate Center, Madison, Wisconsin, date unknown, cited 1995.
Snowdon, C. T. and A. Savage (1989). Psychological well-being of captive primates: General considerations and examples from callitrichids. In *Housing, Care and Psychological Well-being of Captive and Laboratory Primates*, E. F. Segal, ed., Noyes Publications: Park Ridge, NJ, pp.75-88.
DRAFT

Spring, S.E., J.O. Clifford, and D. L. Tomko (1997). Effects of environmental enrichment devices on behaviors of single and group-housed squirrel monkeys.
Contemporary Topics in Laboratory Animal Science 36(3): 72-75.

Thompson, K. V. (1996). Chapter 34: Behavioral development and play. *In Wild Mammals in Captivity: Principles and Techniques*, D.G . Kleiman, M. E. Allen, K. V. Thomson, and S. Lumpkin, eds., University of Chicago Press: Chicago, Illinois, pp. 352-371.

Toates, F. (1995). *Stress: conceptual and biological aspects*. John Wiley and Sons, Ltd.: New York.

Van Vlissingen, J. M. F. (1997) Welfare implications in biomedical research. In Abstracts of the Second EUPREN/EMRG Winter Workshop : *The housing of non-human primates used for experimental and other scientific purposes: Issues for consideration*, Rome 27.09.1996. [Monograph online available from: http://www.dpz.gwdg.de:80/eupren/eupren.htm (March 23, 1998)]. European Primate Resources Network (EUPREN).

Veasey, J. S., N. K. Waron, and R. J. Young (1996). On comparing the behaviour of zoo housed animals with wild conspecifics as a welfare indicator. *Animal Welfare* 5(1): 13-24.

UFAW (1992) *Animal Training: A Review and Commentary on Current Practice.* Universities Federation for Animal Welfare, Potters Bar, UK

Watson, S. L. and C. A. Shively (1996). Effects of cage configuration on behavior of cynomologous macaques. *International Primate Society/American Society of Primatologists Congress Abstract No. 674. IPS Congress Abstracts*, Madison, Wisconsin.

Westergaard, G. C. and D. Munkenbeck-Fragaszy (1985). Effects of manipulatable objects on the activity of captive capuchin monkeys (Cebus apella). *Zoo Biology* 4(4): 317-327.

Whitney, R. A. and E. J. Wickings (1987). Macaques and other old world simians.
In *The UFAW Handbook on the Care and Management of Laboratory Animals* 6th edition, T. B. Poole, ed., Longman Scientific and Technical: Essex, England, pp.599-627.

Williams, L. E. and I. S. Bernstein (1995). *Study of primate social behavior. In Nonhuman Primates in Biomedical Research, Biology, and Management*. B.T. Bennett, C. R. Abee, and R. Henrickson, eds., Academic Press: New York, pp. 77-100.

Woolley, A. P. A. H. (1997). Requirements of biomedical research in terms of housing and husbandry: pharmacology and toxicology. In Abstracts of the Second EUPREN/EMRG Winter Workshop : *The housing of non-human primates used for experimental and other scientific purposes: Issues for consideration, Rome* 27.09.1996. [Monograph online available from:
http://www.dpz.gwdg.de:80/eupren/eupren.htm (March 23, 1998)]. European Primate Resources Network (EUPREN)

MARMOSETS (*Callithrix*) AND TAMARINS (*Saguinus*)

SECTION B: SCIENTIFIC JUSTIFICATION

2.2 Temperature

Background

The allowance for slightly higher temperature than 28 °C takes into account the Callitrichid ecological niches that are geographically distributed in tropical regions (Rylands; 1993, 1997). In experimental conditions, consideration should be given to the effect of environmental temperature on marmosets' core temperature rhythm (Palkova et al., 1999; Petry et al., 1990).

2.3 Humidity

Background

Values above 70% RH will not impact upon the welfare of the animals for the ecological considerations mentioned above.

2.4 Lighting

Background

Marmosets and tamarins are day-light active species (Erkert, 1997) and the period of behavioural activity is 11-12 hours (Stevenson et al., 1988). In order to reduce experimental variation, illumination and intensity of light should be standardized (Wechselberger et al., 1994).

4.1 Social housing

Background

Marmosets and tamarins are highly social animals exhibiting a complex natural behaviour (Caine, 1993; Garber, 1993; Stevenson et al., 1976). The social structure is most often represented by an extended family group with a monogamous mating strategy (Anzenberger, 1992; Dunbar, 1995a; Epple, 1978; Evans et al., 1984). The suppression of subordinate female reproduction is due to hormonal and behavioural mechanisms (Abbott et al., 1993; Porter et al., 1997). In the laboratory conditions, the animals are commonly maintained in breeding pairs with one or more sets of twins (Hubrecht, 1997; Pryce et al., 1997; Tardif et al., 1993). When grouping same-sex animals the success is often unpredictable and may depend on the environment and individual temperament: twins or a parent with offspring are more likely to be compatible (Eckert, 2000). In general, social interactions should be carefully monitored to prevent the outbreak of aggressive behaviours within captive groups (Anzenberger, 1993; Sutcliffe et al., 1984). The infants are reared cooperatively by all family members (Achenbach et al., 1998; Bales et al., 2000; Dunbar, 1995b; Savage et al., 1996; Snowdon, 1996), thus enabling the juveniles to achieve adequate experience as future breeders (Johnson et al., 1986; Missler et al.,1992). However, in tamarins, the eldest set of twins may be rejected as a new offspring is generated.

4.2 Environmental complexity

Background

The natural environment of marmosets and tamarins incorporates features of complexity and unpredictability that stimulate the expression of a complete behavioural repertoire. One of the consequences of laboratory conditions is generally the reduction of space and the impoverishment of the social and physical environment that barely satisfies the behavioural requirements of the animals (Kerl et al., 1996; Kitchen et al., 1996; Schoenfeld, 1989). An environmental enrichment programme for laboratory marmosets and tamarins is unquestionably an essential component in the improvement of their well-being and has the advantage to incorporate natural and artificial elements that enhance either species-typical and potentially adaptive new behavioural patterns. In

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the literature several studies describe various enrichment techniques and their impact on the animal welfare (Box et al., 1993; Buchanan-Smith, 1994,1996,1997; Dettling, 1997; Heath et al., 1993; Poole, 1990; Sambrook et al., 1997; Scott, 1991; Snowdon et al., 1989). The use of different materials and items for cage furniture in callitrichid primates has been proved beneficial to increase their behavioural repertoire, the control over the environment and their foraging propensities (Dettling, 1997; Forster, 1996; Hannaford, 1996; Harrison et al., 1988, 1994; Hosey, 1999; 1998;. Kelly, 1993; McGrew, 1986; Roberts, 1999; Vitale et al. 1997)

4.3 Enclosures- dimensions and flooring

Background

Existing guidelines (European Council Directive, 1986; ILAR guidelines, 1996; Home Office Code of Practice, 1989,1995) provide diverse minimum requirements for cage dimensions of marmosets and tamarins, either for floor area or height. These variations are most probably due to the limited scientific data available on space needs of these species and to the diversity of common practices in various countries.

The present proposals take into account several factors such as: the arboreal nature of the animals, their locomotory patterns and flight reaction, the need for adequate space for social interactions and for the inclusion of enrichment devices. The minimum enclosure/cage sizes for experimental animals allow for sufficient enrichment, although the restriction of space should be limited only to the time required by the experimental procedure. It is more important to provide tamarins with a good volume of space than it is marmosets, if their wellbeing and breeding success are to be maximised, (Prescott and Buchanan-Smith, in press).

4.7 Cleaning

Background

The proposal for cleaning procedures considers the scent marking behaviour of marmosets and tamarins (Bartecki,1990; Epple, 1970, Epple et al, 1986, 1993; Pryce, 1997).

REFERENCES – Marmosets and Tamarins part B

86/609/EEC: Council Directive of 24 November 1986 on the approximation of lows, regulations and administrative provisions of the Member State regarding the protection of animals used for experimental and other scientific purposes.

Abbott DH; Barrett J; George LM (1993): Comparative aspects of the social suppression of reproduction in female marmosets and tamarins. Pp. 152-163 in MARMOSETS AND TAMARINS: SYSTEMATICS, BEHAVIOUR, AND ECOLOGY. A.B. Rylands ed. Oxford, Oxford University Press.

Achenbach GG; Snowdon CT (1998): Response to sibling birth in juvenile cotton-top tamarins (*Saguinus oedipus*). BEHAVIOUR 135(7): 845-862.

Anzenberger G (1992): Monogamous social system and paternity in primates. Pp. 203-224 in PATERNITY IN PRIMATES: GENETIC TESTS AND THEORIES. R.D. Martin; A.F. Dixson; E.J. Wickings ed. Basel, Karger.

Anzenberger G (1993): Social conflict in two monogamous New World primates: Pairs and rivals. Pp. 291-329 in PRIMATE SOCIAL CONFLICT. W.A. Mason; S.P. Mendoza ed. Albany, SUNY Press.

Bales K; Dietz J; Baker A; Miller K; Tardif SD (2000): Effects of allocare-givers on fitness of infants and parents in callitrichid primates. FOLIA PRIMATOLOGICA 71(1-2): 27-38.

Bartecki U; Heymann EW (1990): Field observations on scent-marking behaviour in saddle-back tamarins, Saguinus fuscicollis (Callitrichidae, Primates). JOURNAL OF ZOOLOGY 220(1): 87-99.

Box HO; Rohrhuber B (1993): Differences in behaviour among adult male, female pairs of cotton-top tamarins (*Saguinus oedipus*) in different conditons of housing. ANIMAL TECHNOLOGY 44(1): 19-30.

Buchanan-Smith H (1994): Environmental enrichment in captive marmosets and tamarins. HUMANE INNOVATIONS AND ALTERNATIVES 8: 559-564.

Buchanan-Smith H M (1996): Enriching the lives of marmosets and tamarins in captivity. SHAPE OF ENRICHMENT 5(4): 3-5.

Buchanan-Smith H M (1997): Considerations for the housing and handling of New World primates in the laboratory. Pp. 75-84 in COMFORTABLE QUARTERS FOR LABORATORY ANIMALS. V. Reinhardt ed. Washington, DC, Anim Welfare Inst.

Caine NG (1993): Flexibility and co-operation as unifying themes in Saguinus social organization and behaviour: The role of predation pressures. Pp. 200-219 in MARMOSETS AND TAMARINS: SYSTEMATICS, BEHAVIOUR, AND ECOLOGY. A.B. Rylands ed. Oxford, Oxford University Press.

Dettling A (1997): Physical environment and its influence on behaviour in captive common marmosets (*Callithrix jacchus*). Pp. 54-59 in HANDBOOK: MARMOSETS AND TAMARINS IN BIOLOGICAL AND BIOMEDICAL RESEARCH. Pryce C; Scott L; Schnell C ed. Salisbury, UK, DSSD Imagery.

Dunbar R I M (1995a): The mating system of callitrichid primates: I. Conditions for the coevolution of pair bonding and twinning. ANIMAL BEHAVIOUR 50(4): 1057-1070.

Dunbar R I M (1995b): The mating system of callitrichid primates: II. The impact of helpers. ANIMAL BEHAVIOUR 50(4): 1071-1089.

Eckert K (2000): Same-sex pairing of marmosets: A discussion. LABORATORY PRIMATE NEWSLETTER 39(2): 14.

Epple G (1970): Quantitative studies on scent marking in the marmoset (Callithrix jacchus). FOLIA PRIMATOLOGICA 13: 48-62.

Epple G (1978): Reproductive and social behavior of marmosets with special reference to captive breeding. PRIMATES IN MEDICINE 10: 50-62.

Epple G; Belcher AM; Kuederling I; Zeller U; Scolnick L; Greenfield KL; Smith AB III (1993): Making sense out of scents: Species differences in scent glands, scent-marking behaviour, and scent-mark composition in the Callitrichidae. Pp. 123-151 in MARMOSETS AND TAMARINS: SYSTEMATICS, BEHAVIOUR, AND ECOLOGY. A.B. Rylands ed. Oxford, Oxford University Press.

Epple G; Belcher AM; Smith AB III (1986): Chemical signals in callitrichid monkeys: A comparative review. Pp. 653-672 in CHEMICAL SIGNALS IN VERTEBRATES 4: ECOLOGY, EVOLUTION, AND COMPARATIVE BIOLOGY. D. Duvall; D. Mueller-Schwarze; R.M. Silverstein ed. New York, Plenum Press.

Erkert HG (1997): Circadian rhythms in the marmoset: Their significance for fundamental and applied research. Pp. 128-144 in HANDBOOK: MARMOSETS AND TAMARINS IN BIOLOGICAL AND BIOMEDICAL RESEARCH. Pryce C; Scott L; Schnell C ed. Salisbury, UK, DSSD Imagery.

Evans S; Poole TB (1984): Long-term changes and maintenance of the pair-bond in common marmosets, *Callithrix jacchus jacchus*. FOLIA PRIMATOLOGICA 42(1): 33-41.

Forster FC (1996): Novel objects and learning as enrichment for captive common marmosets (*Callithrix jacchus jacchus*) . AUSTRALIAN PRIMATOLOGY 10 (3): 2-10.

Garber PA (1993): Feeding ecology and behaviour of the genus Saguinus. Pp. 273-295 in MARMOSETS AND TAMARINS: SYSTEMATICS, BEHAVIOUR, AND ECOLOGY. A.B. Rylands ed. Oxford, Oxford University Press.

DRAFT

Hannaford G (1996): Feeding bowl height preferences in captive common marmosets (*Callithrix jacchus jacchus*). AUSTRALIAN PRIMATOLOGY 11(1): 5-13.

Harrison ML; Sherwood RJ; Tardif SD (1988): Differential use of wooden **cage** furniture in callitrichid primates: Implications for psychological well-being. Pp. 40 in THE PSYCHOLOGICAL WELL-BEING OF CAPTIVE PRIMATES, CONFERENCE. ed. Boston, Harvard Medical School,. (Abstract).

Harrison ML; Tardif SD (1994): Social implications of gummivory in marmosets. AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 95(4): 399-408.

Heath M; Libretto SE (1993): Environmental enrichment for large scale marmoset units. ANIMAL TECHNOLOGY 44(3): 163-173.

Heymann EW (1992): Seed ingestion and gastrointestinal health in tamarins? LABORATORY PRIMATE NEWSLETTER 31(3): 15-16.

Home Office (1989) Code of Practice for the Housing and Care of Animals used in Scientific Procedure, HMSO, London.

Home Office (1995) Code of Practice for the Housing and Care of Animals in Designated Breeding and Supply Establishments. HMSO, London.

Hosey GR; Jacques M; Burton M (1999): Allowing captive marmosets to choose the size and position of their nest box. ANIMAL WELFARE 8(3): 281-285.

Hubrecht RC (1997): Current practice in maintaining marmosets: results of a UK survey. Pp. 24-38 in HANDBOOK: MARMOSETS AND TAMARINS IN BIOLOGICAL AND BIOMEDICAL RESEARCH". Pryce C; Scott L; Schnell C (eds), Salisbury, UK, DSSD Imagery.

ILAR Commission on Life Sciences (1996): Guide for the Care and Use of Laboratory Animals, National Academy press, Washington D.

Johnson LD; Petto AJ; Boy DS; Sehgal PK; Beland ME (1986): The effect of perinatal and juvenile mortality on colony-born production at the New England Regional Primate Research Center. Pp. 771-779 in PRIMATES: THE ROAD TO SELF-SUSTAINING POPULATIONS. K. Benirschke ed. New York, Springer-Verlag.

Kelly K (1993): Environmental enrichment for captive wildlife through the simulation of gum-feeding. ANIMAL WELFARE INFORMATION CENTER NEWSLETTER 4(3): 1-2 & 5-10.

Kerl J; Rothe H (1996): Influence of cage size and cage equipment on physiology and behavior of common marmosets (*Callithrix jacchus*). LABORATORY PRIMATE NEWSLETTER 35(3): 10-13.

Kitchen A M; Martin A A (1996): The effects of cage size and complexity on the behaviour of captive common marmosets, *Callithrix jacchus jacchus*. LABORATORY ANIMALS 30(4): 317-326.

McGrew WC; Brennan JA; Russell J (1986): An artificial "gum-tree" for marmosets (*Callithrix j. jacchus*). ZOO BIOLOGY 5(1): 45-50.

Missler M; Wolff JR; Rothe H; Heger W; Merker HJ; Treiber A; Scheid R; Crook GA (1992): Developmental biology of the common marmoset: Proposal for a "postnatal staging". JOURNAL OF MEDICAL PRIMATOLOGY 21(6): 285-298.

Palkova M; Sigmund L; Erkert HG (1999): Effect of ambient temperature on the circadian activity rhythm in common marmosets, *Callithrix j. jacchus* (primates). CHRONOBIOLOGY INTERNATIONAL 16(2): 149-161.

Petry H, Maier J (1990): Radiotelemetric studies on body temperature in marmosets (*Callithrix jacchus*). ZEITSCHRIFT FUER ERNAEHRUNGSWISSENSCHAFT 29(3): 197-207.

Poole TB (1990): Environmental enrichment for marmosets. ANIMAL TECHNOLOGY 41(2): 81-86.

Porter T A; Snowdon C T (1997): Female reproductive status and male pairmate behavior in cotton-top tamarins. ANNALS OF THE NEW YORK ACADEMY OF SCIENCES 807: 556-558.

DRAFT

Prescott MJ and Buchanan-Smith HM (in press),. Cage sizes for tamarins in the laboratory. Animal Welfare.

Pryce C; Samson NA (1997): Integrating marmoset husbandry and research. Pp 39-46 in HANDBOOK: MARMOSETS AND TAMARINS IN BIOLOGICAL AND BIOMEDICAL RESEARCH". Pryce C; Scott L; Schnell C (eds), Salisbury, UK, DSSD Imagery.

Roberts RL; Roytburd LA; Newman JD (1999): Puzzle feeders and gum feeders as environmental enrichment for common marmosets. CONTEMPORARY TOPICS IN LABORATORY ANIMAL SCIENCE 38(5): 27-31.

Rylands AB (1997): The Callitrichidae: A biological overview. Pp. 1-22 in "HANDBOOK: MARMOSETS AND TAMARINS IN BIOLOGICAL AND BIOMEDICAL RESEARCH". Pryce C; Scott L; Schnell C ed. Salisbury, UK, DSSD Imagery.

Rylands AB (ed) (1993) : MARMOSETS AND TAMARINS: SYSTEMATICS, BEHAVIOUR, AND ECOLOGY. Oxford, Oxford University Press.

Sambrook T D; Buchanan-Smith H M (1997): Control and complexity in novel object enrichment. ANIMAL WELFARE 6(3): 207-216.

Savage A; Snowdon C T; Giraldo L H; Soto L H (1996): Parental care patterns and vigilance in wild cotton-top tamarins (*Saguinus oedipus*). Pp. 187-199 & 539 in ADAPTIVE RADIATIONS OF NEOTROPICAL PRIMATES. M.A. Norconk; A.L. Rosenberger; P.A. Garber ed. New York, Plenum Press.

Schoenfeld D (1989): Effects of environmental impoverishment on the social behavior of marmosets (*Callithrix jacchus*). AMERICAN JOURNAL OF PRIMATOLOGY (Suppl. 1): 45-51.

Scott L (1991): Environmental enrichment for single housed common marmosets. Pp. 265-274 in PRIMATE RESPONSES TO ENVIRONMENTAL CHANGE. H.O. Box ed. London, Chapman & Hall.

Snowdon C T (1996): Infant care in cooperatively breeding species. ADVANCES IN THE STUDY OF BEHAVIOR 25: 643-689,. (Book title: Parental Care, J.S. Rosenblatt, et al., eds. San Diego, Academic Press.

Snowdon CT; Savage A (1989): Psychological well-being of captive primates: General considerations and examples from callitrichids. Pp. 75-88 in HOUSING, CARE AND PSYCHOLOGICAL WELLBEING OF CAPTIVE AND LABORATORY PRIMATES. E.F. Segal ed. Park Ridge, New Jersey, Noyes Publications.

Snowdon CT; Savage A; McConnell PB (1985): A breeding colony of cotton-top tamarins (*Saguinus oedipus*). LABORATORY ANIMAL SCIENCE 35(5): 477-480.

Stevenson MF; Poole TB (1976): An ethogram of the common marmoset (*Callithrix jacchus jacchus*): General behavioural repertoire. ANIMAL BEHAVIOUR 24: 428-451.

Stevenson MF; Rylands AB (1988): The marmosets, genus Callithrix. Pp. 131-222 in ECOLOGY AND BEHAVIOR OF NEOTROPICAL PRIMATES, VOL. 2. R.A. Mittermeier; A.B. Rylands; A.F. Coimbra-Filho; G.A.B. Fonseca (eds). Washington, DC, World Wildlife Fund.

Sutcliffe AG; Poole TB (1984): Intragroup agonistic behavior in captive groups of the common marmoset *Callithrix jacchus jacchus*. INTERNATIONAL JOURNAL OF PRIMATOLOGY 5(5): 473-489.

Tardif SD; Clapp NK (1993): Breeding the cotton-top tamarin (*Saguinus oedipus*) in captivity. Pp. 45-53 in A PRIMATE MODEL FOR THE STUDY OF COLITIS AND COLONIC CARCINOMA: THE COTTON-TOP TAMARIN: *SAGUINUS OEDIPUS*. N.K. Clapp ed. Boca Raton, CRC Press.

Vitale A; Queyras A (1997):The response to novel foods in common marmoset (*Callithrix jacchus*): The effects of different social contexts. ETHOLOGY 103(5): 395-403.

Wechselberger E; Erkert HG (1994): Characteristics of the light-induced phase response of circadian activity rhythms in common marmosets, *Callithrix j. jacchus* [Primates-Cebidae]. CHRONOBIOLOGY INTERNATIONAL 11(5): 275-284.

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SQUIRREL MONKEYS (*Saimiri sciureus*)

SECTION B: SCIENTIFIC JUSTIFICATION

The recommendations are based on personal experience gained by the authors (H. Contamin, N. Herrenschmidt, and H.Weber during the past more than 10 years with squirrel monkeys in breeding and experimental colonies of the Pasteur Institute in Cayenne, French Guyana, the Primate Centre at Strasbourg, France and Novartis Pharma Ltd., Basel, Switzerland. They include personal information obtained from G. Dubreuil, CNRS - Marseille and M. Huber, formerly ROCHE, Basel. An overview on squirrel monkeys is published in the book of Rosenblum and Coe,(1985) and their care and management by Mendosa (1999).
Complementary comments on the chapters 1 to 6:

1. Introduction
Background

The taxonomic classification of the various species and subspecies has been undergoing changes throughout the years and has recently been subject to genetic analysis. For practical reasons it seems at present appropriate to differentiate between two subgroups only. In contrast to previous opinion animals of both subspecies can mate and produce viable offspring.

2.2. Temperature:
Background

The squirrel monkeys live in regions with warm climates of the Amazon area to cool climates of the mountain ranges of Peru and Bolivia. Though the species may tolerate a wide range of temperatures, within the habitat of individual colonies, temperatures are not usually subject to sudden substantial variations. Within the forest animals apparently seek the regions with most suitable temperatures in the canopy.

2.4. Lighting:
Background

Little is known about the minimal light requirements for squirrel monkeys ecept that they are daylight active and only feed when there is light. (Parker CE, 1966, Psychon. Sci, Vol. 6, 111-112). Based on current experience and best practice, light intensities of 400 lux and above seem to ensure healthy animals, normal behaviour and reproduction. Provision of UV for upto 1 hour/day has proved useful in in avoiding osteopathogenesis due to possible lack of vitamin D3 in the diet. Animals having access to outdoor enclosures may cater for their needs by moving to the exterior. However, in indoor enclosures, even with large windows, UV exposure is eliminated by the glass. When providing UV with UV lamps time limits and distance to the lamp must be controlled according to the instructions.

3. Health
Background

For the health monitoring of squirrel monkeys reference is made to the FELASA recommendation Health Monitoring of Non-human Primate Colonies (1999), *Laboratory Animals,* 33 (Suppl.1), S1:3 - S1:18.

4.1. Social housing
Background

Saimiris usually live in groups of around 20 individuals, (Robinson and Janson 1987; Emmons and Freer 1990), and, therefore, groups of 5 and more animals, though they may sit together in small subgroups, are preferable to pair housing for any form of permanent housing. The minimal

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enclosure sizes for less than 5 animals are only given for possible cases of incompatibility or if experiments of longer duration require smaller groups than 5.

With their long rearing period, female saimiris produce one offspring (rarely twins) every one to two years. Reproductive performance is rather low compared to macaques, though in stabilised colonies a 50% reproduction rate may be reached. Since, in nature, the leading male may change according to its (“fatty“) reproductive state, an appropriate exchange of males may be considered.

The authors are not aware of literature on the exact ”imprinting“ age of young squirrel monkeys, which may start soon after birth. However, the fact that animals leave their mothers at quite an early stage and are easily adopted by other females and that hand reared infants attach to their foster parents indicates that this takes place within the first six months of life. Nevertheless, if not necessary, weaning and separation from the parent colony should not take place before 6 months of age.

4.3. Enclosures – dimensions and flooring

Background

Although enclosure dimensions for group housing are based on enclosures for two animals, it is recommended - as mentioned for 4.1. to keep squirrel monkeys in larger groups. Fewer than 5 animals in a group should only be kept in cases of incompatibility or experimental requirements. Based on the dimensions given for each additional animal above 6 months of age 5 animals will require 5.5m^3 which is less than the 8m^3 required by the Swiss Animal Protection Act but considered to be sufficient to provide structures and retreat possibilities. The linear space increase given for each additional animal is, therefore to be taken as guidance. Splitting a group may be preferable to simply adding additional space. Judgement should be based on the condition and behaviour of the colony.

The enclosure dimensions suggested for experimental individual or pair housing correspond with two of the present cages recommended by the Appendix A of ETS 123 for unlimited housing of saimiris and with the cages required by the Swiss Animal Welfare Act for temporary housing of monkeys. They are based on allometric measurements and would allow an animal to sit on or under a perch and walk and turn with ease. These cage dimensions are considered as acceptable for experimental conditions where larger enclosures would be contra-productive to animal welfare and/or for a limited time as justified by the experimental protocol..

4.4. Feeding

Background

Like some other South American monkeys, Squirrel Monkeys do not seem able to utilise D2 but rely on D3 which is synthesised in the skin under the influence of UV light. Since UV is absorbed by most types of glass it is recommended, for animals maintained indoors, to ensure a sufficient supply of vitamin D3 in the diet to avoid osteopathogenesis. The risk of vitamin D3 intoxication is low since South American species are known to be very tolerant to excessive vitamin D3. Most commercially available diets are absolutely adequate for feeding to Squirrel Monkeys and for providing sufficient Vitamin C and D3. Feeding meal-worms, crickets or other insects bears the risk of transmitting bacterial diseases.

4.8 Handling:

Background

Squirrel monkeys can be trained to come forward for titbits or drinks as rewards. They are also capable of learning how to solve tasks for reward. For catching for investigation or treatment, animals should be trained to enter gangways with trap cages or individual enclosures

Though animals can be trained to accomplish tasks, attention should be paid to appropriate recovery periods when subjected to experiments repeated at intervals. Task performance requires energy for concentration. Sessions of three to four hours duration seem to be well tolerated by

Squirrell Monkeys. However, single experimental sessions lasting longer should not be repeated more frequenetly than at weekly intervals and the monkeys should be under close veterinary supervision.

GENERAL REFERENCES

Ausmann LM, Gallina DL, Nicolosi RJ (1985) "Nutrition and Metabolism of the Squirrel Monkey" in Handbook of Squirrel Monkey Research. Rosenblum LA and Coe CL eds. Plenum; New York. Pp 447-488.

Bantin GC (1966) "Establishment of a Squirrel Monkey Colony" J. Inst. Animal Tech.. Vol. 17, 66-73

EAZA husbandry guidelines for the Callithricidae. J Bryan Caroll ed, EAZA Bristol Zoological Gardens, 2002

Emmons LH and Freer F (1990) Monkeys (Primates) Neotropical Rainforest Mammals: A Field Guide, pp. 134-153. Chicago: University of Chicago Press.

FELASA (1999) Health Monitoring of Non-human Primate Colonies *Laboratory Animals,* 33 (Suppl.1), S1:3 - S1:18.

Knapka JJ, DE Barnard, KAL Bayne, SM Lewis, BM Mariott & OT Oftedal (1995). Nutrition in Non Human Primates in Biomedical Research; Biology and Management. Academic press, San Diego, USA pp. 211-248

Lehner et al "Biological activities of vitamins D2 and D3 from growing Squirrel Monkeys" Laboratory Animal Care vol. 17, 433-493

Mendoza, S. P. (1999) Chapter 37. Squirrel Monkeys. In Poole, T. (Ed.) *The UFAW Handbook on the Care and Management of Laboratory Animals.* 7th Edition Blackwell Science, Oxford pp. 591-600

Robinson GJ and Janson CH (1987) Capuchins, squirrel monkeys and ateline: Socioecological convergence with Old World primates: In: Smuts BB, Cheney DL, Seyforth RM, Wrangham RW, Strutsaker TT (Eds). Primate Societies, pp 69-82. Chicago; University of Chicago Press.

Rosenblum LA and Coe CL Eds (1985), *Handbook of Squirrel Monkey Research*. Plenum Press, New York

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MACAQUES AND VERVETS

SECTION B REFERENCES

Most of the relevant discussion regarding the basis for the species-specific provisions for Macaques and Vervets and literature has been surveyed in the Introduction, however the following additional references are of value:

Barbe, S. (1999) The influence of environmental enrichment on social behaviour in captive long-tailed macaques (*Macaca fascicularis*). *Folia Primatologica* 70, 193

Baskerville, M. (1999) Chapter 39 Old World Monkeys. In *The UFAW Handbook on the Care and Management of Laboratory Animals* (7th Edition) *Volume 1 Terrestrial Vertebrates* Poole, T. (Ed). Blackwell Science, Oxford pp. 611-635

Bertrand M, (1969) The behaviour of the stumptail macaque. In: *Bibliotheca Primatologica* no 14. Karger, Basel/ NewYork.

Buerge T, B Panoussis and H Weber (1997) : Primate housing facilities for pharmaceutical research in Switzerland (an example). Proceedings of Second EUPREN/ EMRG Winter Workshop. In *Primate Report* 49, Ed. M Schwibbe German Primate Center, Göttingen, pp 19-22

Isbell L, DL Cheney, RM Seyfarth. 1991 Group fusion and minimum group sizes in vervet monkeys. *American Journal of Primatology* 25, 57-65

Lambrechts C, JV Seier, Mdhluli C, 2001, Management and breeding of the vervet monkey in South Africa. In: *Proceedings of VII. FELASA Congress, Mallorca 1999*,
in press

Line SW, KN Morgan, H Markowitz, JA Roberts and M Ridell 1990, Behavioural reponses of female long-tailed macaques (M. fascicuaris) to pair formation. *Laboratory Primate Newsletter*. 29 1-5

Seiers JV, 1986, Breeding vervet monkeys in a closed environment. *Journal of Medical Primatology* 15, 339-349

Seiers JV. and PW de Lange, 1996, A mobile cage facilitates periodic social contact and exercise for singly caged adult vervet monkeys. *Journal of Medical Primatology* 25, 64-68.

SECTION B: EXPLANATORY PART AND REFERENCES

1. Introduction:
Background

The species nomenclature is based on commonly accepted terms (Jolly C.E. 1993). Other names, occasionally to be found in some publications are not considered to be correct and may be due to the fact that hybridisation of species can occur.

3. Health:
Background

Like all Old World species, baboons are very susceptible to tuberculosis and have to be protected from contamination through humans. They are also susceptible to the Ebola viruses and - if originating from certain African areas - may be carriers of yellow fever virus for which, however, vectors for transmission are missing in Europe. Simian immunodeficiency virus (SIV) could be of concern in transplantation studies and transmission to Asian species may induce clinical symptoms in the same.

4.1. Social housing/ breeding
Background

The proportion males to females and group sizes recommended for breeding groups are based on the average subgroup sizes observed in the wild and ensure adequate reproduction However, other group sizes with a single male may also be acceptable. Since both species mentioned are not organised as harems, females may mate with different males. Therefore, if the genetic background is relevant, single male groups will be appropriate.

With about 4 months infants start walking on their own when the troop is on the move. Between the age of 8 to 14 months the infants begin to interact with other animals of the colony with increasing independence and, therefore, can be separated and integrated into peer groups with less stress. For animals that have to be separated from their mothers below the age of 8 months for veterinary reasons adoption by other adult females in stable colonies may be considered. This may be problematic with older infants, in which case housing with peers is recommended if the infant cannot be left in the group. Animals foreseen for breeding should be left in their maternal colony in order to acquire parental skills and complete social competence.

It is evident that for most experimental purposes and stock single sex groups will be formed. It is preferable to have groups of 4 and more animals in order to avoid bullying of individual animals. Since conflicts due to competition may occur when males and females are housed in the same room the sexes should be housed in separate rooms.

4.3. Enclosures - dimensions and flooring
Background

The proposed dimensions are based on morphometric characteristics of growing and adult male baboons allowing them to sit upright on and under two perches and move at least 4 steps in one direction. For experimental purposes the dimensions required could also be provided with two or more interconnected modules with the minimal volume per animal. This would have the advantage of providing retreat if required or temporarily separating individuals.

Baboons have a growth spurt up to the age of 4-5 years. After 5 years size (but not body weight) does not increase much. Sexual maturity is attained between 4.5 (females) and 7 (males) years. In immature animals the time budget is dominated by play. From 4 years onward play regresses

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and conflicts may occur especially in confined conditions, thus requiring careful observation of the group. In such cases groups of three may not be ideal. Therefore, baboons older than 3 years may have to be kept in compatible pairs or preferably in larger same sex groups.

Determination of age: At the age of 4 years both male and female baboons have attained a weight of approximately 9 kg. Since, however, weight may differ more than size, depending on nutritional conditions during development, other criteria may be more helpful in determining the age of animals for which the birth date is not known. In baboons the testicles of males drop between the age of two to three years. Dentition/ tooth eruption may also be helpful in as much as at the age of 3 years the first two secondary incisivi as well as most premolars and molars have erupted. The canines in males erupt between 3 and 4 years of age.

Indoor/Outdoor enclosures In most European regions it is necessary to ensure that all animals have access to an indoor enclosure with the recommended minimal space allowances to accommodate all the animals of a group should detrimental weather conditions force to prevent them from moving to an additionally provided outdoor enclosure. In some parts of Europe it is entirely possible to provide the minimum space allowance by an outdoor enclosure. Nevertheless, under such circumstances additional accommodation must be provided to allow shelter from inclement weather, shade from the sun and overnight sleeping quarters. The size of such sheltered or indoor areas should be large enough to accommodate all of the animals harmoniously for their resting periods.

4.8. Handling

Background

Young baboons are often easier to handle than macaques or vervets. With adult male baboons, though they too may be trained to come forward for the minor manipulations mentioned in section A safety precautions for personnel may be necessary in view of their strength and large canines.

SELECTED REFERENCES:

Alberts SC and Altmann J (2001) Immigration and hybridization patterns of Yellow and Anubis baboons in and around Amboseli, Kenya. American Journal of Primatology, 53: 139- 154.

Altmann J (1980) Baboon mothers and infants. Cambridge, MA, USA Harvard University Press.

Altmann J, Altmann SA, Hausfater G and McCuskey SA (1977) Life history of yellow baboons, physical development, reproductive parameters and infant mortality. Primates 18, 315 - 330

Altmann SA and Altmann J (1970) Baboon ecology: African field research. Bibliotheca Primatologica, No. 12

Castracane DV, KC Copeland, P Reyes and TJ Kuehl (1986) Pubertal endocrinology of Yellow Baboons (*Papio cynocephalus)*: Plasma tesosteron, testis size, body weight and crown rump length in males, Amer. J. Primatol. 11, 263-270

Coelho, A.M. Jr. (1985). Baboon dimorphism: growth in weight, length and adiposity from birth to 8 years of age. In Watts, E.S. (ed.), Nonhuman primate models for human growth and development, Alan R. Liss,New York, pp. 125-159.

Coelho, A.M. Jr. and Rutenberg, G.W. (1989). Neonatal nutrition and longitudinal growth in baboons: adiposity measured by skinfold thickness. Am. J. Hum. Biol. 1: 429-442.

Glassman, D.M., Coelho, A.M. Jr., Dee Carey, K., & Bamblett, C.A. (1984). Weight growth in savannah baboons: a longitudinal study from birth to adulthood. Growth 48: 425-433.

DRAFT

Hausfater G (1975) Dominance and reproduction in baboons (Papio cynocephalus): a quantitative analysis. Contrib. Primatol. Vol. 7 Basel, S. Karger

Jolly CJ, (1993) Species, subspecies, and baboon systematics. In: „Species, concepts and primate evolution." Kimbel WH and LB Martin (eds.) Plenum, New York pp 67-107

Kingdon J (1997) The kingdon field guide to African mammals. San Diego, Academic Press

Kummer H (1968) Social organisation of Hamadryas baboons: a field study. Basel, S. Karger

Muehlenbein MP, BC Campbell, CM Philippi, MA Murchinson, RJ Richards, F Svec and L Myers (2001) Reproductive maturation in a sample of captive male baboons. J. Med. Primatol. **30**, 273-282

Ransom TW and Rowell TE (1972) Early social development of feral baboons. In: "Primate socialization" FE Poirrier ed. New York, Random House, pp 105-144

Rhine RJ, GW Norton, BJ Westlund (1984) The waning of dependence in infant free ranging yellow baboons *(Papio cynocephalus)* Mikumi National Park. Amer. J. Primatol. **9,** 213-228

Shohoji, T. & Sasaki, H. 1987. An aspect of growth analysis of weight in savannah baboons. II Gender comparison by adjusting age. Growth 51:425-431

Sigg H, Stoiba A, Abblegen JJ, Dasser V, (1982) Life history of hamadryas baboons: physical development, infant mortality, reproductive parameters and family relationships- Primates **23**, 473-487

Stammbach E (1987) Desert, forest and mountain baboons: multilevel societies. In: „Primate societies" BB Smuts, DL Cheney, RM Seyfarth, RW Wrangham and TT Struhsaker eds. Chicago, the Chicago University Press. pp 112-120

Strum SC, and Western JD (1982) Variatins in fecundity with age and environment in olive baboons (Papio anubis). Am. J Primatol. **3**, 61-76.

Swindler DR (2002) Primate dentition; an introduction to the teeth of non-human primates. Cambridge University Press, Cambridge, UK

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GT 123 (2004) 15

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)

8th Meeting of the Working Party
Strasbourg, 22-24 September 2004

Species specific provisions for Reptiles

Background information for the proposals presented by the Group of Experts on Amphibians and Reptiles

PART B

This document will not be distributed at the meeting. Please bring this copy.

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Background information

On the species-specific proposals for reptiles

Presented by the Expert Group on Amphibians and Reptiles

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C o n t e n t s

Preamble

This document contains species-specific proposals for amendments to the Appendix A of the Council of Europe's Convention ETS 123 dealing with the protection of animals used or intended for use in any experimental or other scientific procedure which may cause pain, suffering, distress or lasting harm.

In 1997, the Council of Europe established working groups with the aim of advising the Council whether, how and to what extent the Appendix A of the Council Convention ETS 123 needed revision. The expert group appointed to deal with species-specific aspects of amphibians and reptiles was set by representatives of the following international organizations:

European Science Foundation, *ESF*
European Federation of Pharmaceutical Industries and Associations, *EFPIA*
European Federation of Animal Technologists, *EFAT*
Eurogroup for Animal Welfare, *EUROGROUP*
Federation of European Laboratory Animal Science Associations, *FELASA*
Canadian Council of Animal Care, *CCAC*

Representatives were:

Prof. Dr. Jörg-Peter Ewert (Co-ordinator)
ESF

Prof. John E. Cooper
FELASA

Dr. Tom Langton
EUROGROUP

Prof. Dr. Gilbert Matz
ESF

Kathryn Reilly
EFPIA

Dr. Helen Schwantje
CCAC

The Group was complete in September, 2000. Unlike expected, no representative from the *EFAT* participated in this Group. Yet Mr. Chambers of *EFAT* let the Council's Secretariat know that *EFAT* remain very interested in the work of the Group and will give comments and suggestions on the proposal to be made by the Group.

The general tasks of the Group were defined as follows:

a. listing, for the species concerned, the main questions to be answered with a view to revising Appendix A;
b. examining results already available and practical experience acquired which could possibly answer these questions;
c. identifying areas where further research would be needed;
d. making proposals for amendments to Appendix A, providing information in particular to the ethological and physiological needs of the animals. These proposals (Part A) should be supported by background information in an explanatory report (Part B), presenting scientific evidence and/or practical experience.

The Group was expected to send a first draft of the proposal for the revision of the species-specific parts of Appendix A of the ETS 123 by 15 January 2001.

By the middle of November, 2000, the General Coordinator Dr. Wim de Leeuw suggested that Prof. Dr. J.-P. Ewert be the Coordinator of the Expert Group on Amphibians and Reptiles. Since there were no objections, Ewert accepted to do this job, presented a preliminary draft of a proposal to the group members on December 12, 2000, and asked for suggestions for improvements. In this draft, the presentation of the consensus proposals made by the Group of Experts on Rodents and Rabbits (Strasbourg, 21 February 2000) and the Standard Format for Species Specific Sections was used formally as a basis. The Resolution on the Accommodation and Care of Laboratory Animals adopted by the Multilateral Consultation on 30 May, 1997, was taken into account where appropriate. Furthermore, the Guide to the Care and Use of Experimental Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press, Washington, D.C., 1996) and the Guide of the Swedish Board of Agriculture (Department for Animal Production and Health, Animal Welfare Division) were considered. Since there were no suggestions for improvements on the draft proposal by the group members, the Coordinator sent the consensus proposal to the European Council's Drafting Group in due time by 14 January, 2001.

The original draft proposal for the revision of Appendix A concerning amphibians and reptiles presented by the Expert Group was sent to the members of the Drafting Group for consultation [see paragraphs 236 and 237 of Summary of proceedings GT123(2000)39]. In agreement with the General Coordinator, the Chairman of the Working Group and the members of the Drafting Group, and in order to bring the document in line with the presentation previously adopted by other groups of experts, the document was divided into two parts: Part A containing paragraphs with proposals for Appendix A [doc. GT123(2001)1], and Part B providing detailed scientific background information supporting these proposals [doc. GT123(2001)23]. Paragraphs transferred into the Part A are in boxes (bold face italics).

Part A. The species-specific proposals of Part A, concerning amphibians and reptiles, were revised based on suggestions and comments provided by the Drafting Group (*) and the representatives and observers at the meetings of the Working Party at Strasbourgh from 2001 through 2003 [cf. also Summary Proceedings of the Working Party GT123(2001)35, GT123(2002)41, GT123(2003)40, GT123(2003)41, GT123(2003)72, and of the Drafting Group GT123(2003)57 rev].

Amphibians & Reptiles:
GT 123 (2001) 1)*
GT.123 (2001) 23)[A&B]*
GT 123 (2001) 30
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GT 123 (2002) 10)*
GT 123 (2002) 25
GT 123 (2002) 40)*
GT 123 (2002) 56
GT 123 (2002) 59
GT 123 (2002) 62
GT 123 (2002) 64
GT 123 (2002) 69
GT 123 (2003) 15)*
GT 123 (2003) 27
GT 123 (2003) 33
GT 123 (2003) 35
GT 123 (2001) 1E rev3)*

Reptiles :
GT 123 (2003) 34E)*
GT 123 (2003) 55)*
GT 123 (2003) 61
GT 123 (2003) 65
GT 123 (2003) 67
GT 123.(2004) 1E)*
[Rev. Appendix]

The revision of the proposals proceeded preferably via e-mail communication in the Expert Group on Amphibians and Reptiles. On September 10th and 11th 2001, a meeting of the Expert Group was organized by Kathryn Reilly at Harlow/Essex UK. This meeting was supported by the Merck Sharp & Dohme Company. Furthermore, the Coordinator participated in meetings at the *Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft* organized by the representative of Germany of the Working Party at February 19th and November 6th ,2003.

At the 6th meeting of the Working Party in March 2003 it was decided to separate the proposals in two documents: *Species-specific Provisions for Amphibians* and *Species-specific Provisions for Reptiles.* Both documents were adopted and finalized at the 7th and 8th meeting of the Working Party in December 2003 and September 2004.

Part B. The present background information provides, where possible, scientific evidence for the *Species-specific Provisions for Reptiles*. Where this is not available, they take account of established good laboratory practice, based both on the experience of the members of the Expert Group and also on consultations with other experts. Additional comments and suggestions from members of the Working Party are considered and incorporated in Part B where appropriate. The revised Part B – including Part A – was submitted to the Council of Europe in advance of the 8th meeting of the Working Party.

In reply to the general tasks a-d:

a. *Listing, for reptiles, the main questions to be answered:*

(1) Both reptiles and amphibians are significant bio-indicators of the environmental health. In view of world-wide declines of populations of reptiles [cf. Section 1.1], a selection of suitable species for the use in scientific procedures should be recommended. "Suitable" means that captive breeding programs for this species already exist, and/or the population of this species is not in danger. Captive breeding programs are required and should be promoted.
(2) Recommendations for housing reptiles (minimum cage sizes, heights) under consideration of the natural biotope and the species-specific needs are required.
(3) Reptiles are ectothermic and, thus strongly adapted to their different biotopes. This requires species-specific considerations regarding temperature and humidity preferences, homeostatic capabilities, and seasonal activity patterns.
(4) Knowledge on diseases of reptiles and their treatments should be incorporated.

b. *Examining results already available and practical experience acquired which could answer these questions:*

Examples of reptile species from main habitats (aquatic, semi-aquatic, semi-terrestrial and arboreal) frequently used in experimental and other scientific procedures are listed. A reference list is provided. Information on caging reptiles (cage dimensions, temperature/humidity preferences) is provided in Section 4.3 and in an Appendix.

c. *Investigating what research is being carried out within the field and identifying areas where further research would be needed*:

An Internet MEDLINE search on research activities in reptiles among different science disciplines is provided in Section 1.2. See also list of references.

d. *Providing information in particular to the ethological and physiological needs of reptiles:*

The standard format of the species-specific Sections 2-4 is provided with explanatory reports for the proposals and recommendations and these are supported by scientific evidence and practical experience.

Reptiles

J. Species-specific provisions for reptiles

1. Introduction

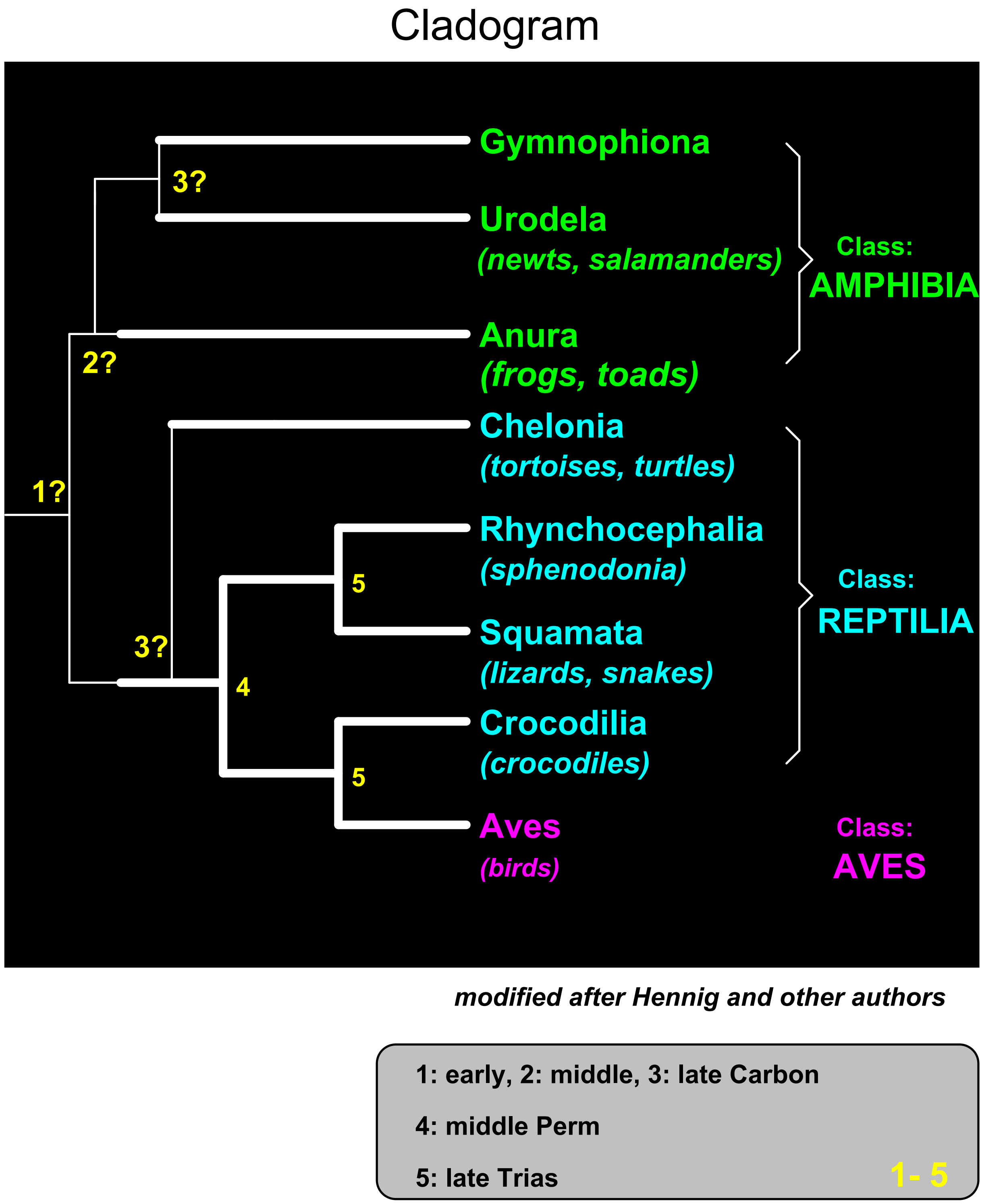


Fig. 1. Cladogram of amphibians and sauropsides (birds and reptiles).

According to morphological systematics, reptiles include the main orders Rhynchocephalia (tuatara), Squamata (lizards, snakes), Chelonia (tortoises, turtles, and terrapins), and Crocodilia (alligators, crocodiles, caimans, and gavials). They differ greatly in their patterns of geographic distribution and in the diversity of living types.

In the cladogram, shown above reptiles and amphibians are traced back to a common ancestor living in early Carbon. Lizards and snakes, on the one hand, and crocodiles and birds, on the other hand, can be traced back to a common ancestor living in the middle of Perm. The common ancestor of crocodiles and birds probably lived in late Trias. (For a phylogenetic analysis of body-form evolution in anguid lizards, see Wiens & Slingluff, 2001). Among the reptiles, Chelonia and Squamata will be considered in the present proposals.

In contrast to the more or less smooth and moist skin seen in amphibians, reptiles have a skin protected by overlapping scales (snakes, lizards), by a box-like shell (chelonians), or by bone plates in the skin (crocodiles, alligators, and caimans). The thick skin is an adaptation to better protect reptiles from the water loss that occurs with the permeable skin of amphibians.

Reptiles and amphibians are different in many other respects of which some will be mentioned. An ontogenetic developmental aspect points to the fact that reptiles – like birds and mammals – belong to the Amniota due to the existence of embryonic sheaths. Amphibians are Anamniota. The eggs of reptiles are mostly laid on land and develop into animals already adapted for land life (see also Sever & Hamlett, 2002). Amphibian eggs are mostly laid in the water and develop into tadpoles which after metamorphosis mature to the adults.

From an evolutionary point of view, basic research both in amphibians and reptiles is of fundamental interest in order to evaluate comparable functional principles in mammals and, thus, also in humans (e.g., see Ewert, 1998). Phylogenetically, reptiles and mammals can be traced back to a common ancestor from that amphibians are derived. Current research suggests that certain fundamental morphological and physiological principles, present in amphibians and reptiles, were conserved during evolution up to the primates, in an appropriate differentiation and specification. A hypothesis on brain evolution, for example, suggests that the mammalian telencephalic neocortex (isocortex) is derived from a structure that is homologous to the amphibian telencephalic dorsal pallium (Northcutt & Kaas, 1995; cf. also Ewert, 1998; Reiner, 2000; Super & Uylings, 2001; Guirado & Davila, 2002). Unlike in amphibians, the telencephalon of reptiles is characterized by a structure called the dorsal ventricular ridge which plays an important role for various sensorimotor functions. Another hypothesis suggests that a part of the mammalian neocortex is homologous to a structure derived phylogenitically from the dorsal ventricular ridge of a common precursor (Northcutt & Kaas, 1995; Aboitiz, 1995; 1999; Aboitiz et al., 2002). For evolutionary perspectives on the basal ganglia see Reiner et al. (1998) and Smeets et al. (2000) and on the limbic system see Bruce & Neary (1995).

1.1. Declining reptile populations

Both reptiles and amphibians are bio-indicators of environmental contaminants. Health effects of endocrine-disrupting chemicals on wildlife population – particularly in the aquatic environment – is a potential global problem. In reptiles, such effects vary from feminized or masculinised sex organs, changed sexual behaviour and altered immune function. Embryonic exposure to natural hormones and man-made chemicals (such as PCBs and common herbicides) can permanently alter the functioning of the reproductive system (Crain & Guilette, 1998; Vos et al., 2000). Furthermore, egg-shell thinning caused population declines. Distorted sex organ development and function in alligators has been related to a major pesticide spill into a lake in Florida. Estrogenic/anti-androgenic effects in eggs in this reptile have been causally linked to the DDT complex (Vos et al., 2000).

All major groups of reptiles contain some endangered species, such as marine turtles, crocodilians and constricting snakes (family Bordae). *Sphenodon*, the sole surviving member of the order Rhynchocephalia, is limited to the island Cook Strait in New Zealand. As pointed out for amphibians, future goals must include the establishment of activities of scientific research that cover all areas of the world where reptiles live, in order to discover which species are rare or declining and to investigate the reasons behind such declines (cf. causes of reptile decline established by PARC in 1999).

The EMBL Reptile Database is intended to provide information on the classification of all living reptiles by listing all species and their pertinent higher taxa. The database therefore covers all living turtles, tortoises, snakes, lizards, and crocodiles. It is supposed to be a source of taxonomic data, thus providing primarily (scientific) names, synonyms, distributions and related data.

On protected reptile species, see:
http://www.CITES.org

Where possible, reptiles used for experimental or other scientific purposes should be procured from reputable suppliers.

A main question to be addressed with a view to revising species-specific aspects in Appendix A to the convention ETS 123 concerns the problem of declining reptile populations, on the one side, and the consumption of reptiles for the use in experimental or other scientific procedures, on the other side. One answer to this question is a selection of species under the aspects of protection and breeding programs that maintain the population of reptiles in captivity.

1.2. Selection of species: Examples of species from two main habitats

Table J.1. lists two very general habitat categories of reptiles and examples of species of each habitat frequently used for experimental and other scientific purposes. The following proposals provide details on the basic housing and care conditions recommended for species found within these habitats. Specific procedures may require the use of certain other species which do not fall into these categories, such as semi-aquatic, arboreal or rock-climbing reptiles. Should behavioural or breeding problems occur, or should further information on specific requirements for other species be required, advice should be sought from experts specialised in the species concerned and care staff, to ensure that any particular species' needs are adequately addressed. Additional information on species and habitats is available in the background information document by the expert group.

Table J.1. Two habitat categories and examples of reptile species of each habitat frequently used

Habitat	***Species***	***Size***	***Original geographic distribution/ Biotope***	***Optimal temperature***	***Relative humidity***	***Main period of activity***
Aquatic	***Red-eared terrapin*** ***Trachemys scripta elegans***	***20-28 cm***	***Mississippi Valley drainage/ Quiete water with muddy bottom***	***20-25°C***	***80-100%***	***Day***
Terrestrial	***Common garter snake*** ***Thamnophis sirtalis***	***40-70 cm***	***North America/ Woodland, wet areas***	***22-27°C***	***60-80%***	***Day***

Examples of species of other habitat categories include:

HABITAT	Reptile Species	Size	Original geographic distribution/ Biotope	Optimal temperature	Relative humidity	Main activity
SEMI-AQUATIC Tortoises	Golden Greek tortoise *Testudo graeca*	30-40 cm	Central South Europe/ Grass dry areas	25-35°C	40-80%	Day
TERRESTRIAL Lizards	Common lizard *Lacerta vivipara*	10-18 cm	North Europe/ Grass, heath lands	15-20°C	40-60%	Day
ARBOREAL Lizards	Green anole *Anolis carolinensis*	17-22 cm	South-Eastern USA/ Moist forest, trees, shrubs, walls	28-30°C	50-80%	Day

Any project requiring consumptive use of wild caught animals from a species or population which is rare or in danger of extinction should require rigorous justification before an institutional animal care committee allows to proceed research. Captive breeding programs should be encouraged. For reproductive biology and diseases of captive reptiles see Murphy & Collins (1980). A large number of species of snakes already are being bred in captivity, especially Colubridae (*Elaphe* spp., *Lampropeltis* spp. and others) and Boidae (*Boa* spp., *Epicrates* spp., *Python* spp. and others), but also Viperidae and Elapidae. The Reptile Breeding Institute at Picton (Ontario, Canada) has developed techniques for breeding several species of constructing snakes (Boidae) in connection with a program to attempt needs for captives of rare or endangered constructor species without exploiting wild populations. Many species of lizards are also being bred in large numbers. Concerning turtles *Trachemys scripta elegans* is bred in large numbers.

1.3. Current research

An internet MEDLINE search comparing the number of publications (original research papers) on amphibians in relation to those on reptiles and on mammals (rhodents, such as rats and mice) shows that amphibians and reptiles are much less frequently used (Fig.2). Among anuran amphibians *Xenopus* spp. is the most frequently used genus. The frequent use of *Xenopus* spp. as research subject explains the dominance in the number of research papers on amphibians compared to those on reptiles.

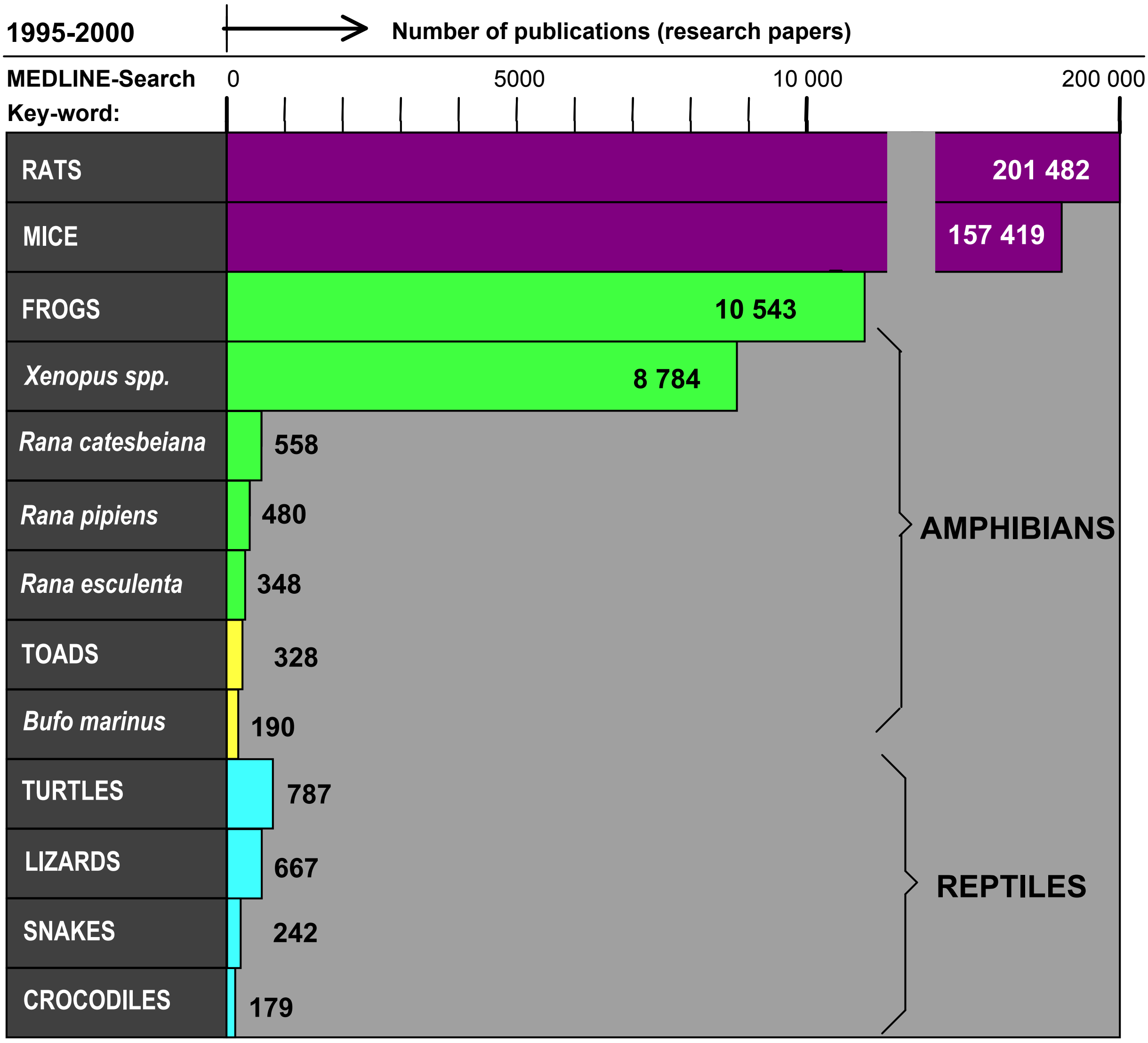


Fig. 2. Internet search for scientific publications related to rhodents, amphibians, and reptiles.

The following table shows the result of an Internet MEDLINE search dealing with research activities in different scientific disciplines on reptiles (key words: reptilia, reptiles) in comparison to amphibians (key words: amphibia, amphibians) for the time segment 1995-2000:

Research Discipline	Amphibians Publications 1995-2000 %	Reptiles Publications 1995-2000 %
Cellbiology	**17.0**	4.8
Vegetative Physiology	**16.9**	**20.6**
Genetics	**14.7**	**11.5**
Morphology	**14.2**	8.0
Brain Research	**11.3**	8.0
Ecology	6.4	3.8
Sensory Physiology	6.1	1.9
Biochemistry	5.2	**16.5**
Parasitology	4.9	**14.7**
Pharmacology	2.0	4.0
Ethology	1.0	2.7
Housing	0.3	3.5

First of all, it is worth mentioning that the overall research activity in amphibians is greater than in reptiles (Fig.2). A relative comparison between literature on amphibians and reptiles shows comparable high research activities in Vegetative Physiology and Genetics. Brain Research and particularly Morphology and Cellbiology clearly dominate in amphibians, whereas Biochemistry and Parasitology vice versa. Ecological and ethological research and studies on housing captive reptiles and amphibians are relatively scarce and should be promoted [see also Section 3.3].

2. The environment and its control

First of all, experience from *good laboratory practice* (GLP) should be considered. In 1990 *Greendale Laboratories* UK became the first dedicated laboratory to achieve full GLP compliance for all its disciplines. The GLP scheme became administered by the *Medicines Control Agency of the Department of Health*. Having GLP means that every aspect of the laboratory from automatic analysers right down to the smallest piece of equipment has calibration, maintenance and test methodologies rigorously controlled and checked both prior and during their working lives. It is internationally recognized that GLP is one of the highest quality assurance accreditations that a laboratory can attain. GLP is concerned with the organisational processes and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported. Adherence by laboratories to the principle of GLP ensures the proper planning of studies and the provision of adequate means to carry them out. It facilitates the proper conduct of studies, promotes their full and accurate reporting, and provides a means whereby the integrity of studies can be verified.

Cooper (1984) working on Exotic, Zoological and Wildlife species, is a Consultant Pathologist with Wildlife Health Services UK (see also Cunningham et al., 1996; Cooper & Cooper, 2003).

With reference to the different ecological adaptations of reptiles, the diversity of living types, and the apparent survival difficulties of many species of reptiles in nature, the necessity must be emphasized to accumulate sufficient information on the biology of their behaviour and the environmental needs of the species to be maintained and studied in captivity [cf. Section 1.2]. This Section of the proposal can only provide some aspects of the environmental conditions required in the laboratory use. For details see, e.g., Steward, 1969; Greenberg, 1977; Greenberg & MacLean, 1978; Matz, 1983; Warwick, 1990a,b; Beynon et al., 1992; Schaeffer et al., 1992; Zug, 1993; Warwick et al., 1995; Barnard, 1996; Matz & Weber, 2002; O'Rourke, 2002; Kaplan, 2002; Cooper & Cooper, 2003; Matz & Vanderhaege, 2003; Bolhuis & Giraldeau, 2004).

Laboratory reptiles are accommodated in a vivarium (e.g., terrarium, aqua-terrarium, tank). A vivarium is any room, building or other facility in which live, vertebrate animals are housed for periods of time exceeding 24 hours. The housing of animals should conform to the *NIH Guide for the Care and Use of Laboratory Animals in Research* (NIH publication No. 85-23, 1985 or succeeding editions). A new vivarium to be constructed should conform to the recommendations of this guide. Although desirable, it is impractical to require older vivaria to meet all of these standards. Remodels of older facilities, however, should attempt to bring the facilities more nearly in compliance with these standards. Animals may be held in laboratories or other locations outside vivaria for periods of time not to exceed 12 hours, after which time they should be returned to a vivarium. Exceptions to this policy need to be justified. It is advisable to locate vivaria in close proximity to the laboratories.

2.1. Ventilation

Enclosures of reptiles should be adequately ventilated. To prevent animals from escaping, ventilation should be screen-covered.

For most reptiles neither "open" units, such as wire-mesh cages, nor tightly closed units, are advantageous. Open units, however, are beneficial for terrestrial turtles. They should not be exposed to draught. Ventilation ports should be screen-covered and be placed at opposite sides of a terrarium (cf. Fig. 3A). Cages for arboreal reptiles tend to be high and often narrow. Adequate ventilation must be provided.

Housing venomous snakes requires ventilation ports with a double layer of screening which should be separated by a space wider than the length of the snakes' fangs to provide effective protection. It should also be taken into account that cobras (*Naja* spp.) and other venomous snakes spit out at the eyes of their enemies at a distance.

The water in the pool area of terraria (semi-aquatic, terrestrial or arboreal) and tanks (aquatic) should be renewed about twice a week [see also Section 4.5]. The water of tanks should be circulated, filtered, and aerated.

2.2. Temperature

Reptiles are ectothermic. In order to maintain their body temperatures, under natural conditions they will select microenvironments in which they can gain or lose heat. Therefore, enclosures should offer to the animals areas of different temperatures (temperature gradient).

Temperature requirements of different species vary considerably and may even fluctuate in the same species at different times of the year. In the laboratory, room and water temperatures should be controlled. In many reptiles, sex determination and gonadal differentiation are temperature-dependent.

An incandescent lamp positioned over the platform provided as a resting board will allow basking reptiles to increase their body temperature. When the lights are turned off, a flat heating device may be used. Terraria of snakes or lizards from tropical biotopes should be furnished with at least one warmth-plate. Heating devices must be thermostatically-controlled to prevent the animals from overheating and burning.

Like amphibians, reptiles are ectotherms ("cold-blooded" animals). Unlike endotherms ("warm-blooded" animals), their body temperature is dependent on the ambient environment. The advantage of ectothermy is that the resting metabolic rate and general energy requirements are less than those for mammals or birds of comparable size since no metabolic energy is spent on warming or cooling the body, and less energy is spent on food intake because less food is required to meet the body's low energy demands. The disadvantage of ectothermy, however, is that the ambient temperature determines the animal's metabolic processes and behaviour. The animal must actively seek temperatures that will allow it to feed, digest food, hibernate, etc. Reptiles adapt their body temperatures by finding the appropriate thermal environment through basking, burrowing, hiding under logs or leaves, or entering water.

In many respects ectothermic animals are more interactive with their environments than endotherms. At the same time, they tend to have greater problems adapting to changes in their species-typical environment. Therefore, the design of their artificial habitats demands special care, since research-biasing stress and distress responses to species-inadequate environmental conditions are to be avoided.

Reptiles depend on the temperature around them to control their internal body temperature. Thermal requirements of reptiles in captivity are reported, e.g., by Laszlo (1979). A reptile's ability to digest food and use energy, and its ability to protect itself from disease, are dependent upon reaching the correct body temperature. Reptiles can change their body temperature by moving back and forth from a warmer part of the cage to a cooler part and vice versa. A warmer area of the cage can be provided by special lamps, heated rocks and/or under the tank heaters. Keeping the temperatures in the optimal ranges and providing gradient temperatures throughout the cage is best (cf. Fig. 3B). If a reptile is kept in temperatures which are too warm or too cold, this places stress on their immune system and can lead to problems such as dehydration and problems with their body temperatures. Thermoregulatory aspects in reptiles are discussed by Greenberg (1976, 1980).

Reptiles in captivity require an environmental temperature at or near their optimal body temperature, which for most reptiles can be 25°C or even 35 to 40°C, especially in certain species of lizards, and below 13°C in sphenodon. Therefore, room and water temperatures must be regulated. Water temperatures for most North American freshwater turtles, for example, should be held at about 25°C.

Incandescent lamps are the most used systems. Heat sources that allow direct contact between the reptile and the source should not reach a temperature of more than 40°C. Even when there is opportunity to move away from a focal heat source within the cage, it is not uncommon for a reptile to remain in direct contact with that heat source until third degree burns have been inflicted.

Thermally-induced changes in heart rate and blood flow in reptiles are believed to be of selective advantage by allowing an animal to exert some control over rates of heating and cooling. This notion has become one of the principal paradigms in reptilian thermal physiology (Seebacher, 2000). Nocturnal snakes *Hoplocephalus bungaroides* select body temperatures of 28.1° to 31.1°C in laboratory thermal gradients. Accuracy of prey-catching and strike speed increase at higher body temperatures. The thermal biology of digestion in rubber boas is described by Doreas et al. (1997). For effects of temperature on the metabolic responses to feeding in snakes see Wang et al. (2002; cf. also Wang et al., 2001; Klein et al., 2002; Schumacher, 2003). Data on disease-associated preferred body temperatures in reptiles are reviewed by Kaplan (2002g; cf. also Warwick, 1991). High body temperature enhances the snake's fitness for a short time (Webb & Shine, 1998).

During the development, temperature influences the embryo's heart rate (Bichard & Reiber, 1996) and energetics (Booth, 1998). O'Steen (1998) has shown that embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles (see also O'Steen & Janzen, 1999). For temperature-dependent sex determination see Section 3.3.

A number of reptiles have cyclic behaviour, becoming active or inactive with the type of the coming season or are active all year around despite seasonal change (Abe, 1995). In South America, in savannah-like vegetation, during the dry season reptiles retreat into shelter deep enough to avoid temperature fluctuations during aestivation or reduce metabolic response to temperature. This seems to be a strategy common to many aestivating reptiles.

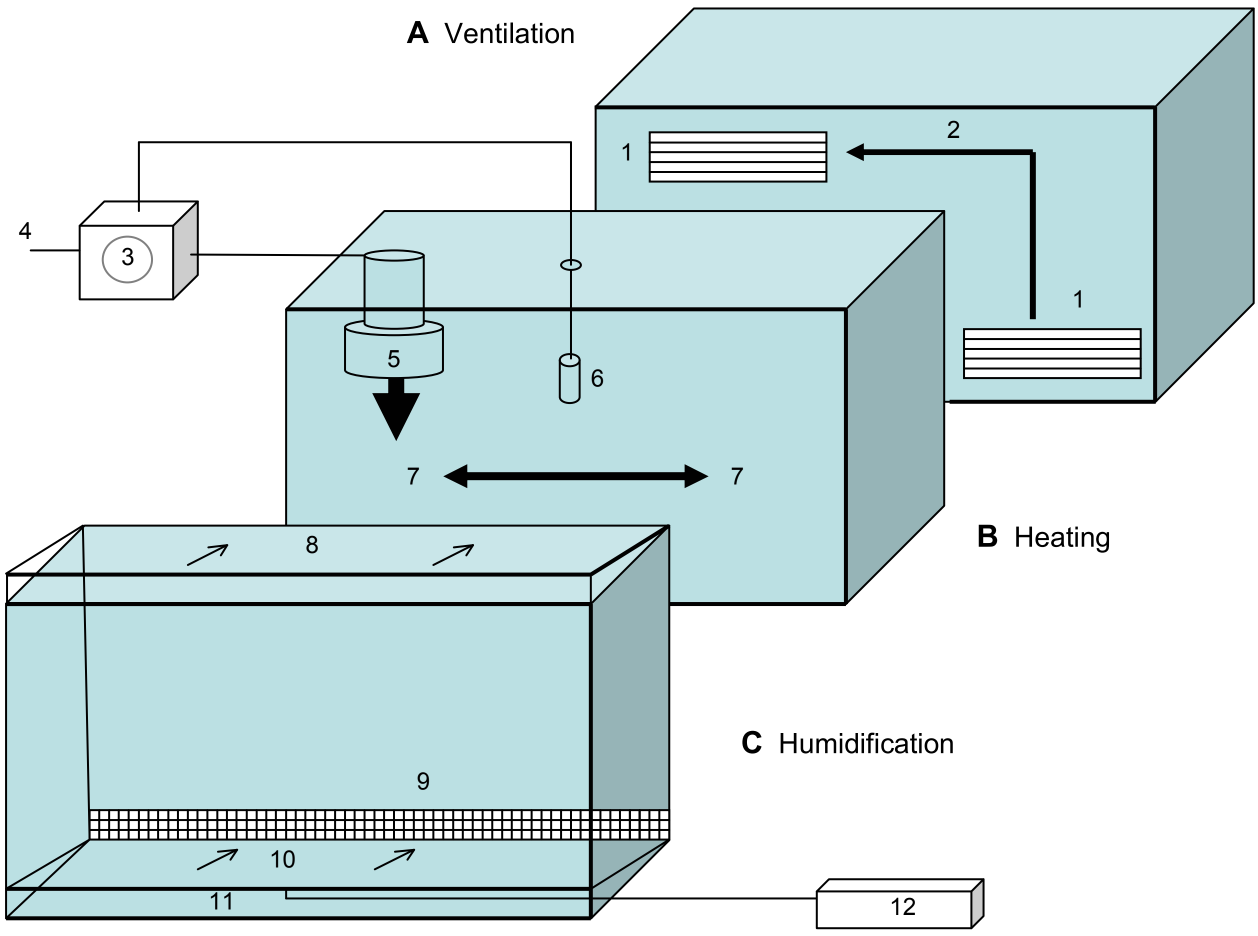
Winter survival for numerous ectothermic animals includes freeze tolerance, namely the ability to endure conversion of as much as 65% of total body water into extracellular ice. There are certain genes and proteins that are up-regulated by freezing or thawing in both freeze-tolerant reptiles (Storey, 1996, 1999; see also Constanzo et al., 1995; Voituran et al., 2002). For winter advisory in captive reptiles see, e.g., Kaplan (2002i). Temperature preferences of different species of snakes, lizards, and chelonians living on ground, sand, or rocks, in water or trees, respectively, are listed in an Appendix.

2.3. Humidity

In order to regulate humidity, it will also be necessary to regulate the ventilation rate. A relative humidity of 70-90% can be maintained by evaporating water from a container placed near the heater. The provision of areas of different humidity (humidity gradient) is beneficial.

The thick skin of reptiles better protects them from water loss and absorption of noxious substances from their environment than does the moist, permeable skin of amphibians. Therefore, reptiles can withstand lower humidity, except small individuals and species adapted to humid, tropical conditions. Nevertheless, ventilation should never be blocked completely. A high humidity can be reached with partial blocking. An example for high humid conditions is shown in Fig. 3C. To reduce air exchange, ventilation ports should be covered with a porous paper cover.

Snakes which live normally under tropical conditions, will require a relative humidity of between 60 and 90% in their terraria. Lower humidity may result in an inability to shed the skin completely. Such humidity levels can be maintained by watering by spray connected to a timer. Terrestrial turtles should not be exposed to high humidity for longer than 30-60 min per day. This can be achieved by using a humidifier in the room for 30 min daily.



1 Ventilation grid
2 Air flow
3 Thermostat
4 Power supply
5 Heating device
6 Probe
7 Thermal gradient
8 Sloping roof
9 Ventilation grid
10 Sloping floor
11 Water tray
12 Water heater

Figure 3 (legend on p.16)

Fig. 3. Examples of some basic requirements for the installation of a vivarium suitable to housing reptiles. A) *Ventilation.* At least two vents placed at either end of the vivarium at different height allow the air to circulate (2) from the ventilation grid (1) at the bottom through the ventilation grid at the top [details see Section 2.1]. B) *Heating*. The heating device (5) should be placed in a manner that allows for thermal gradients (7). Sufficient space between heating device and roof prevents scorching. The heater must be thermostat (3) -controlled using a sensor (6) placed near the centre of the vivarium. The capacity of the heater should be adjusted to the dimensions of the vivarium. [Further details see Section 2.2. *Lighting* cf. Section 2.4]. C) *Humidification.* The humidity in the vivarium should be adjusted to the requirements of the reptilian species [cf. Section 2.3]. The design, shown here, is suitable to keep relatively high humid conditions (for terrestrial reptiles), also allowing for different gradients of humidity: a heater (12) heats the water of a tray (11) that – at the vivarium bottom – evaporates to increase the humidity; a sloping floor (10) allows water to run down (see arrows) into a tray near a ventilation grid (9) placed at the opposite side of the vivarium. A sloping roof (8) at the top allows water to run down (see arrows) the back of the vivarium's wall. A vivarium providing high humid conditions should not be made, for example, of wood or melamine, rather of glass, perspex, fibreglass or of other appropriate material. All electrical connections must be waterproof.

An appropriate water source for animals which will drink water or will submerge must be available [see also Section 4.5]. For several lizards and snakes a little covered plastic container with humid substrate and a hole should be installed to carry out slough. It is important to mist the reptile's habitat and allow water to collect on foliage. This raises the humidity as well as allows an animal to drink fresh water from the leaves and vines. Most tree dwelling reptiles, such as anoles and chameleons prefer to drink droplets of water from plants and cage walls. When misting, a very light fine warm spray should be used. It will help to clean its eyes, remove shedding skin, and help with keeping it hydrated. Water can be provided in a bowl or misted with a spray bottle. For lizards and tortoises, the water bowl should be low enough that the animal can easily drink, and not so deep that it can lead to drowning. Water should be changed daily [see also Section 4.5].

Reptiles not receiving or drinking enough water can become dehydrated. Signs of dehydration include weakness, wrinkling of skin when pulled, sunken eyes, and thick mucous in the mouth. Animals suffering from dehydration should be soaked in water and re-hydrated as soon as it is possible. Dehydrated animals lose electrolytes and replacement is critical to their survival. These animals are often weak and will not eat. Dehydration can lead to death. An appetite stimulant can be used to re-hydrate the reptile and boost its appetite.

Humidity preferences of different species of reptiles living on ground, on sand, on rocks, in water or in trees, respectively, are listed in an Appendix.

2.4. Lighting

Appropriate light:dark regimes for each species, life stage, and time of the year should be provided. Reptiles should have the opportunity to withdraw to shaded areas within the enclosure. Light or sun lamps should not be the sole source of heat. The provision of ultraviolet radiation is necessary to stimulate the organism's production of vitamin D.

Various investigators have explored the effects of light on a variety of physiological processes in reptiles (e.g., see Gehrmann, 1987, 1994a,b; 1996, 1997). The influence of photoperiod and position of a light source on behavioural thermoregulation was investigated by Sievert (1991). If possible, the animal should have access to light comparable to natural sunlight. Filtered light, either by glass or plastic, will block out beneficial ultraviolet rays and should be avoided. A few hours of natural sunlight each month will suffice, if the reptile is supplied with full-spectrum artificial light the rest of the time. Reptiles should not be kept in areas of direct sunlight without an area of shade, especially in the summer months.

Full spectrum lighting offers a large selection of lamps including incandescent, halogen, energy-efficient par and compact fluorescent lamps, as well as, a full line of linear fluorescent lamps (see also Laszlo, 1969). There are two main types of artificial light sources commonly available for reptiles, incandescent and fluorescent. Incandescent light provides both heat and visible light. The effectiveness of a lamp will depend on the species, the length of exposure time, the distance to the lamp as well as dietary and thermal factors. The ideal lighting system for daylight preferring reptiles should include both incandescent and fluorescent lights. To choose the right colour right fluorescent lamp for a specific application, considers two things: Colour rendering index and colour temperature. For example, "Life-Lite" lamps deliver both the colour spectrum of sunlight plus the healthful ultraviolet of natural outdoor light that is important to a reptile's well-being. The term full-spectrum light refers to any lamp that had a colour-rendering index CRI >90, a correlated colour temperature CCT=5500 to 6800 K, and a spectral power distribution for visible and UV light similar to that of open-sky natural daylight.

Colour Rendering Index (CRI) is a numerical system that rates the "colour rendering" ability of fluorescent light in comparison with natural daylight, which has CRI=100. This means that a lamp with CRI=91 shows colours more naturally than a lamp with CRI=62.

The colour appearance of a light source is typically defined in terms of the source's CCT. The CCT of a light source, or lamp, is a specification of the colour appearance of the light emitted by the lamp, relating its colour to the colour of light from a reference source when heated to a particular temperature, which is measured in degrees Kelvin (K). The CCT rating for a lamp is a general warmth or coolness measure of its appearance. However, opposite to the temperature scale, lamps with a CCT rating below 3200 K are usually considered warm sources, while those with a CCT above 4000 K are usually considered cool in appearance. Some typical colour temperatures are:

3000 K: 100 W incandescent lamp [CRI=100]; *3200 K*: sunrise/sunset; *5500 K*: sunny daylight around noon; *5500 K*: 40 W "Life-Lite" lamp [CRI=91]; *6500 K*: "Daylite 65" lamp [CRI=92]; *9000-12000 K*: blue sky

The UV transmission through the skin is in reptiles much less (about 1%) than in humans (about 30%) or in amphibians. UV radiation is divided into UVA (long-wave UVA-1: 340-400 nm; UVA-2: 320-340 nm), UVB (mid-wave 280-320 nm), and UVC (short-wave 100-280 nm). Blacklights are a strong source of UVA.

UVA can influence agonistic, reproductive, and signalling behaviours in some species of lizards (Gehrmann, 1994) as well as inhibit growth in female panther chameleons, *Chamaeleo pardalis* (Ferguson et al., 1996). A UVA requirement for long-term health and reproduction of captive reptiles has not been demonstrated. However, because UVA is a component of natural light in many environments, some researchers may use a blacklight in conjunction with some other visible light.

UVB is noted for its ability to promote the synthesis of vitamin D3 in the skin. It is therefore recommended to irradiate the vivarium with a UV lamp from about 1m distance for 10 min three times a week. If the lid of the terrarium is made of quartz glass, the UV will traverse it. The extent to which dietary D3 can compensate for an insufficiency of UVB-synthesized D3 remains problematic. *Iguana iguana*, appear to be able to utilize UVB-synthesized D3 better than dietary D3 (Bernard, 1995; Ferguson, et al. 1996; see also Dierenfeldt et al., 1996). Jones et al. (1995) reported that female panther chameleons, *C. pardalis*, receiving low levels of dietary D3 will behaviourally increase their exposure to UV light, compared to controls, in a UV gradient. While UV light is necessary to provide Vitamin D3 for most reptiles, veterinarians are divided about the need for UV light for snakes. This is because snakes consume whole prey as the diet, and the prey is "nutritionally balanced" for snakes. However, providing UV light to snakes would not be harmful and may be beneficial, so it would probably be wise to provide some type of UVB light.

Gehrmann (1996) mentioned the importance of infrared radiation (heat) for thermoregulation. Some species, particularly nocturnal forms, may preferentially utilize heat radiated from the substrate rather than from sunlight. Diurnal basking species, however, receive both light and infrared radiation when they bask. It may be beneficial to the reptiles to use a reflector lamp as a combined heat and light source, particularly for basking species, with adjunct light sources. Light lamps should not be the sole source of heat and heating lamps should not be the sole source of light [see also Section 2.2].

2.5. Noise

Reptiles are very sensitive to acoustic noise (airborne stimuli) and to vibratory noise (substrate-borne stimuli) and are disturbed by any new, unexpected stimulus. Therefore, such extraneous disturbances should be minimised.

Keeping a reptile in a room where there is a lot of traffic, people coming and going often, or where they can see people frequently causes stress to the animal [see also Sections 4.7 and 4.8].

2.6. Alarm systems (See also paragraph 2.6. of the General Section)

Adequate alarm systems should be provided if water circulation systems are used and/or aeration is required.

Cages should be routinely inspected for sick, dead or missing animals. If alarm systems are used, they should be "silent", so that they do not disturb the animals.

3. Health (See also paragraph 4.1. of the General Section)

Care is needed when housing different species of possible different health status.

Various criteria account for a healthy reptile:

- Looks rounded bodied
- Breathing normally
- Eyes are clear and normal
- Snout should not be damaged or discoloured
- Skin and shell are free of injuries, sores, bumps, ticks and mites
- Joints are not swollen
- No missing toes
- Tails and hips are well flushed (no protruding bones)

3.1. Animal supply

In the appropriate season of the year, reptiles should be ordered from commercial institutions, so that records about the number of individuals are available for book keeping. New reptiles need to be acclimated to the novel environment [see also Section 4.8]. Cages should be set up with all necessary enclosures in advance. Often reptiles will first be stressed and will not eat or drink. The temperature and humidity should be checked to be sure that the range is right and there is adequate water [cf. Sections 2.2 and 2.3].

3.2. Animal transportation

Reptiles should be obtained from those commercial institutions which follow the recommendations of adequate animal transportation: the *European Convention on the Protection of Animals During International Transport*, the *International Air Transport Association*, and the *Animal Air Transport Association.* After arrival, animals must be unpacked without delay. Animals which arrived in a sick condition and which do not have a chance to recover should be sacrificed at once by a human method [Section 4.6]. The sender should be informed. Newly acquired aquatic animals should be isolated for at least two weeks and checked for infections with *Salmonella*, *Pseudomonas* or *Aeromonas* as they pose a serious threat to the health of other reptiles or humans [see Section 3.4.1]. For quarantine protocols and preventive medicine procedures in reptiles see Miller (1996).

Before venomous snakes are received, proper training of all personal exposed to them is necessary (e.g., Russel et al., 1997). The institutional or other appropriate medical authorities should be informed of the types of venomous snakes to be kept, so that the appropriate precautious (stock of antisera, etc.) are available in advance of any possible emergency.

3.3. Reproduction

In species which undergo winter torpor (e.g., garter snakes), re-warming and activation will induce mating behaviour. In species which will not usually experience a sharply changing climatic cycle (warm temperature or tropical species), sexual maturation seems to be governed by intrinsic rhythms. Reptiles are laying leathery-shelled eggs on land (a protected side or nest) or are showing ovoviviparous development. Several species are parthenogenetic (e.g., Darevesky, 1966; Maslin, 1971). For reproductive cycles in lizards and snakes, e.g., see Fitch (1970) and Greenberg & Hake (1990). Breeding season basics are summarized, e.g., by Kaplan (2002d,e; see also Greenberg et al., 1984b; Greenberg & Wingfield, 1987).

Internal fertilization and oviparity most likely are symplesiomorphies for modern reptiles, and viviparity has evolved numerous times in Sauria and Serpentes. Oviductal sperm storage is known in females of all the above taxa except Rhynchocephalia and Amphisbaenia (Sever & Hamlett, 2002). Sperm storage in crocodilians, however, is poorly studied, and chelonians, saurians, and serpentians differ from each other in the anatomy of the oviduct and the location of sperm storage tubules. These differences coupled with the controversial nature of sister groupings make it difficult to infer the ancestral character states for sperm storage in reptiles. Thus, female sperm storage may have evolved independently within the three clades: Chelonia, Crocodilia, and Squamata (see Sever & Hamlett, 2000, *XVIIIth (New Int. Congr.Zool., Athens*).

Whereas the morphological development of the gonads appears to be conserved evolutionary among the Amniota (reptiles, birds and mammals), sex determination can be different. In mammals, which exhibit chromosomal sex determination, testis development is initiated by activity of the Y-chromosome linked gene SRY. Reptiles do not have sex-dimorphic chromosomes. Different from amphibians, temperature-dependent sex determination (TSD) dominates among reptiles (e.g., Westen & Sinclair, 2001; Pieau et al., 2001). For example, under laboratory conditions, in snapping turtles eggs at 21.5°C produced both sexes, at 24.5°C all males, at 27.5°C both sexes, and at 30.5°C all females (O'Steen & Janzen, 2000). The temperature of incubation during a critical thermo-sensitive period preceding sexual differentiation determines the future sex of the embryo, probably by altering the expression of temperature-dependent regulatory factors (Kettlewell et al., 2000). In the alligator, expression of the anti-Mullerian hormone (AMH) gene precedes testis differentiation (Western et al., 2000). Temperature signals may be transduced into steroid hormone signals with estrogens directing ovarian differentiation in which the expression of the steroidogenic factor 1 (SF-1) seems to be involved (Fleming et al., 2000; Picau et al., 1999; Jeasuria and Place, 1998). Estrogen-estrogen receptor-dependent mechanisms appear to play a key role in female sex determination, since administration of exogeneous estrogen during the temperature-sensitive period to embryos incubating at a male-producing temperature can override the temperature effects, so that females will be produced (Bergeron et al., 1998). For the role of estrogen in turtle sex determination see also Crews et al. (1995), Crews (1996), and Fleming et al. (1999).

In viviparous lizards, too, sex-determination may depend on temperature. The spotted skink, *Niveoscincus ocellatu*, – found in Tasmania – gives birth to life young. Females store sperm in the oviducts before ovulation occurs in spring. Embryos develop by means of a placenta (viviparous) with a gestation period of 14-16 weeks. Usually from 1-6 young are born in January or February, depending on the climate over summer. Is the climate rather warm, mainly *big* young lizards of female sex will be born. Years, however, in which the summers are cool stimulate the production of *small* males which show a reproductive advantage compared to the big females (Wapstra et al., 2004, *Proc.Royal.Soc.:Biol.Lett.*).

3.4. Diseases

Diseases in reptiles (e.g., Keymer, 1978; Cooper & Jackson, 1981; Davies, 1981; Cooper, 1984; Jarchow, 1988; Klingenberg, 1988; 1994; Frye, 1991; Mader, 1991; 1996; Kaplan, 2002a,b,c,f) and wild animal medicine (Burke, 1978; Beynon & Cooper, 1999; Harkewicz, 2001; Zwart, 2001; Brown & Sleemann, 2002; Cooper & Cooper, 2003) are better investigated than in amphibians (cf. also Miller, 1996; Ippen & Zwart, 1996; Davies & Johnston, 2000). For medicine and surgery in captive reptiles see, e.g., Frye (1973), Marcus (1977, 1981), Schultz et al. (1996), Hernandez-Divers (2001), Redrobe (2002), and Schumacher (2003).

3.4.1. Aquatic reptiles

Aquatic reptiles are particularly prone to superficial bacterial infections which may be treated with Tetracycline. Fungal infections can be controlled by daily bathing with 1:100.000 potassium permanganate solution for up to four days. *Saprolegnia* fungal infections (common in turtles) can be treated by 3% sodium chloride. Myocotic dermatosis can be controlled by treatment, e.g., with Nystatin or Fungicidin or topically with Providone-iodine or Lugols solution.

Bacteria that thrive in water (*Pseudomonas, Aeromonas, Salmonella*) constitute the major threat to free living aquatic reptiles. They cause systemic or local infections, in captivity mostly induced by dirty holding tanks. Signs are not specific, lethargy is common. Animals may just be found dead. For a study on salmonellosis in captive wildlife see Gopee (2000). Since *Salmonella* infection is a serious disease also in humans, captive aquatic reptiles (particularly fresh water terrapins and turtles) must be checked for the presence of *Salmonella*. Treatment of these infections is difficult. Flow-through water supplies and frequent careful cleaning of water tanks will help to prevent infection.

3.4.2. Terrestrial reptiles

Mite infestations, frequently shown up in snakes and lizards by white appearing areas (at neck and legs) of densely packed mites, usually arise from the eggs of mites surviving in the substrate in the terrarium. Insecticides such as Pyrethrin or Rotenone are suitable for treatment, accompanied by sanitization of the terrarium and replacement of the substrate.

Necrotic stomatitis (mouth rot) is a bacterial infection of the oral mucosa seen in snakes and resulting from injury to the mouth during feeding or from accidental injection of substrate (For nutritional diseases see Jackson & Cooper, 1981). Signs are reddened, irritated areas on lips and gums, followed by ulceration and the appearance of necrotic plaques. Severely affected animals will cease feeding and usually die. Early treatment involves – under anesthesia – removal of deposits, cleaning, desinfection, topical application of antibiotics (e.g., Streptomycin). Transfer of the animal to a cage with a non-particulate substrate such as absorbent paper or clean synthetic carpet (Recommendations for antibiotic therapy in reptiles see, e.g., Bush et al., 1980).

Zoonoses involve infections with the *Salmonella* bacterium (e.g., Mitchell & Shane, 2000; Morrison, 2001; Warwick et al., 2001; Bandy et al., 2003; Pasmans et al., 2003), the Western equine encephalitis virus (extremely seldom) (Burton et al., 1966), and the *Alaria* trematode (often) (Freeman & Fallis, 1973; Freeman et al., 1976; Fernandez et al., 1976). The latter two have been identified in garter snakes, the *Alaria* infection probably resulting from eating frogs (For a study on gnathostomiasis in frog-catching snakes see Ishiwata et al., 1997). In humans, *Salmonella* serotype Marina infection is a potentially serious illness associated with iguana exposure (Mermin et al., 1997). There is a study on an outbreak of salmonellosis (*Salmonella enteritides*) among children attending a reptile Komodo dragon exhibit at a zoo (Friedman et al., 1998); 52% of the patients only touched the wooden barrier that surrounded the dragon pen. Washing hands at the zoo after visiting the dragons was highly protective (e.g., see Greene et al., 2002; Rosen & Jablon, 2003). For a study on iguanas and *Salmonella* infection in children see also Mermin et al. (1997) and Sanyal et al. (1997).

Housing of rat snakes *Elaphe obsoleta* with other reptiles can enhance transmission of *Cryptosporidium* to snakes, and therefore should be avoided (Graczyk & Cranfield, 1998). Persons dissecting freshly killed garter snakes should wear disposable gloves, wash their hands and desinfect the instruments used. Infectious and parasitic diseases are reviewed by Ippen & Zwart (1996) in tabulated form with a number of references for those seeking more detailed information.

Difficulties in skin shedding in captive snakes may result from insufficient humidity and suboptimal temperatures in the terrarium and may be reduced by maintaining snakes at humidities at 75% or greater and temperatures of 30 to 35°C [see Section 2.3]. In animals which have failed to shed or have shed incompletely, the unshed skin should be removed manually by: confining the snake in a large vessel, filling the vessel with a 25 to 30°C solution of 2% liquid detergent and 2% glycerin, soaking the animal for an hour or more, and rubbing with the fingers (or picking with blunt forceps), to remove the skin. If shedding has failed around the head, then the eyes, the facial or the labial infrared-sensory pits (in vipers, boids) should be examined carefully (eventually under magnification) and unshed pieces removed. Venomous and highly irritable snakes should be immobilized by narcotics or by cooling to torpor-like 4°C (e.g., North American vipers) or to 10 to 12°C (tropical snakes) [see also Section 4.6.1].

4. Housing, enrichment and care

4.1. Social housing (See paragraph 4.5.2. of the General Section)

Accumulation of as much information as possible on the ethological needs of the species is a necessary prerequisite for accommodating reptiles in groups. By far not all species of reptiles are typically communal and only a few species will actually do well – even in a large cage – if kept together. Even under these circumstances stress is still present and they need to be monitored (for reports on social stress in reptiles see, e.g. Greenberg, 1983, 1985, 1990, 2002, 2003; Greenberg & Crews, 1983, 1990; Greenberg et al., 1984a; Greenberg & Wingfield, 1987; Summers et al., 1995; 1998) [cf. also Section 4.8]. The housing of different species of reptiles together may enhance the transmission of pathogens, and therefore should be avoided.

4.2. Environmental complexity

The habitat of reptiles should be structured to include, for example, natural or artificial branches, leaves, pieces of bark and stones. Reptiles benefit from such environmental enrichment in different ways: for example, such inclusions allow animals to hide, and provide labels for visual and spatial orientation. To prevent collision with clear glass, the side walls of the terraria should be patterned to provide a structured surface.

The biotope of reptiles is textured/structured. It is advisable to paint the side walls of the vivaria (e.g., terraria) in a patterned fashion. Hiding places that are appropriate to the reptile's nature are essential to minimize stress. Therefore, the provision of shelters adapted to the need of the different species is recommended.

4.3. Enclosures – dimensions and flooring

Enclosures and enclosure furniture should have smooth surfaces and rounded edges to minimise the risk of injury, and in the most sensitive species opaque materials should be used.

A terrarium should be furnished, for example, with artificial branches, leaves, pieces of bark and stones. The smooth surfaces of such enclosures allow skin shedding in snakes. Recommendations for flooring the cages of different species of reptiles are listed in an Appendix.

4.3.1. Enclosures for aquatic reptiles

Aquatic reptiles should be accommodated in water-circulated, filtered, and aerated tanks. The water should be renewed about twice per week. To minimise the bacterial contamination of the water, water temperatures should not exceed 25°C. Water levels should be sufficient for reptiles to submerge.

A platform should be provided as a resting board on which the reptiles can haul out or under which take shelter. Such platforms should be made of suitable materials, such as wood, so that animals are able to get a purchase with their claws in order to pull themselves out of the water. Platforms should be replaced at intervals as necessary. Platforms made of epoxy or polyurethane may not serve this function and will deteriorate quickly under continuous warm temperatures.

Table J.2. Aquatic chelonians, e.g., Trachemys spp.: Minimum enclosure dimensions and space allowances

Body length[1)] (cm)	***Minimum water surface area (cm^2)***	***Minimum water surface area for each additional animal in group holding (cm^2)***	***Minimum water depth (cm)***
= 5	***600***	***100***	***10***
6 - 10	***1600***	***300***	***15***
11 - 15	***3500***	***600***	***20***
16 - 20	***6000***	***1200***	***30***
21 - 30	***10000***	***2000***	***35***
31 - 40	***20000***	***5000***	***40***

[1)] measured in a straight line from the front edge to the back edge of the shell

4.3.2. Enclosures for terrestrial reptiles

Terrestrial reptiles should be kept in enclosures consisting of an appropriate terrestrial part and an aquatic part. The water area of the terrarium should allow animals to submerge. It is advisable to renew the water at least twice a week, except in the case of a flow-through system.

Terraria should be transparent, have tight seams, with all holes securely screened, and be provided with well-fitted lids or doors that can be securely fastened down. All doors and lids should be fitted with latches, hooks or hasps. It is advisable to construct doors and lids, so that the entire top or an entire end or side opens to facilitate cleaning (exception: venomous reptiles). For some species, except for the front wall, all side walls including the top should be opaque. In case of highly irritable or easily frightened reptiles, the clear wall can be provided with a removable covering. For housing venomous snakes, certain security criteria must be fulfilled.

The provision of appropriate shelter is important for all terrestrial reptiles, both in which to hide and also sometimes to feed. A shelter-box, such as a tube of clay simulates the darkness of a burrow.

A terrarium (vivarium) suitable to housing venomous reptiles could be constructed, so that only the lid could open and the cage should be deep enough to at least slow down any attempt by the snake to climb to the top. Removable opaque covers should be fitted to the outside of the viewing walls, since many venomous snakes are highly irritable. Walls should be constructed of a non-reflecting material, rather than glass, to ensure against shattering and possible escape. For example, a chameleon in a cage of reflective glass will see its own reflections and will attack its mirror image due to “social stress” [see also Sections 4.1 and 4.8]. Many species of reptiles are stressed by their reflection because they see it as a trespassing competitor that never goes away. Also having other reptiles within eye sight can be very stressful because they may see the other reptiles as predators. A venomous reptile terrarium should be equipped with a sign with a warning and the name of the species kept.

Many lizard species burrow in loose sand and should be provided with this sort of substrate. Absorbent paper (cage-pan lining paper) or a piece of synthetic fibre indoor/outdoor carpet may serve a solid substrate.

The terraria must be equipped with water supplies, since most snakes, terrestrial turtles, and lizards – other than some desert-adapted species – need, and will drink, standing water. Many snakes will even submerge themselves in water, especially prior to shedding. In this case, the water container should be large enough. For small terrestrial reptiles, a dish of water (petri-dish cover) holding a water-soaked sponge or filled with a layer of absorbent cotton soaked with water will provide appropriate water sources.

Minimum space requirements for semi-terrestrial and terrestrial chelonians

Body length[1)] (cm)	Minimum floor area (cm^2)	Minimum area for each additional animal in group holding (cm^2)	Minimum cage height (cm)
≤ 10	1200	400	20
11 - 15	3000	1000	25
16 - 20	5000	1600	30
21 - 30	11.000	3600	40
31 - 40	20.000	6600	50
41 - 50	40.000	13.300	60
> 50	80.000	26.000	80

[1)] measured in a straight line from the front edge to the back edge of the shell

Minimum space requirements for terrestrial lizards

Body length[1)] (cm)	Minimum floor area (cm^2)	Minimum area for each immature[2)] additional animal in group holding (cm^2)	Minimum area for each mature[3)] additional animal in group holding (cm^2)	Minimum cage height[4)] (cm)
≤ 10	600	75	300	20
11 - 15	900	110	450	20
16 - 20	1600	200	800	30
21 - 30	3600	450	1500	30
31 - 40	6400	800	3200	40
41 - 50	9000	1500	4500	50
51 - 75	18.000	3000	9000	60
76 - 100	25.000	6000	12.500	75
101 - 125	30.000	10.000	15.000	90
126 - 150	40.000	20.000	20.000	100
151 - 200	50.000	25.000	25.000	100

[1)] measured from snout to tail; if tail is broken (autotomy) or regenerated, the original length of the tail will be approximated
[2)] a lizard is considered to be immature until it reaches half the length of the adult animal, and before it shows signs of sex-related rivalry and aggressiveness against conspecifics
[3)] a lizard is considered to be mature after it reaches more than half of the length of the adult animal, or if it before that shows signs of sex-related rivalry and aggressiveness against conspecifics
[4)] measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the cage should be adapted to the interior design including, e.g., shelves and large artificial branches

Table J.3. Terrestrial snakes, e.g. Thamnophis spp: Minimum enclosure dimensions and space allowances

Body length[1)] ***(cm)***	***Minimum floor area*** ***(cm²)***	***Minimum area for each additional animal in group-holding (cm²)***	***Minimum enclosure height[2)]*** ***(cm)***
= 30	***300***	***150***	***10***
31 - 40	***400***	***200***	***12***
41 - 50	***600***	***300***	***15***
51 - 75	***1200***	***600***	***20***
76 - 100	***2500***	***1200***	***28***

1) measured from snout to tail
2) measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosure should be adapted to the interior design including, e.g., shelves and large artificial branches

4.3.3. Arboreal caging and caging for rock climbing species

Having regard for the behaviour of different species, every effort should be made to allow for this by the provision of appropriate structures (e.g., shelves and branches) for climbing and resting by arboreal species. Shelters or the ability to use camouflage (e.g., artificial leaves) adapted to the need of the different species should be provided. In addition, it may be necessary to provide water in which they can submerge themselves or seek a higher humidity. If water dishes are used, they should be arranged in a way that they are easy to enter or to leave by the reptiles**.**

Minimum space requirements for arboreal and rock climbing lizards

Body length[1)] (cm)	Minimum floor area (cm²)	Minimum area for each immature[2)] additional animal in group holding (cm²)	Minimum area for each mature[3)] animal in group holding (cm²)	Minimum cage height[4)] (cm)
≤ 15	600	75	300	25
16 - 20	1200	150	600	35
21 - 30	2500	300	1200	50
31 - 40	5000	600	2500	70
41 - 50	8100	1500	4000	90
51 - 75	15.000	3000	7500	120
76 - 100	21.000	5000	10.000	140
101 - 125	25.000	12.500	12.500	160
126 - 150	30.000	15.000	15.000	170
151 - 200	40.000	20.000	20.000	180

1) measured from snout to tail; if tail is broken (autotomy) or regenerated, the original length of the tail will be approximated
2) a lizard is considered to be immature until it reaches half the length of the adult animal, and before it shows signs of sex-related rivalry and aggressiveness against conspecifics
3) a lizard is considered to be mature after it reaches more than half of the length of the adult animal, or if it before that shows signs of sex-related rivalry and aggressiveness against conspecifics
4) measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the cage should be adapted to the interior design including, e.g., shelves, large artificial branches, and structures for climbing

Fig.4. Example of a vivarium quipped with various enclosures suitable to housing arboreal lizards. [Source: www.cagesbydesign.com/testimonials.asp].

4.4. Feeding

Captive reptiles should be maintained on their natural foods, foodstuffs or commercial diets approximating those of their natural diets. Many reptiles are carnivores (all snakes and crocodiles, most lizards, and some turtles), but some are vegetarian and others are omnivores. Some species exhibit very narrow and specific feeding habits. Reptiles, except for some snakes, can be trained to feed on dead prey. Therefore, it should normally not be necessary to feed on live vertebrates. When dead vertebrates are used, they should have been humanely killed using a method that avoids the risk of toxicity to the reptiles. Daily feeding is not advisable for adult reptiles, but two to three times weekly to satiation at each feeding is recommended.

Not only natural diets are recommended. There are commercial diets for several species that are very good vitamin and mineral balanced. Vitamin supplements include the fat-soluble vitamins A, D, E and K and the water soluble vitamins B and C. Excess water soluble vitamins are excreted from the body, while excess fat-soluble vitamins can be deposited in body fat and can thus build up to toxic levels. Because some reptiles cannot form vitamin A from beta-carotene, most reptiles supplements do contain some vitamin A. Since vitamins are unstable when exposed to water, air and light, they should be closed tightly in containers and stored in a cool, dry place.

Mineral supplements, such as calcium, phosphorus, magnesium, iron, potassium, manganese, cobalt and selenium are required by reptiles. Calcium and phosphorus play major roles. Metabolic bone disease, MBD*,* is the most common mineral related problem in captive reptiles. MBD – common in young, fast growing reptiles – can be caused by unbalance of calcium and phosphorous, lack of vitamin D or calcium. Symptoms of MBD are uncontrollable muscle twitching (especially of limbs), jerky movements, swelling of the limbs or soft, uneven shell growth on turtles. Affected animals require an immediate dietary.

The required ratio of calcium (Ca) to phosphorus (P) is 1 : 1 to 1.5 : 1, although it can be as high as 6 : 1 in some reptiles, such as tortoises (for details, e.g., see Dacke, 1979; Frye, 1997). As a general rule, all fruits, insects and meat without bone are low in calcium and have a negative Ca/P ratio. Many dark green vegetables are good sources of calcium and contain a positive Ca/P ratio. Most of the foods contain plenty of phosphorus but not nearly enough calcium:

Food items	Calcium	Phosphorus
Crickets	1	4
Mealworms	1	8
Waxworms	1	13
Meat	1	16
beef heart	1	38
Adult mice	1.5	1
Pinkies	1.1	1
Fuzies	1	2
Apples	1	2

Food items	Calcium	Phosphorus
bananas	1	4
grapes	1	2
mushrooms	1	7
peas	1	4
collard greens	3	1
kale	3	1
mustard greens	3.5	1
turnip greens	7	1

A calcium supplement can be used to correct this imbalance.

4.4.1. Turtles

The aquatic turtles are carnivorous or omnivorous and can be fed earthworms, guppies, goldfish and bait fish. Many sliders, pond and painted turtles tend to be more omnivorous as they mature and will readily accept vegetables and fruits. Aquatic turtles enjoy also a wide variety of insects which, however, are deficient in vitamins and minerals. Consequently, insects should be a minor part of their diet. Carnivorous species of turtles, e.g., snapping turtles *Chelydra*, soft shells *Trionyx*, and pond turtles *Pseudemys*) feed in the water and will thus accept in captivity dead food (fish, pieces of liver, heart and meat). Since calcium deficiency diseases are generally due to diets (e.g., meat without bone), the diet of the turtle requires a Ca/P ratio of at least 1:1 (e.g., Dacke, 1979; Frye, 1997).

The terrestrial tortoises, such as red-footed and yellow-footed tortoises are omnivorous. All other tortoises, with few exceptions, are herbivores and should be fed a good mixture of vegetables and roughage (such as alfalfa, clover, etc.). Both herbivorous and omnivorous terrestrial turtles (e.g. *Terapene, Gopherus, Testudo*) will all feed on soft fruits and leafy green vegetables which provide adequate Ca:P ratios. Several species of freshwater turtles *Trachemys scripta elegans* (Emydidae) undergo an ontogenetic dietary shift. As juvenile turtles mature, they change from a primarily carnivorous to a primarily herbivorous diet, which is not a result of body size (McCauley & Bjorndal, 1999).

4.4.2. Lizards

Lizards can be divided into four categories depending upon their diet preferences: insectivores, carnivores, herbivore, and omnivores.

Insectivores represent the most popular category of lizards. They include most geckos, bearded dragons, old world chameleons, anoles, water dragons, skinks, armadillo lizards, curly-tailed lizards, plated lizards, swifts and small monitors. Commercially available insects, such as crickets, earthworms, mealworms, and wax worms are a major part of their diet. Food specialists with restricted diet tolerance are, for example, small arboreal gekkos *Hemidectylum* and *Gonatodes* (feeding on flying fruitflies and houseflies), Western alligator lizard *Gherronotus caeruleus* (preferring spiders), and horned lizards *Phrynosoma* (accepting only ants). Nutritional aspects of insectivores are reported, e.g., by Allen (1989).

Many commercially available insects are low in calcium and high in phosphorus, so that these insects must be properly prepared before being offered. First, insects should be fed (gut load) with a high calcium and low phosphorus diet. A "gut loading" diet can be prepared using assorted vegetables and greens mixed with a cricket dust. Insects should be fed "gut loading" diet for 24 hours before being offered to the reptile. Second, it is advisable to dust all insects with a vitamin and mineral supplement before being fed to the reptile. Uneaten insects should be removed within 30 minutes of being dusted and offered later. It is recommended not to leave insects overnight, as they can harm the reptiles.

Juvenile insectivorous lizards should be fed daily and adults 3-4 times per week. Although some insectivorous lizards will only eat invertebrates (insects, spiders, etc.) others will supplement their (invertebrates) diet with an occasional pinkie, fruit or vegetable. The food items offered should be small enough for the young lizard to fit its mouth:

food-width = ½ mouth-width; food-length < head-length

The diet of herbivores often consists of a wide variety of vegetables and fruits. The following are recommended vegetables and fruits for herbivores:

Green vegetables	**Other vegetables**	**Fruits**
Kale	frozen mixed vegs.	apples
mustard greens	broccoli	peaches
Turnip greens	carrots	grapes
collard greens	peas	melons

Green vegetables	**Other vegetables**	**Fruits**
dandelions	beans	strawberries
parsley	zucchini	bananas
spinach	mushrooms	blueberries
romaine lettuce	cauliflower	pears

It is advisable, to remove all seeds from fruits. Different vegetables and fruits as possible should be used. Herbivorous lizard food, for example, should contain 60% to 70% green vegetables, 20% to 30% other vegetables and 5% to 10% fruits and/or a commercially prepared diet. Herbivorous lizards are prone to nutritional problems. Therefore a vitamin and mineral supplement should be offered. All uneaten fruit and vegetables should be discarded within twelve hours.

4.4.3. Snakes

Snakes are predatory. However, a number of snakes held as captives (such as garter snakes) will also accept dead foods, such as pieces of fish fillet and dead whole frozen minnows. The latter, however, may cause problems of sickness due to nutritional Vitamin B deficiency. Furthermore, feeding water snakes on certain kinds of fish (*Osmerus* spp., *Gadus* spp.) which contain high levels of thiaminase may induce thiamine deficiency (hypovitaminosis B). For nutritional diseases from feeding see also Wallach (1978).

Constrictors like boa constrictor (*Constrictor constrictor*), ground boa (*Epicrates* spp.) anaconda (*Eunectes murinus*), and pythons (*Phyton* spp.) – next to garter snakes the most commonly encountered captive snakes – are predators on live small mammals and birds. By means of infrared-sensory pits in their upper and/or lower labial scutes, most of them detect and locate prey in the dark. In captivity, constrictors may be trained in stages first to feed on a moved dead warm mammal, then a warm non-moved one, and finally on a non-moved one at room temperature.

Pit vipers, such as rattle snakes (*Crotalus, Sistrurus*), are predators on small live mammals. They are difficult to feed in captivity. Most captive vipers are often so irritable that they assume defensive postures toward any stimulus, including food. Highly irritable rattle snakes may not feed at all, or only under conditions of darkness in isolation. It must be emphasized that force feeding risks various kinds of injuries to the animal and eventually to the handler, too. Some irritable rattlers are adaptable to feed on warm carcasses of mammals, if the room (or the terrarium) is dark. Least adaptable to feed in captivity is, e.g., the Western diamondback rattler (*Crotalus atrox*), among the moderately adaptable is the prairie and pacific rattler (*Crotalus viridis, Crotalus viridis oreganus*) and best adaptable is, e.g., the massasauga (*Sistrurus catenatus catenatus*).

Among the colubrid snakes there are small mammal predators (bullsnake *Pituophis*, rat snake *Elaphe*, racer *Coluber*). Kingsnakes (*Lampropeltis*), ringnecked snakes (*Diadophis*), and hognose snakes (*Heterodon*) feed on amphibians and reptiles, *Heterodon* showing a preference for toads. Brown snakes (*Storeria*) feed on slugs, earthworms and larval insects, and green snakes (*Opheodrys*) are insectivores.

Although it is considered that snakes prefer lived preys, there are several techniques for doing that they accept dead preys. If frozen mice are available, first of all they should be defrosted under an infrared lamp. Mouse should be washed and rinsed thoroughly and impregnated with organic substances coming from their natural preys as reptiles, small mammalians, amphibians and others to each case. Then, mice will be offered using a very large forceps, for avoiding hazards, with the cranium opened.

Although it is recommended maintaining snakes isolated, if there were any problem of feeding acceptance, housing snakes in the same terrarium may encourage them to swallow food due to competition.

It is important to note that pregnant females often do not accept food. Furthermore, snakes will never eat during sloughing, however, immediately thereafter.

4.5. Watering

Drinking water should be provided for all reptiles.

The water in the tanks and aquaria could circulate and be filtered and aerated. The water in the pool area of the terraria should be renewed by uncontaminated water about twice a week, and the drinking dishes daily. The terraria should be equipped with water supplies, since most snakes, terrestrial turtles, and lizards – other than some desert-adapted species – need, and will drink, standing water. Many snakes will even submerge themselves in water, especially prior to shedding. In this case, the water container should be large enough. For small terrestrial reptiles, a dish of water (petri-dish cover) holding a water-soaked sponge or filled with a layer of absorbent cotton soaked with water will provide appropriate water sources.

Some arboreal snakes and lizards are better adapted to taking rainwater rather than drinking from a water source on the ground. Setting up a misting system or using a hand-held spray bottle can accomplish this [see also Section 2.3]. Many species of lizards, turtles and tortoises should also have access to water to submerge in. Tortoises should have water enough to be able to wade, while turtles of species that live in swamps should have water to swim in.

4.6. Substrate, litter, bedding and nesting material

A variety of substrates may be used for terraria, depending on the requirements of the species. Fine sawdust and any other small-particle substrate should be avoided, as this may cause serious mouth or internal injuries or bowel obstruction, particularly in snakes.

4.7. Cleaning (See paragraph 4.9. of the General Section)

In order to avoid diseases, the land and pool areas of the terraria must be carefully cleaned to remove dirt, excrements, and food particles. The same holds for the tanks of aquatic animals; in these tanks the water should circulate. It should be avoided to use aggressive detergents. Reptiles do explore their cages and will choose a favourite place (e.g., a stone, a branch or piece of bark) for basking on, drinking from, and sleeping on. They become used to the enclosures of the cage. Therefore after cleaning the cage it is advisable to place each branch and all other enclosures in the cage in the same positions they were before cleaning [see also Section 4.8].

4.8. Handling

Care is needed when handling reptiles, as they can be easily injured. For example, some lizards may shed their tails (autotomy) if handled in an inappropriate way, and other species can easily be traumatized.

Before handling reptiles, it is necessary to accumulate as much information as possible on the behavioural biology of the species to be accommodated under laboratory conditions. Ganesh & Yajurvedi (2002), for example, report that stressors (like handling, chasing, and noise) applied randomly five times per day for one month or longer during the recrudescence phase of the ovarian cycle caused a significant reduction in mean number of oocytes when compared to those of controls. It is also reported that gentle handling of lizards elevated the body temperature, producing an "emotional fever". Heart rate, another indicator of emotion, was accelerated by gentle handling from 70 to 110 beats/min, and fading in about 10 min. The role of "sensory pleasure" in decision making in iguanas was investigated by Cabanac (1999). The psychological well-being of reptiles is reviewed by Kreger (1993).

Based on data from ample experimental analyses, Greenberg (1985, 1990, 2002, 2003) points out that any changes in a reptile's environment can evoke highly adaptive responses in the reptile that are coordinated by the neural and endocrine mechanisms of the "stress response-axes". Acute responses involve catecholamines released in varying proportion at different sites along their symphathetic neural pathways. They may interact with and be complemented by chronic responses, involving the hypothalamic-adrenocortical system. The central and systemic consequences of these actions include apparent changes in cognition, affect, and motivation which, in concert, result in altered responses to environmental stimuli including the social stimuli provided by male and female conspecifics (e.g., inter-male, inter-female interactions) or by handling of captive reptiles. Long-term captivity-related stress and its associated increase in the production of adrenalin and other stress related hormones may result in disruption of normal body functions. Unfortunately, many of the responses mounted by reptiles to deal with stress are ineffective when dealing with the long-term, artificial stresses of captivity (e.g., see also Frye, 1973, 1991; Greenberg & Crews, 1984a; Greenberg & Wingfield, 1987; Warwick, 1990ab; Lance, 1992; Summers & Greenberg, 1995; Warwick et al., 1995; Bernard, 1996; Ewert, 1998; Summers et al., 1998; Kaplan, 2002a; Ganesh & Yajurvedi, 2002).

No matter how tame ("socialized", "domesticated") they may seem to be, reptiles are still wild animals. As such, they may not react the way we expect they would. Handling stress is an area that is often overlooked or even ignored. In fact, being physically picked up and/or restrained may be extremely stressful to reptiles. For this season, it elicits the same physiological and behavioural responses as threats from a natural predator. However, unlike in nature the handler – a perceived putative 'predator' – will present an ongoing 'threat' in an unnatural context. While some reptiles occasionally engage in what could be described as social behaviour, most are generally considered asocial, as physical contact typically occurs in specific circumstances and for many is not a routine part of they daily lives. Handling stress thus may be a contributing factor in the development of diseases in reptiles. Stressors – via the hypothalamic-adrenocortical axis – may significantly weaken their immune system. The inability to satisfactorily cope with chronic stress can result in a general deterioration of health. This is usually referred to as "maladaptation syndrome" and may also involve a decreased ability to cope with natural parasitic organisms due to a breakdown in the symbiotic relationship between parasite and host.

Whereas some anecdotal reports suggest that individual members of a few reptile species (e.g., green iguana) may become limitedly "habituated" to physical contact, the detrimental impact of handling and its effect on reptile health and well-being should not be underestimated. Actually, it is difficult to figure out the mechanisms behind such putative "stress habituation". A failure of the reptile to respond to stress stimuli caused by handling must not result from a decrement in stimulus-responses due to habituation, i.e., non-associative learning (stimulus-specific habituation; e.g., see Ewert et al., 2001; Bolhuis & Giraldeau, 2005; Ewert, 2004). Rather, the failure of a reptile's responses to stress stimuli caused by handling may express endocrine long-term effects from long-term stress.

In fact, there are only a few species of reptiles that may deal with being handled on a regular basis. The majority of reptiles will get stressed over being handled excessively. It is important to note that stress in reptiles may even kill them. Therefore, if necessary, reptiles should be handled as quietly as possible with the minimum personal necessary. Darkened conditions tend to calm the reptile and reduce stress reactions. The following notes provide some general points to be considered for proper handling reptiles:

Don't (–) turn them upside down; (–) dangle them in the air; (–) hold them with two fingers; (–) squeal no matter how weird or creepy it feels; (–) hold a prey animal in front of or in line of sight of a perceived predator; (–) relinquish control when passing the reptile to someone until you are certain they are holding it properly; (–) smell like prey.

Do (+) support their body weight and length; (+) let them get comfortable on you; (+) move calmly and smoothly, avoiding abrupt hand movements or changes in direction; (+) be aware of what is going on around you and what may be stressful or alarming to the reptile, and either move away or make the situation go away; (+) wash your hands before and after handling, cleaning, or servicing reptile and prey animal enclosures.

Reptiles of the different orders require an appropriate careful handling:

Lizards: The handler should place the index finger under lizard's chin and the thumb on top of lizard's neck, while supporting the body in the palm of the hand. It is advisable never to grab a lizard by the tail and to avoid sharp claws.

Turtles and tortoises: The handler should grasp the shell from the side of the top. The head of the animal should face away from the handler. A turtle or tortoise should never be held upside down for any length of time. Sharp beaks and claws should be avoided.

Old world chameleons: These reptiles should not be grabbed, but rather coaxed onto the hand or a stick.

Snakes: The handler must try to convince the snake that it is safe. It is advisable to always support the snake's body and to avoid fast movements. Furthermore techniques should be avoided which restrict a snake's movement. Also public situations, which draw attention to the animal, should be avoided. A "nervous" snake may occasionally bite. It should be kept in mind that snakes bite for two main reasons: they have mistaken the handler as food or they are in fear of their safety.

Venomous reptiles: Investigators must be informed and take the necessary precautions when handling venomous reptiles. Gentle handling of non-venomous reptiles can make the individual animal more docile and tractable during minor procedures, such as cleaning of the enclosure, transferring to another area, veterinary inspection and blood collection.

4.9. Humane killing (See also paragraph 4.11. of the General section)

An appropriate method of killing is by an overdose of a suitable anaesthetic.

Invasive, potentially painful procedures should be accompanied both by analgesia and anaesthesia (e.g., see Mader, 1996; Heard, 2001). Various methods are suitable for killing reptiles after experiments have been finished in a procedure. Methods which provide least stress and no pain to the animal are an overdose of an appropriate anaesthetic by injection or inhalation, respectively. For an evaluation of pain and stress in reptiles see Lance (1992). All methods used need to be in conformity with the *European Commission's report on Euthanasia of Experimental Animals*.

4.10. Records (See paragraph 4.12. of the General Section)

The person responsible for an animal facility should keep a diary in which all events and activities are noticed: feeding, watering, cleaning, actual count of animals per tank or terrarium; admissions, loss by death; cases of disease; unusual disturbances; identification and marking of experimental animals.

4.11. Identification

Where animals must be identified individually, for example, if an animal stays in an experiment that is repeated daily, various methods can be used: transponders; enclosure labels for individually housed animals; monitoring individual skin patterns (according to colour, skin damages, etc.); pen markings require renewal after skin shedding; small labels at the toes by coloured thread. Toe clipping is deleterious and should not be done.

5. Transport

During transport reptiles should be provided with adequate air and moisture and, if necessary, appropriate devices included to maintain the required temperature and humidity.

In the appropriate season of the year, reptiles should be ordered from dealers which follow the recommendations of: the *European Convention on the Protection of Animals during International Transport*, the *International Air Transport Association*, and the *Animal Air Transport Association*. Semi-terrestrial reptiles should be singly packed in boxes of adequate size and provided sufficiently with air and moisture. Transportation of tropic species – depending on the local climate – requires an accommodation with appropriate heating devices. After arrival, animals must be unpacked without delay. Animals which arrived in a sick condition, and which do not have a chance to recover, should be sacrificed at once by a human method [Section 4.9]. The sender should be informed.

Appendix: Biotopes, flooring, temperature and humidity of reptiles[1])

[1]) Source: Swedish Board of Agriculture, Department of Animal Production and Health, Animal Welfare Division

Snakes

A,	Biotope	**1** - in trees **2** - on ground
B,	Flooring: sand and water	**1** - sand as floor covering **2** - water (pool) **3** - drinking water, dropping or spraying
C,	General temperature during the day	**1** - cool (+20 to 24°C) **2** - middle warm (+24 to 27°C) **3** - warm (+27 to 30°C)
D,	Atmospheric humidity	**1** - low (< 50% RH) **2** - middle (50 to 70% RH) **3** - high (70 - 100% RH)

Temperature in the terraria is somewhere between the temperature interval. Furthermore, the terrarium floor should be furnished with at least one warmth-plate at a local temperature between 35 and 50°C. At night time, the general temperatures fall more or less, depending on the snake species.

Species	A	B	C	D
Acrantophis spp.	2	2	3	3
Agkistrodon bilineatus	2	2	2-3	2
Agkistrodon contortrix	2		2-3	2
Agkistrodon piscivorus	2	2	2	2
Ahaetulla spp.	1	3	3	3
Antaresia childreni	2	2	3	2
Apodora papuana	2	2	3	3
Aspidelaps spp.	2	1	2-3	1-2
Aspidites melanocephalus	2	2	3	2
Aspidites ramsayi	2	2	3	1
Atheris superciliaris	2		2	2
Cerastes vipera	2	1,3	3	1
Botrochilus boa	2	2	3	3
Bitis arietans	2		3	2
Bitis caudalis	2	1,3	3	1
Bitis cornuta	2	1,3	3	1
Bitis gabonica	2		2-3	2-3
Bitis nasicornis	2	2	2-3	2-3
Bitis peringueyi	2	1,3	3	1
Bitis schneideri	2	1,3	3	1
Boa constrictor ssp.	2	2	3	3
Boa mandrita	1	3	3	3
Boaedon spp.	2	2	2-3	1-2
Bogertophis subocularis	2		2	1
Boiga cyanea	1	3	3	3
Boiga dendrophila	1	3	3	3
Boiga irregularis	1	3	2	3
Bothriechis schlegelii	1	3	3	3
Bothrops alternatus	2	2	2-3	2-3
Bothrops atrox	1	2	2-3	2-3
Botrochilus boa	2	2	3	3
Calloselasma rhodostoma	2		2-3	3
Candoia aspera	2	2	3	3
Candoia bibroni	1	2	3	3
Candoia carinata	1	2	3	3
Causus spp.	2	2	2-3	2
Cerastes cerastes	2	1,3	3	1
Cerastes vipera	2	1,3	3	1
Charina bottae	2	1	2	1
Chondropython viridis	1	3	3	3
Chrysopelea spp.	1	3	3	3
Coluber spp.	2		2	2
Corallus caninus	1	3	3	3
Corallus enydris	1	3	3	3
Crotalus adamanteus	2		3	2
Crotalus atrox	2		3	1
Crotalus cerastes	2	1,3	3	1
Crotalus durissus	2		3	2
Daboia russelli	2		2-3	1-2
Dasypeltis spp.	2		3	1
Dendroaspis spp.	1		2-3	2-3
Deniagkistrodon acutus	2	2	2	2
Dispholidus typus	1		3	2
Drymarchon corais	1	2	2-3	1-2
Echis spp.	2	1,3	3	1
Elaphe dione	2		2	2
Elaphe guttata	2		2	2
Elaphe helena	2		2	2
Elaphe longissima	2		2	2

Elaphe mandarina	2		1	2
Elaphe obsoleta lindheimeri	2		2	2
Elaphe obsoleta obsoleta	2		2	2
Elaphe obsoleta rossalleni	2		2	2
Elaphe obsoleta quadrivittata	2		2	2
Elaphe quatuorlineata	2		2	2
Elaphe scalaris	2		2	2
Elaphe schrencki	2		2	2
Elaphe situla	2		2	2
Elaphe taeniura	1		3	2
Elaphe vulpina	2		2	2
Epicrates angulifer	1	2	3	2
Epicrates cenchria	2	2	3	3
Epicrates inornatus	2	2	3	2
Epicrates striatus	1	2	3	2
Eristicophis macmahonii	2	1,3	3	1
Eryx spp.	2	1	2	1
Eunectes murinus	2	2	3	3
Eunectes notaeus	2	2	2	2
Gonyosoma oxycephala	1		3	3
Hemachatus haemachatus	2	2	2-3	1-2
Heterodon spp.	2	1,2	1	2
Hydrodynastes gigas	2	2	3	3
Hypnale spp.	2		2-3	2-3
Lachesis muta	2		2	3
Lampropeltis getula ssp.	2		2	2
Lampropeltis pyromelana	2		1	1
Lampropeltis triangulum ssp.	2		2	2
Lamprophis spp.	2	2	2-3	1-2
Leiopython albertisii	2	2	3	3
Liasis macktoli	2	2	3	3
Liasis olivaceus	2	2	2	2
Lichanura spp.	2		2	1
Vipera ammodytes	2		2-3	2
Vipera aspis	2		2-3	2
Vipera berus	2		2	2

Macrovipera lebetina	2		2-3	1-2
Malpolon spp.	1		2-3	1
Morelia amethistina	2	2	3	2
Morelia spilotes spilotes	2	2	2	2
Morelia spilotes variegata	2	2	2	2
Morelia viridis	1	3	3	3
Naja annulifera	2		2-3	1-2
Natrix spp.	2	2	2	2
Nerodia spp.	2	2	2	2
Opheodrys spp.	1	2	2	2
Oxybelis spp.	1	3	3	3
Pituophis spp.	2		2	2
Porthidium spp.	2		2-3	2
Psammophis spp.	2		2-3	1
Pseudaspis cana	2	1,2	2-3	1-2
Pseudocerastes spp.	2	1,3	3	1
Ptyas mucosus	1	2	2	2
Python curtus	2	2	2	3
Python molurus	2	2	2	2
Python regius	2	2	2	2
Python reticulates	2	2	2	2
Python sebae	2	2	3	2
Rhabdophis spp.	2	2	2	2
Sistrurus spp.	2		2-3	1-2
Thamnophis spp.	2	2	2	2
Trimeresurus albolabris	1	3	3	3
Trimeresurus popeorum	1	3	3	3
Trimeresurus purpureomaculatus	2	3	2-3	2-3
Trimeresurus stejnegeri	1	3	2-3	2-3
Trimeresurus trigonocephalus	1	2	2-3	2-3
Trimeresurus wagleri	1	3	3	3

Lizards		
A,	Biotope	**1** - in trees **2** - on rock **3** - on ground
B,	Flooring: sand and water	**1** - sand as floor covering **2** - water (pool) **3** - drinking water, dropping or spraying
C,	General temperature during the day	**1** - cool (+20 to 24°C) **2** - middle warm (+24 to 27°C) **3** - warm (+27 to 31°C)
D,	Atmospheric humidity	**1** - low (< 50% RH) **2** - middle (50 to 70% RH) **3** - high (70 to 100% RH)

Temperature in the terraria is somewhere between the temperature interval. Furthermore, the terrarium floor should be furnished with at least one warmth-plate at a local temperature between 35 and 50°C. At night time, the general temperatures fall more or less, depending on the lizard species.

Species	**A**	**B**	**C**	**D**
Acanthodactylus cantoris	3		3	1
Acanthodactylus scutellatus	3		3	1
Acontias spp.	2		3	1
Agama agama	3		3	2
Agama stellio	2		3	2
Algyroides nigropunctatus	3		2	2
Ameiva ameiva	3		3	2
Anguis fragilis	3		2	2
Anolis carolinensis	1	3	2	3
Anolis conspersus	1	2	3	3
Anolis equestris	1	3	2	3
Anolis sagrei	1	3	3	3
Basiliscus basiliscus	1	2	3	3
Basiliscus plumifrons	1	2	3	3
Basiliscus vittatus	1	2	3	3
Bronchocela cristatellus	1	3	3	3
Brookesia thieli	2	3	1	3
Callisaurus draconoides	3		2	1
Callopistes spp.	3		2	2
Calotes versicolor	1	3	3	3
Chalcides chalcides	3		2	2
Chamaeleo calyptratus	1	3	2	3
Chamaeleo chamaeleon	1	3	2	2
Chamaeleo dilepis	1	3	2	2
Chamaeleo fischeri	1	3	2	3
Chamaeleo jacksoni	1	3	2	3
Chamaeleo lateralis	1	3	3	3
Chamaeleo pardalis	1	3	3	3
Chondrodactylus angulifer	3	1,3	3	1
Cnemaspis africana	2	3	3	3
Cnemidophorus lemniscatus	3		3	2
Coleonyx variegatus	3		2	1
Cordylus giganteus	3	3	3	1
Corucia zebrata	1		3	3
Corytophanes cristatus	1	3	2	3
Crotaphyrus collaris	3		3	1
Crotaphytus wislizenii	3		3	2
Ctenosaura pectinata	3		2	2
Cyclura cornuta	3		3	1
Cyrtodactylus spp.	2		2	2
Dasia smaragdina	1	3	3	3
Diplodactylus vittatus	2	3	3	1
Dipsosaurus dorsalis	3	3	3	1
Dracaena guianensis	3	2	3	3
Emoia spp.	3		3	3
Eremias spp.	3	1	3	1
Eublepharis macularius	3		3	1
Eumeces fasciatus	3		2	2
Eumeces obsoletus	3		2	2
Eumeces schneideri	3		2	2
Eumeces schneideri algeriensis	3		2	2
Gallotia galloti	3		2	1
Geckonia chazaliae	3	3	3	1
Gekko gecko	1	3	2	3
Gekko vittatus	1	3	2	3
Gerrhonotus spp.	3		2	2
Gerrhosaurus flavigularis	3		2	2
Gerrhosaurus major	3		2	2
Gonocephalus abbotti	1	3	3	3
Gonocephalus bellii	1	3	3	3
Heloderma horridum	3	1,2	3	1
Heloderma suspectum	3	1,2	3	1
Hemidactylus spp.	2		2	2
Hemitheconyx caudicinctus	3		2	1
Holaspis guentheri	3		3	3
Hydrosaurus amboinensis	1	2	3	3

Hydrosaurus pustulatus	1	2	3	3
Hydrosaurus weberi	1	2	3	3
Iguana iguana	1	2	3	3
Japalura spp.	3	2	2	2
Lacerta agilis	3	1	2	2
Lacerta lepida	3		2	2
Lacerta trilineata	3		2	2
Lacerta viridis	3		2	2
Latastia longicaudata	3		2	3
Leamacatus lonigipes	1		3	3
Leiocephalus spp.	3	1	3	2
Leiolepis spp.	3	1	3	1
Liolaemus chiliensis	3		2	2
Lygodactylus capensis	1	3	3	3
Lygosoma spp.	3		2	2
Mabuya multifasciata	3		2	2
Mabuya quinquetaeniata	3		2	2
Mabuya striata	3		2	2
Mabuya vittata	3		2	2
Ophiomorus tridactylus	3		2	1
Ophisaurus spp.	3		2	2
Ophryoessoides spp.	3	1	3	1
Oplurus sebae	3		2	2
Pachydactylus spp.	1	3	3	3
Phelsuma madagascariensis	1	3	3	3
Phelsuma quadriocellata	1	3	3	3
Phrynocephalus mystaceus	3	1,3	3	1
Phrynosoma spp.	3	1,3	3	1
Physignathus cocincinus	1	2	3	3
Physignathus lesueurii	1	2	3	3
Platysaurus spp.	2	3	3	1
Podarcis muralis	3	1	2	2
Podarcis taurica	3	1	2	2
Pogona vitticeps	3	1	3	1
Ptychozoon kuhli	1	3	3	3

Ptydactylus hasselqvistii	2	3	2	2
Rhacodactylus spp.	1		2	2
Riopa fernandi	3		3	3
Sauromalus obesus	3	1	3	2
Sceloporus clarki	2		3	2
Sceloporus cyanogenus	2		3	2
Sceloporus magister	3	3	3	1
Sceloporus malachiticus	2		2	2
Sceloporus occidentalis	3		3	2
Sceloporus poinsettii	3		3	1
Sceloporus undulatus	3		3	2
Sceloporus woodi	3		3	2
Scincus spp.	3	1,3	3	1
Stenocercus spp.	3	1	3	1
Stenodactylus petrii	1		2	2
Takydromus sexlineatus	1	3	2	2
Tarentola mauritanica	2		2	2
Teratoscincus scincus	3		2	1
Tiliqua gigas	3		3	1
Tiliqua scincoides	3		3	1
Tupinambis spp.	3		2	2
Uma spp.	3	1,3	3	1
Uromastyx acanthinurus	3	1	3	1
Uromastyx aegypticus	3	1	3	1
Varanus albigularis	3		3	1
Varanus dumerilii	3		3	3
Varanus exanthematicus	3		2	2
Varanus griseus	3		3	1
Varanus indicus	3		3	3
Varanus niloticus	3	2	3	3
Varanus prasinus	1		3	3
Varanus salvator	3	2	3	3
Zonosaurus spp.	3		3	1

Turtles (tortoises)

A, Biotope — **1** - land; **2** - swamp; **3** - water

B, Facility for turtles — **1** - possibility to dig in the soft ground

Species	**A**	**B**
Annamemys annamensis	2-3	
Chelodina spp.	3	
Chelus fimbriatus	3	
Chelydra serpentina	3	
Chinemys reevesi	3	
Chitra indica	3	1
Chrysemys picta	3	
Claudius angustatus	3	
Clemmys guttata	2-3	
Clemmys insculpta	2	
Clemmys marmorata	2-3	
Cuora amboinensis	2-3	
Cyclanorbis spp.	3	1
Cyclemys spp.	2-3	
Cycloderma spp.	3	1
Deirochelys reticularia	3	
Elseya spp.	3	
Emydoidea blandingii	2-3	
Emydura spp.	3	
Emys orbicularis	2-3	
Erymnochelys madagascariensis	3	
Geochelone carbonaria	1	
Geochelone chilensis	1	
Geochelone denticulata	1	
Geochelone elegans	1	
Geochelone pardalis	1	
Geochelone sulcara	1	
Geoclemys hamiltonii	3	
Geoemyda japonica	1-2	
Geoemyda spengleri	1-2	
Gopherus spp.	1	
Graptemys spp.	3	
Heosemys grandis	1-2	
Heosemys spinosa	1-2	
Hieremys annandalii	3	
Hydromedusa spp.	3	
Indotestudo elongata	1	
Kachuga spp.	2-3	
Kinixys belliana	1	
Kinixys erosa	1-2	
Kinixys homeana	1	
Kinosternon spp.	3	
Lissemys punctata	3	1
Macroclemys temmincki	3	
Malaclemys terrapin	3	
Malacochersus tornieri	1	
Malayemys subtrijuga	3	
Manouria spp.	1-2	
Mauremys caspica	3	
Melanochelys trijuga	2-3	
Morenia ocellata	3	
Notochelys platynota	3	
Ocadia sinensis	3	
Orlitia borneensis	3	
Pelochelys bibroni	3	1
Pelomedusa subrufa	3	
Peltocephalus dumeriliana	3	
Pelusios spp.	3	
Phrynops spp.	3	
Platysternon megacephalum	3	
Podocnemis spp.	3	
Psammobates spp.	1	
Pseudemys spp.	3	
Pyxidea mouhotii	1-2	
Rhinoclemmys annulata	1-2	
Rhinoclemmys areolata	1-2	
Rhinoclermmys funerea	3	
Rhinoclemmys melanosterna	3	
Rhinoclemmys nasuta	3	
Rhinoclemmys pulcherrima	1-2	
Rhinoclemmys punctularia	3	
Rhinoclemmys rubida	1-2	
Sacalia spp.	2-3	
Siebenrockiella crassicollis	3	
Staurotypus spp.	3	
Sternotherus spp.	3	
Terrapene carolina	1-2	
Terrapene ornata	1-2	
Testudo graeca	1	
Testudo hermanni	1	
Testudo horsfieldii	1	
Testudo kleinmanni	1	
Testudo marginata	1	
Trachemys scripta elegans	3	
Trionyx triungius	3	1

References

Aboitiz F. (1995) Homology in the evolution of the cerebral hemispheres. The case of reptilian dorsal ventricular ridge and its possible correspondence with mammalian neocortex. J. Hirnforsch. 36(4): 461-472
Aboitiz F. (1999) Comparative development of the mammalian isocortex and the reptilian dorsal ventricular ridge. Evolution considerations. Cereb. Cortex. 9(8): 783-791
Aboitiz F., Montiel J., Morales D., Concha M. (2002) Evolutionary divergence of the reptilian and mammalian brains: considerations on connectivity and development. Brain Res. Rev. 39(2-3): 141-153
Aleksic S., Heinzerling F., Bockemuhl J. (1996) Human infection caused by *Salmonellae* of subspecies II to VI in Germany, 1977-1992. Zentralbl. Bakteriol. 283(3): 391-398
Allen, M.E. (1989) Nutritional Aspects of Insectivore. Ph.D. Dissertation, Michigan State University
Bandy U., McCarthy H., Hannafin C. (2003) Reptile-associated salmonellosis: a preventable pediatric infection. Med. Health. R. I. 86(1): 27-29
Bergeron J.M., Gahr M., Horan K., Wibbels T., Crews D. (1998) Cloning and in situ hybridization analysis of estrogen receptor in the developing gonad of the red-eared slider turtle, a species with temperature-dependent sex determination. Dev. Growth Differ. 40(2): 243-254
Bernard, J.B. (1995) Spectral Irradiance of Fluorescent Lamps and their Efficacy for Promoting Vitamin D Synthesis in Herbivorous Reptiles. Ph.D. Dissertation, Michigan State University
Bernard M. (1996) Reptile Keeper's Handbook. Krieger Publishing Company, Malabar FL.
Beynon P.H., Cooper J.E. (1994) Manual of exotic pets. Iowa State University Press
Beynon P.H., Cooper J.E. (1999) Bsava: Manual animales exotics. Elsevier Science
Beynon P., Lawton M.P.C, Cooper J.E. (1992) Manual of reptiles. Cheltenham, Glos.: British Small Animal Veterinary Association
Bichard G.F., Reiber C.L. (1996) Heart rate during development in the turtle embryo: effect of temperature. J. Comp. Physiol. B. 166(8): 461-466
Blair T.A., Cree A., Skeaff C.M., Grimmond N.M. (2000) Physiological effects of a fish oil supplement on captive juvenile tuatara (*Sphenodon punctatus*). Physiol. Biochem. Zool. 73(2): 177-191
Bolhuis J.J., Giraldeau L.-G. (eds.) (2004) Principles of Animal Behavior. Blackwell Publ.
Booth D.T. (1998) Effects of incubation temperature on the energetics of embryonic development and hatching morphology in Brisbane river turtle *Emydura signata*. J. Comp. Physiol. B 168(5): 399-404
Brown J.D., Sleemann J.M. (2002) Morbidity and mortality of reptiles admitted to the Wildlife Center of Virginia, 1991 to 2000. J. Wildl. Dis. 38(4): 699-705
Bruce L.L., Neary T.J. (1995) The limbic system of tetrapods: a comparative analysis of cortical and amygdalar populations. Brain. Behav. Evol. 46(4-5): 224-234
Burke T.J. (1978) Reptiles. In: Zoo and Wild Animal Medicine (M.E. Fowler, ed.). W.B. Saunders & Co., Philadelphia, PA. pp. 134-137
Bursey C.R., Goldberg S.R. (1999) *Spauligodon ovifilus* n. sp. (Nematoda: Pharyngodonidae) and other helminths from *Diplodactylus stenodactylus* (Reptilia: Gekkonidae) from Austalia. J. Parasitol. 85(5): 898-902
Burton A.N., McLintock J., Rempel J.G. (1966) Western equine encephalitis virus in Saskatchewan garter snakes and leopard frogs. Science 154: 1023
Bush M., Custer R.S., Smeller J.M., Charache P. (1980) Recommendations for antibiotic therapy in reptiles. In: Reproductive Biology and Diseases of Captive Reptiles (J.B. Murphy, J.T. Collins, eds.). Symp. Soc. for the Study of Amphibians and Reptiles, Oxford, OH.
Cabanac M. (1999) Emotion and phylogeny. Jpn. J. Physiol. 49(1): 1-10
Cain D.A., Guillette L.J. jr. (1998) Reptiles as models of contaminant-induced endocrine disruption. Anim. Reprod. Sci. 53(1-4): 77-86
Conant R.M. (1975) A field guide to reptiles and amphibians of Eastern and Central North America (2nd Edn.). Houghton Mifflin Co., Boston, MA.
Conley A.J., Elf P., Corbin C.J., Dubowsky S., Fivizzani A., Lang J.W. (1997) Yolk steroids decline during sexual differentiation in the alligator. Gen. Comp. Endocrinol. 107(2): 191-200
Cooper J.E. (1984) The First Edward Elkan Memorial Lecture. International Colloquium on the Pathology of Reptiles and Amphibians. Nottingham UK
Cooper J.E. (1985) Manual of exotic pets. Revised edn. J.E. Cooper, M.F. Hutchison, O.F. Jackson, R.J. Maurice. Cheltenham, BSAVA
Cooper J.E., Cooper M.E. (2003) Wildlife Health Services. Wellingborough, Northants UK
Cooper J.E., Jackson O.F. (1981) Diseases of the reptilia. London, New York, Academic Press
Cooper J.E., Jackson O.F. (eds.) (1981) Diseases of the Reptilia (Vol. 1 and 2). Academic Press, Toronto, Ont.
Costanzo J.P., Lee R.E. Jr., DeVries A.L., Wang T., Layne J.R. Jr. (1995) Survival mechanisms of vertebrate ectotherms at subfreezing temperatures: applications in cryomedicine. Faseb. J. 9(5): 351-358
Crews D. (1996) Temperature-dependent sex determination: the interplay of steroid hormones and temperature. Zoolog. Sci. 13(1): 1-13
Dacke C.G. (1979) Calcium Regulation in Sub Mammalian Vertebrates. Academic Press, London, UK.
Darevesky I.S. (1966) Natural parthenogenesis in a polymorphic group of caucasian rock lizards related to *Lacerta saxicola* Eversmann. J. Ohio Herpetol. Soc. 5: 115
Davies A.J., Johnston M.R. (2000) The biology of some intraerythrocytic parasites of fishes, amphibia and reptiles. Adv. Parasitol. 45: 1-107

Davies P.M. (1981) Anatomy and physiology. In: Diseases of the Reptilia Vol.1 (J.E. Cooper, O.F. Jackson, eds.). Academic Press, London, UK. pp. 9-67

Dorcas M.E., Peterson C.R., Flint M.E. (1997) The thermal biology of digestion in rubber boas (*Charina bottae*): physiology, behavior, and environmental constraints. Physiol. Zool. 70(3): 292-300

Ewert, J.-P. (1998) Neurobiologie des Verhaltens. Huber Verlag, Bern

Ewert J.-P. (2004) Stimulus perception. Chapter 2; in: Principles of Animal Behavior (J.J. Bolhuis, L.-A. Giraldeau, eds.). Blackwell Publ.

Ewert J.-P., Buxbaum-Conradi H., Dreisvogt F., Glagow M., Merkel-Harff C., Röttgen A., Schürg-Pfeiffer E., Schwippert W.W. (2001) Neural modulation of visuomotor functions underlying prey-catching behaviour in anurans: Perception, attention, motor performance, learning. Comp. Biochem. Physiol. A. Physiol. 128(3): 417-461

Ferguson, G.W., Jones, J.R., Gehrmann, W.H., Hammack, S.H., Talent, L.G., Hudson, R.D., Dierenfeld, E.S., Fitzpatrick, M.P., Frye, F.L., Holick, M.F., Chen, T.C., Lu, Z., Gross, T.S., Vogel, J.J. (1996) Indoor husbandry of the panther chameleon *Chantaeleo pardalis*: effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. Zoo Biology 15: 279-299

Fernandes B.J., Cooper J.D., Cullen J.B. et al. (1976) Systemic infection with *Alaria americana* (Trematoda). Can. Med. Assoc. J. 115: 1111

Frye F.L. (1991) Infectious disease. In: Biomedical and Surgical Aspects of Captive Reptile Husbandry, 2nd edn., Vol. I. Krieger Publishing, Melbourne

Fitch H.S. (1970) Reproduce Cycles of Lizards and Snakes. Misc. Pub. No. 52. Univ. Kansas Mus. Nat. Hist., Lawrence, KS.

Fleming A., Wibbels T., Skipper J.K., Crews D. (1999) Developmental expression of steroidogenic factor 1 in a turtle with temperature-dependent sex determination. Gen. Comp. Endorcrinol. 116(3): 336-346

Fowler M.E. (1978) Metabolic bone disease. In: Zoo and Wild Animal Medicine (M.E. Fowler, ed.). W.B. Saunders and Co., Philadelphia, PA. pp. 55-76

Freeman R.S., Fallis, A.M. (1973) International Larval Trematode. Transactions of the American Academy of Ophthalmology and Otolaryngology Vol. 77, pp. 784-991

Freeman R.S., Stuart P.F., Cullen, J.P., et al. (1976) Fatal human infection with mesocercariae of the trematode *Alaria Americana*. J. Trop. Med. Hyg. 25: 803

Friedman C.R., Torigian C., Shillam P.J., Hoffman R.E., Heltzel D., Beebe J.L., Malcolm G., DeWitt W.E., Hutwagner L., Griffin P.M. (1998) An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. J. Pediatr. 132(5): 802-807

Frye F.F. (1997) The importance of calcium in relation to phosphorus, especially in folivorous reptiles. Proc. Nutr. Soc. 56(3): 1105-1117

Frye F.L. (1973) Husbandry, Medicine and Surgery in Captive Reptiles. Veterinary Medical Publishing Inc., Bonner Spring, KS.

Ganesh C.B., Yajurvedi H.N. (2002) Stress inhibits seasonal and FSH-induced ovarian recrudescence in the lizard *Mabuya carinata*. J. Exp. Zool. 292(7): 640-648

Gehrmann, W.C. (1997) Reptile lighting: a current perspective. The Viviarum 8(2): 44-45, 62

Gehrmann, W.H. (1987) Ultraviolet irradiances of various lamps used in animal husbandry. Zoo Biology 6: 117-127

Gehrmann, W.H. (1994A) Light requirements of captive amphibians and reptiles. In: Captive Management and Conservation of Amphibians and Reptiles (J.B. Murphy, K. Adler, J.T. Collins, eds.). Soc. Study Amphib. Reptiles

Gehrmann, W.H. (1994B) Spectral characteristics of lamps commonly used in herpetoculture. The Vivarium 5: 16-21

Gehrmann, W.H. (1996) Lizard-saver light support. The Vivarium 7: 49

Girling J.E., Cree A. (1995) Plasma corticosterone levels are not significantly related to reproductive stage in female common geckos. Gen. Comp. Endocrinol. 100(3): 273-281

Gopee N.V., Adesiyun A.A., Caesar K. (2000) Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. J. Wildl. Dis. 36(2): 284-293

Graczyk T.K., Cranfield M.R. (1998) Experimental transmission of *Cryptosporidium* oocyst isolates from mammals, birds and reptiles to captive snakes. Vet. Res. 29(2): 187-195

Greenberg N. (1976) Thermoregulatory aspects of behavior in the blue spiny lizard, *Sceloporus cyanogenys* (Sauria, Iguanidae). Behaviour 59: 1-21

Greenberg N. (1977) A neuroethological investigation of display behavior in the lizard, *Anolis carolinensis* (Lacertilia, Iguanidae). American Zoologist 17(1): 191-201

Greenberg N. (1980) Physiological and behavioral thermoregulation in living reptiles. In: A Cold Look at the Warm-Blooded Dinosaurs (R.D.K. Thomas, E.C. Olson, eds.). AAAS Selected Symposium, Washington 28: 141-166

Greenberg N. (1983) Central and autonomic aspects of aggression and dominance in reptiles. In: Advances in Vertebrate Neuroethology (J.-P. Ewert, R.R. Capranica, D.J. Ingle, eds.). Plenum Press, New York

Greenberg N. (1985) Exploratory behavior and stress in the lizard, *Anolis carolinensis*. Z. Tierpsychol. 70: 89-102.

Greenberg N. (1977) An ethogram of the blue spiny lizard, *Sceloporus cyanogenys* (Reptilia, Lacertilia, Iguanidae). Journal of Herpetology 11(2): 177-195

Greenberg N. (1990) The behavioral endocrinology of physiological stress in a lizard. J. Exper. Zool. 4: 170-173

Greenberg N. (2002) Ethological aspects of stress in a model lizard, *Anolis carolinensis*. J. Int. Comp. Biol. 42(3): 526-540

Greenberg N. (2003) Sociality, stress, and the corpus striatum of the green anolis lizard. Physiol. Behav. 79(3): 429-440

Greenberg N., Chen T., Crews D. (1984a) Social status, gonadal state, and the adrenal stress response in the lizard, *Anolis carolinensis*. Hormones and Behavior 18: 1-11

Greenberg N., Crews D. (1983) Physiological ethology of aggression in amphibians and reptiles. In: Hormones and Aggressive Behavior (B. Svare, ed.). Plenum Press, New York

Greenberg N., Crews D. (1990) Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. Gen. Comp. Endocrinol. 77: 1-10

Greenberg N., Hake L. (1990) Hatching and neonatal behavior of the lizard, *Anolis carolinensis*. J. Herpetol. 24(4): 402-405

Greenberg N., MacLean P.D. (eds.) (1978) Behavior and Neurology in Lizards. NIMH, Rockville, MD

Greenberg N., Scott M., Crews D. (1984). Role of the amygdala in the aggressive and reproductive behavior of the lizard, *Anolis carolinensis*. Physiol. Behav. 32(1): 147-151

Greenberg N., Wingfield J. (1987) Stress and reproduction: Reciprocal relationships. In: Reproductive Endocrinology of Fish, Amphibians, and Reptiles (D.O. Norris, R.E. Jones, eds.). Plenum Press, New York

Greene M.J., Stark S.L., Mason R.T. (2002) Predatory response of brown tree snakes to chemical stimuli from human skin. J. Chem. Ecol. 28(12): 2465-2473

Guillette L.J. Jr., Crain D.A., Rooney A.A., Pickford D.B. (1995) Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environ Health Perspect. 103 Suppl. 7: 157-164

Guirado S., Davila J.C. (2002) Thalamo-telencephalic connections: new insights on the cortical organization in reptiles. Brain Res. Bull. 57(3-4): 451-454

Harkewicz K.A. (2001) Dermatology of reptiles: a clinical approach to diagnosis and treatment. Veterinary Clin. North Am. Exot. Anim. Pract. 4(2): 441-461

Heard D.J. (2001) Reptile anesthesia. Veterinary Clin. North Am. Exot. Anim. Pract. 4(1): 83-117

Hernandez-Divers S.J. (2001) Clinical aspects of reptile behavior. Veterinary Clin. North Am. Exot. Anim. Prakt. 4(3): 599-612

Holt P.E. (1981) Drugs and dosages. In: Diseases of the Reptilia Vol. 2 (J.E. Cooper, O.F. Jackson, eds.). Academic Press, London, UK. pp. 551-584

Holz P., Barker, I.K., Burger J.P., Crashaw G.J., Conlon P.D. (1997) The effect of the renal portal system on pharmacokinetic parameters in the red-eared slider (*Trachemys scripta elegans*). J. Zool. Wildl. Med. 28(4): 386-393

Ippen R., Zwart P. (1996) Infectious and parasitic disease of captive reptiles and amphibians, with special amphasis on husbandry practices which prevent or promote diseases. Rev. Sci. Tech. 15(1): 43-54

Ishiwata K., Nakao H., Nose R., Komiya M., Hanada S. Enomoto Y., Nawa Y. (1997) Gnathostomiasis in frog-eating snakes in Japan. J. Wildl. Dis. 33(4): 877-879

Jackson O.F. (1974) Reptiles and the general practitioner. Vet. Rec. 95: 11

Jackson O.F., Cooper, J.E. (1981) Nutritional diseases. In: Diseases of the Reptilia Vol. 2 (J.E. Cooper, O.F. Jackson, eds.). Academic Press, London, UK. pp. 409-428

Jarchow J. (1988) Hospital care of the reptile patient. In: Exotic Animals (E. Jacobson, G. Kollias jr., eds.). Churchill Livingstone, New York

Jeyasuria P., Place A.R. (1998) Embryonic brain-gonadal axis in temperature-dependent sex determination of reptiles: a role for P450 aromatase (CYP19). J. Exp. Zool. 281(5): 428-449

Jones, J.R., Ferguson, G.W., Gehrmann, W.H., Holick, M.F., Chen, T.C., Lu, Z. (1996) Vitamin D nutritional status influences voluntary behavioral photoregulation in a lizard. In: Biologic Effects of Light. (M.F. Holick, E.G. Jung, eds.). Walter de Gruyter, N.Y.

Kaplan M. (2002) Melissa Kaplan's Herp Care Collection.
(2002a) Signs of illness and stress in reptiles. – (2002b) Fluid and fluid therapy in reptiles, with information of the administration of oral and injectible medications. – (2002c) Guidelines for medicating sick herps [Reptiles 5(3): 84-85]. – (2002d) Iguana breeding season basics. – (2002e) Dealing with male green iguana breeding aggression. – (2002f) Lethargy in reptiles. – (2002g) Commentary: observations on disease-associated preferred body temperatures in reptiles [Appl. Anim. Behav. Sci. 28(1991): 375-380]. – (2002h) Reptile skin shedding; snakes, lizards and chelonians. – (2002i) Winter advisory.

Kemp T.S. (1982) Mammal-like reptiles and the origin of mammals. Academic Press, Toronto, Ont.

Kettlewell J.R., Raymond C.S., Zarkower D. (2000) Temperature-dependent expression of turtle Dmrt 1 prior to sexual differentiation. Genesis 26(3): 174-178

Keymer I.F. (1978) Disease of chelonians: (2) Necropsy survey of terrapins and turtles. Vet. Rec. 103: 577

Klein W., Andrade D.V., Wang T., Taylor E.W. (2002) Effects of temperature and hypercapnia on ventilation and breathing pattern in the lizard *Uromastyx aegyptius*. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132(4): 847-859

Klingenberg R.J. (1988) Anorexia in reptiles. Proceedings of the 12th International Symposium on Captive Propagation and Husbandry. New York-New Jersey Metopolitan Area, pp. 621-634

Klingenberg R.J. (1994) Basic principles of therapeutics used in reptile medicine. Proceedings Association of Reptilian and Amphibian Veterinarians, Pittsburgh, PA., Oct. pp. 22-24

Kluge A.G., Eckardt M.J. (1969) *Hemidactylus garnotii* Dumeril and Bibron, a triploid all-female species of gekkonid lizard. Copeia 1969(4): 651

Kreger M.D. (1993) The psychological well-being of reptiles. Humane Innovationss and Alternatives, pp. 519-523

Lance V.A. (1992) Evaluation pain and stress in reptiles. In: The Care and Use of Amphibians, Reptiles and Fish in Research (D.O. Schaeffer, K.M. Klienow, L. Krulisch, eds.). Scientists Center for Animal Welfare, Bethesda, MD, pp. 101-106

Laszlo, J. (1969) Observations on two new artificial lights for reptile displays. International Zoo Yearbook 9: 12-13

Laszlo, J. (1979) Notes on thermal requirements of reptiles and amphibians in captivity. In: 3rd Annual Reptile Symposium on Captive Propagation and Husbandry, Knoxville, Tennessee

MacLaughlin, J.A., Anderson, R.R., Holick, M. F. (1982) Spectral character of sunlight modulates photosynthesis of pre-vitamin D, and its photoisomers in human skin. Science 216: 1001-1003

Mader D. (1996) Reptile Medicine and Surgery. W.B. Saunders Company, Philiadephia, PA., p. 512

Mader D. (ed.) (1996) Reptile Medicine and Surgery. W.B. Saunders, Philadelphia, PA, pp. 301-302

Mader D.M. (1991) Antibiotic therapy. In: Biomedical and Surgical Aspects of Captive Reptile Husbandry, 2nd ed., Vol. II (F.L. Frye, ed.). Krieger Publishing, Melbourne, FL.

Marcus L.C. (1977) Parasitic diseases of captive reptiles. In: Current Veterinary Therapy, Vol.6 (R.W. Kirk, ed.. W.B. Saunders Co., Philadelphia, PA. pp. 801-806

Marcus L.C. (1981) Veterinary Biology and Medicine of Captive Amphibians and Reptiles. Lea and Febiger, Philadelphia, PA.

Matz G. (1983) Amphibien und Reptilien. BLV-Verlagsgesellschaft, München

Matz G., Weber D. (2002) Guide des amphibiens et reptiles d'Europe. Les Guides du Naturaliste. Delachaux & Niestle

Matz G., Vanderhaege M. (2003) Terrarium. Guides du Naturaliste. Delachaux & Niestle

McCauley S.J., Bjorndal K.A. (1999) Response to dietary dilution in an omnivorous freshwater turtle: implications for ontogenetic dietary shifts. Physiol. Biochem. Zool. 72(1): 101-108

Maslin T.P. (1971) Conclusive evidence of parthenogenesis in three species of *Cnemidophorus* (Teiidae). Copeia 1971: 156

Mermin J., Hoar B., Angula F.J. (1997) Iguanas and *Salmonella marina* infection in children: a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics 99(3): 399-402

Miller R.E. (1996) Quarantine protocols and preventive medicine procedures for reptiles, birds and mammals in zoos. Rev. Sci. Tech. 15(1): 183-189

Mitchell M.A., Shane S.M. (2000) Preliminary findings of *Salmonella* spp. in captive green iguanas (*Iguana iguana*) and their environment. Prev. Vet. Med. 45(3-4): 297-304

Morrison G. (2001) Zoonotic infections from pets. Understanding the risks and treatment. Postgrad-Med. 110(1): 24-26, 29-30, 35-36

Murphy J.T., Collins, J.B. (eds.) (1980) Reproductive biology and diseases of captive reptiles. Proc. Symp. Soc. for the Study of Amphibians and Reptiles, Oxford, OH.

Northcutt R.G., Kaas H. (1995) The emergence and evolution of mammalian neocortex. Trends Neurosci. 18: 373-379

Orlans F.B. (1977) Reptiles. In: Animal Care from Protozoa to Small Mammals. Addison-Wesley Publ. Co., Don Mills, Ont. pp. 214-241

O'Rourke D.P. (2002) Reptiles and amphibians as laboratory animals. Lab. Anim. (NY) 31(6): 43-47

O'Steen S. (1998) Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. J. Exp. Biol. 201(3): 439-449

O'Steen S., Janzen, F.J. (1999) Embryonic temperature affects metabolic compensation and thyroid hormones in hatching snapping turtles. Physiol. Biochem. Zool. 72(5): 520-533

Pasmans F., Van Immerseel F., Van den Broeck W., Bottreau E., Velge P., Ducatelle R., Haesebrouck F. (2003) Interactions of *Salmonella enterica* subsp. *enterica serovar* Muenchen with intestinal explants of the turtle *Trachemys scripta scripta*. J. Comp. Pathol. 128(2-3): 119-126.

Peterson C.C., Walton B.M., Bennett A.F. (1998) Intrapopulation variation in ecological energetics of the garter snake *Thamnophis sirtalis*, with analysis of the precision of doubly labeled water measurements. Physiol. Zool. 71(4): 333-349

Pieau C., Dorizzi M., Richard-Mercier N. (1999) Temperature-dependent sex determination and gonadal differentiation in reptiles. Cell Mol. Life Sci. 55(6-7): 887-900

Pieau C., Dorizzi M., Richard-Mercier N. (2001) Temperature-dependent sex determination and gonadal differentiation in reptiles. EXS 91: 117-141

Porter K.R. (1972) Herpetology. W.B. Saunders, Toronto, Ont.

Redrobe S. (2002) Reptiles and disease-keeping the risks to a minimum. J. Small. Anim. Pract. 43(10): 471-472

Reiner A.J. (2000) A hypothesis as to the organization of cerebral cortex in the common amniote ancestor of modern reptiles and mammals. Novartis. Found. Symp. 228: 83-102, discussion 102-113

Reiner A., Medina L., Veenman C.L. (1998) Structural and functional evolution of the basal ganglia in vertebrates. Brain Res. Brain Res. Rev. 28(3): 235-285

Romer A.S. (1966) Vertebrate Paleontology. Univ. of Chicago Press, Chicago, IL.

Rosen T., Jablon J. (2003) Infectious threats from exotic pets: dermatological implications. Dermatol. Clin. 21(2): 229-236

Russell F.E., Walter F.G., Bey T.A., Fernandez M.C. (1997) Snakes and snakebite in Central America. Toxicon. 35(10): 1469-1522

Saehoong P., Wonsawad C. (1997) Helminths in house lizards (Reptilia: Gekkonidae). Southeast-Asian-J-Trop-Med-Public-Health 28 Suppl. 1: 184-189

Sanyal D., Douglas T., Roberts R. (1997) *Salmonella* infection acquired from reptilian pets. Arch. Dis. Child. 77(4): 345-346

Schaeffer D.O., Klienow K.M., Krulisch L. (eds.)(1992) The Care and Use of Amphibians, Reptiles and Fish in Research. Scientists Center for Animal Welfare, Bethesda, MD

Schmidt K.P., Inger R.F. (1957) Living reptiles of the world. Doubleday and Co., Toronto, Ont.

Schultz D.J., Hough I.J., Boardman W. (1996) Special challenges of maintaining wild animals in captivity in Australia and New Zealand: prevention of infectious and parasitic diseases. Rev. Sci. Tech. 15(1): 289-308

Schumacher J. (2003) Fungal diseases of reptiles. Veterinary Clin. Nort. Am. Exot. Anim. Pract. 6(2): 327-335

Schumacher J. (2003) Reptile respiratory medicine. Veterinary Clin. North Am. Exot. Anim. Pract. 6(1): 213-231

Seebacher F. (2000) Heat transfer in a microvascular network: the effect of heart rate on heating and cooling in reptiles (*Pogona harbata* and *Varanus varius*). J. Theor. Biol. 203(2): 97-109

Sever D.M., Hamlett W.C. (2002) Female sperm storage in reptiles. J. Exp. Zool. 292(2): 187-199

Siegmund O.H. (ed.) (1979) The Merck Veterinary Manual (5th Ed.). Merck & Co. Inc., Rahway, NJ

Sievert, L.M. (1991) The influence of photoperiod and position of a light source on behavioral thermoregulation. Copeia 1991: 105-110

Smeets W.J., Marìn O., Gonzaléz A. (2000) Evolution of the basal ganglia: new perspectives through a comparative approach. J. Anat. 196 (Pt.4): 501-517

Stebbins R.C. (1966) Anatomy and physiology. In: A Field Guide to Western Reptiles and Amphibians (2nd Edn.). Houghton Mifflin Co., Boston, MA. pp. 279

Stewart J.R., Thompson, M.B. (1998) Placental ontogeny of the Australian scincid lizards *Niveoscincus coventryi* and *Pseudemonia spenceri*. J. Exp. Zool. 282(4-5): 535-559

Stewart J.W. (1969) Care and management of amphibians, reptiles and fish in the laboratory. In: IAT Manual of Laboratory Animal Practice and Techniques (2nd Edn.) (D.J. Short, D.P. Woodnott, eds.). Chas . C. Thomas, Springfield, IL

Storey K.B. (1996) Metabolic adaptation supporting anoxia tolerance in reptiles recent advances. Comp. Biochem. Physiol. B. Biochem Mol. Biol. 113(1): 23-35

Storey K.B. (1999) Living in the cold: freeze-induced gene responses in freeze-tolerant vertebrates. Clin. Exp. Pharmacol. Physiol. 26(1): 57-63

Striedter G.F. (1997) The telencephalon of tetrapods in evolution. Brain Behav. Evol. 49(4): 179-213

Summers C.H., Greenberg N. (1995) Activation of central biogenic amines following aggressive interactions in male lizards, *Anolis carolinensis*. Brain Behav. Evol. 45: 339-349

Summers C.H., Larson E.T., Summers T.R., Renner K.J., Greenberg N. (1998) Regional and temporal separation of serotonergic activity mediating social stress. Neurosci. 87(2): 489-496

Super H., Uylings H.B. (2001) The early differentiation on the neocortex: a hypothesis on neocortical evolution. Cereb. Cortex. 11(12): 1101-1109

Thomas R. (1965) The smaller teeid lizards (*Gymnophthalmus* and *Bachia*) of the Southeastern Carribbean. Proc. Biol. Soc. of Washington 78: 141

Tyrrell C.L., Cree A. (1998) Relationships between corticosterone concentration and season, time of day and confinement in a wild reptile (*Sphenodon punctatus*). Gen. Comp. Endocrinol. 110(2): 97-108

Voituron Y., Storey J.M., Grenot C., Storey K.B. (2002) Freezing survival, body ice content and blood composition of the freeze-tolerant European common lizard, *Lacaerta vivipara*. J. Comp. Physiol. [B] 172(1): 71-76

Vos J.G., Dybing E., Greim H.A., Ladefoged O., Lambre C., Tarazona J.V., Brandt I., Vethaak A.D. (2000) Health effects of endocrine-disrupting chemical on wildlife, with special reference to the European situation. Crit. Rev. Toxicol. 30(1): 71-133

Walker C.H. (1998) Biomarker strategies to evaluate the environmental effects of chemicals. Environ. Health Perspect. 106(2): 613-620

Wallach J.D. (1978) Feeding and nutritional diseases. In: Zoo and Wild Animal Medicine (M.E. Fowler, ed.). W.B. Saunders and Co., Philadelphia, PA. pp. 123-128

Wang T., Busk M., Overgaard J. (2001) The respiratory consequences of feeding in amphibians and reptiles. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 128(3): 535-549

Wang T., Zaar M., Arvedsen S., Vedel-Smith C., Overgaard J. (2002) Effects of temperature on the metabolic response to feeding in *Python molurus*. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 133(3): 519-527

Warwick C. (1990a) Reptilian ethology in captivity: Observations of some problems and an evaluation of their aetiology. Appl. Anim. Behav. Sci. 26: 1-13

Warwick C. (1990b) Important ethology and other considerations of the study and maintenance of reptiles in captivity. Appl. Anim. Behav. Sci. 27(4): 363-366

Warwick C. (1991) Observations on disease-associated preferred body temperatures in reptiles. Appl. Anim. Behav. Sci. 28(4): 375-380

Warwick C., Frye F.L., Murphy J.B. (1995) Health and welfare of captive reptiles. Chapman & Hall, London

Warwick C., Lambiris A.J., Westwood D., Steedman C. (2001) Reptile-related salmonellosis. J. R. Soc. Med. 94(3): 124-126

Webb J.K., Shine R. (1998) Thermoregulation by a nocturnal elapid snake (*Hoplocephalus bungaroides*) in Southeastern Australia. Physiol. Zool. 71(6): 680-692

Western P.S., Sinclair A.H. (2001) Sex, genes, and heat: triggers of diversity. J. Exp. Zool. 290(6): 624-631

Western P.S., Harry J.L., Graves J.A., Sinclair A.H. (1999) Temperature-dependent sex determination in the American alligator: AMH precedes SOX9 expression. Dev. Dyn. 216(4-5): 411-419

Wiens J.J., Slingluff J.L. (2001) How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. Evolution Int. J. Org. Evolution 55(11): 2303-2318

Zug G.R. (1993) Herpetology: An Introductory Biology of Amphibians and Reptiles. Academic Press, New York

Zwart P. (2001) Assessment of the husbandry problems of reptiles on the basis of pathophysiological findings: a review. Vet. Q. 123(4): 140-147

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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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Background information

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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 (27.1.99, 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.

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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).

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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; Huldt 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 (Friis 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*

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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.

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"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."

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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.

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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 littermates, 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, 2000).

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 subdominants 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.

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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-Rowland et al, 1987b).

- 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 following 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 degeneration, 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 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). Sudden, loud noise can cause audiogenic seizures in rodents (Iturrian, 1973). Sound may have many adverse 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 individually ventilated cages.

The group proposes that the rodents' general considerations section should contain the following 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 laboratory 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 acoustic 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 facility, but be audible to man.

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

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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), Holson 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. "*

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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

	CoE ETS 123, 1986 Appendix A		**UK Home Office, Code of Practice, 1989 Scientific Procedures**		
	floor area	height	floor area	height	remarks
Mouse	180 cm^2	12 cm	200 cm^2	12 cm	
Rat	350 cm^2	14 cm	500 cm^2	18 cm	250-450g: 700 cm^2 / 20 cm; >450g: 800 cm^2 / 20 cm
Gerbil	-	-	500 cm^2	18 cm	
Hamster	180 cm^2	12 cm	300 cm^2	15 cm	
Guinea pig	600 cm^2	18 cm	700 cm^2	20 cm	250-550g: 900 cm^2 / 23 cm; 550-650g: 1000 cm^2 / 23 cm; > 650g: 1250 cm^2 / 23 cm

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Table 2 Minimum space requirements for breeding rodents (mother and litter)

	CoE ETS 123, 1986 Appendix A		**UK Home Office, Code of Practice, 1995 Breeding and Supplying Establishments**		
	floor area	height	floor area	height	remarks
Mouse	200 cm^2	12 cm	300 cm^2	12 cm	pair (inbred/outbred) or trio (inbred)
Rat	800 cm^2	14 cm	900 cm^2	18 cm	also for monogamous pair
Hamster	650 cm^2	12 cm	650 cm^2	15 cm	also for monogamous pair
Guinea pig	1200 cm^2	18 cm	1500 cm^2	23 cm	also for monogamous pair
in harem	1000 cm^2	18 cm	1000 cm^2	23 cm	per female

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 fulfilment 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 cm^2 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 (Würbel 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 3rd 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.

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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]

	Body weight gms	*Minimum floor area cm^2*	*Minimum cage height cm*	*Floor area per animal cm^2*
In stock and during pro-cedure	*<20 21-25 26-30 > 30*	*330 330 330 330*	*12 12 12 12*	*60 70 80 100*
Breeding		*330 For a monogamous pair (out-bred/inbred) or trio (inbred). For each additional female plus litter 180cm^2 should be added.*	*12*	
*Stock at breeders**	*< 20 < 20*	*950 1500*	*12 12*	*40 30*

** 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, 1999).

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 cm^2, 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 cm^2 cage contains enough space for adequate enrichment at higher

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stocking densities. Therefore, increased stocking density should only be allowed if the minimum floor space is increased to 1500 cm^2. 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]

	Body weight gms	*Minimum floor area cm^2*	*Minimum cage height cm*	*Floor area per animal cm^2*
In stock and during procedure	*< 200 201-300 301-400 401-600 > 600*	*800 800 800 800 1500*	*18 18 18 18 18*	*200 250 350 450 600*
Breeding		*800 Mother and litter. For each additional adult animal permanently added to the cage add 400 cm^2*	*18*	
*Stock at breeders**	*< 50 51-100 101-150 151-200*	*1500 1500 1500 1500*	*18 18 18 18*	*100 125 150 175*
*Stock at breeders**	*< 100 101-150 151-200*	*2500 2500 2500*	*18 18 18*	*100 125 150*

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 nest, 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]

	Body weight gms	*Minimum Floor area cm^2*	*Minimum cage height cm*	*Floor area per animal cm^2*
In stock and during procedure	*< 40* *> 40*	*1200* *1200*	*18* *18*	*150* *250*
Breeding		*1200 Monogamous pair or trio with off-spring*	*18*	

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

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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]

	Body weight gms	*Minimum floor area cm^2*	*Minimum cage height cm*	*Floor area per animal cm^2*
In stock and during procedure	*< 60* *60-100* *> 100*	*800* *800* *800*	*14* *14* *14*	*150* *200* *250*
Breeding		*800* *Mother or monogamous pair with litter*	*14*	
*Stock at breeders**	*< 60*	*1500*	*14*	*100*

** 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.

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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.***
[GT 123 (2000) 57, Rodents, Table 5]

	Body weight gms	*Minimum floor area cm^2*	*Minimum Cage height cm*	*Floor area per animal cm^2*
In stock and during procedure	*< 200*	*1800*	*23*	*200*
	200-300	*1800*	*23*	*350*
	301-450	*1800*	*23*	*500*
	451-700	*2500*	*23*	*700*
	> 700	*2500*	*23*	*900*
Breeding		*2500 Pair with litter. For each additional breeding female add 1000 cm^2*	*23*	

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, non-toxic 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).

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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.

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Table 3 Marking methods for rodents

Non Invasive	
Labels:	Cages or pen labels for individually housed animals.
Marker pens:	Tail or coat, short term, depending on grooming and on housing conditions.
Hair clipping:	Lasts 2 to 6 weeks.
Dyes:	Sheep markers may be used, last longer but require renewal after moulting.
Permanent Methods	
Microchips:	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.
Tail tattooing:	Rats, mice, (ears –guinea pigs) – local anaesthetics should be used to minimise discomfort. Requires trained competent staff to ensure legibility.
Ear notching/punching:	Only sharp punches should be used to avoid tearing the tissue and damaging veins.
Ear tags:	Difficult to read, can fall out or get caught in caging.
Freeze marking:	With spots of liquid nitrogen, useful for pigmented strains

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).

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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.

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Table 4 Minimum space requirements for rabbits in stock, and during procedure

weight	CoE ETS 123, 1986 Appendix A		UK Home Office, Code of Practice, 1989 Scientific Procedures			
	when housed singly		when housed singly		when housed in groups	
	floor area	height	floor area	height	floor area	height
up to 2000 g	1400 cm^2	30 cm	2000 cm^2	40 cm	1300 cm^2	40 cm
up to 3000 g	2000 cm^2	30 cm				
up to 4000 g	2500 cm^2	35 cm	4000 cm^2	45 cm	2600 cm^2	45 cm
up to 5000 g	3000 cm^2	40 cm				
above 5000 g	3600 cm^2	40 cm				
up to 6000 g			5400 cm^2	45 cm	3300 cm^2	45 cm
above 6000 g			6000 cm^2	45 cm	4000 cm^2	45 cm

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

weight	CoE ETS 123, 1986 Appendix A			UK Home Office, Code of Practice, 1995 Breeding & Supplying Establishments		
	floor area	height	nestbox inclusive	floor area	height	remarks
up to 2000 g	3000 cm^2	30 cm	1000 cm^2			
up to 3000 g	3500 cm^2	30 cm	1000 cm^2	4300 cm^2	45 cm	no specific nestbox
above 3000 g				6400 cm^2	45 cm	size recommendations
up to 4000 g	4000 cm^2	35 cm	1200 cm^2			
up to 5000 g	4500 cm^2	40 cm	1200 cm^2			
above 5000 g	5000 cm^2	40 cm	1400 cm^2			

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 cm^2 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 remove and to clean. 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 round 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 manoeuvre. 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]

The final body weight kg that any rabbit will reach in this housing	*Minimum floor area for one or two socially harmonious animals cm²*	*Minimum height cm*
< 3	*3500*	*45*
3-5	*4200*	*45*
> 5	*5400*	*60*

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]

Doe weight kg	*Minimum floor area cm²*	*Addition for nestboxes cm²*	*Minimum height cm*
<3 *3-5* *>5*	*3500* *4200* *5400*	*1000* *1200* *1400*	*45* *45* *60*

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]

Age	*Minimum floor area cm²*	*Maximum number of animals on minimum floor area*	*For each additional animal add cm²*	*Minimum height*
Weaning - 7 weeks old	*4000*	*5*	*800*	*40*
8-10 weeks	*4000*	*3*	*1200*	*40*

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]

Age (weeks)	*Final body weight (kg)*	*Approximate size (cm x cm)*	*Approximate height above cage floor (cm)*
< 10	*-*	*55 x 25*	*-*
> 10	*<3*	*55 x 25*	*25*
	3-5	*55 x 30*	*25*
	>5	*60 x 35*	*30*

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.

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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

Non Invasive	
Coat colour:	Only usable with white or mixed coloured strains.
Felt marker / marker dye:	On ear, needs regular renewal, can cause skin irritation. Coats can also be marked but require regular renewal.
Leg rings:	Numbered, aluminium or plastic. Usually fitted at weaning. Can cause irritation, need regular checking to ensure they do not become too tight.
Fur clipping:	Grows out quite quickly, needs regular renewal.
Permanent Methods	
Microchips:	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.
Ear tattooing:	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.
Ear tags:	Can fall out, get caught in caging or become infected. Can be painful to insert.

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REFERENCES

Ader R, Friedman SB: Social factors affecting emotionality and resistance to disease in animals. IV. Differential housing, emotionality, and Walker 256 carcinosarcoma in the rat. Psychological Reports 15, 535-541, 1964.

Agren G: Pair formation in the Mongolian gerbil. Anim. Behav. 32, 528-535, 1984.

Agren G: Sociosexual behaviour in the Mongolian Gerbil, Meriones unguiculatus. PhD Thesis, University of Stockholm, Sweden, 1978.

Albonetti ME, Dessi-Fulgheri F, Farabollini F: Interfemale agonistic interactions in the domestic rabbit. Aggressive Behaviour 16, 77-86, 1990.

Andersen AA, Hanson RP: Intrauterine infection of mice with St. Louis encephalitis virus: immunological, physiological, neurological, and behavioral effects on progeny. Infect. Immun. 12(5):1173-83, 1975.

Anzaldo AJ, Harrison PC, Riskowski GL, Sebek LA, Maghirang RG, Stricklin WR, Gonyou HW: Increasing welfare of laboratory rats with the help of spatially enhanced cages. Animal Welfare Information Centre Newsletter 5, 1-5, 1994.

Armario A, Castellanos JM, Balasch J: Chronic noise stress and insulin secretion in male rats. Physiol. Behav. 34, 359-361, 1985.

Arnold C., Gillaspy S: Assessing laboratory life for golden hamsters: social preference caging selection and human interaction, Lab. Anim. 28, 34-37, 1994.

Arnold CE, Estep DQ: Laboratory caging preferences in golden hamsters (Mesocricetus auratus), Lab. Anim. 28(3), 232238, 1994.

Arnold EC, Estep OD: Effects of housing on social preference and behaviour in male golden hamsters (Mesocricetus auratus). Appl. Anim. Behav. Sci. 27, 253-261, 1990.

Ashley MP, Neoh SH, Kotlarski I, Hardy D: Local and systemic effects in the non-specific tumor resistance induced by attenuated Salmonella enteritidis 11RX in mice, Aust. J. Exp. Biol. Med. Sci., 54, 157-168, 1976.

Baer H: Long-term isolation stress and its effects on drug response in rodents. Lab. Anim. Sci. 21, 341-349, 1971.

Baer J.F. In: Second nature, environmental enrichment for captive animals. Shepherdson D.J., Mellen JD, Hutchins M (eds.), Smithsonian Institution Press, 277-301, 1998.

Barnard C.J., Behnke J.M., Sewell J: Social behaviour and susceptibility to infection in house mice: effects of group size, aggressive behaviour and status-related hormonal responses prior to infection on resistance to Babesia microti. Parasitology 108, 487-496, 1994.

Barthold S: Research complications and state of knowledge of rodent coronaviruses, In Complications of Viral and Mycoplasma Infections in Rodents to Toxicology Research, Hamm Jr. TE (ed.), Chapt. 4, Hemisphere Press, Washington D. C., 1985.

Barthold SW, Jonas AM: Morphogenesis of early 1,2-dimethylhydrazine-induced lesions and latent period reduction of colon carcinogenesis in mice by a variant of Citrobacter freundii, Cancer Res. 37, 4352-4360, 1977.

Barthold SW, Brownstein DG: The effect of selected viruses on Corynebacterium kutscheri, Lab. Anim. Sci. 38, 580-583, 1988.

Batchelor GR: Group housing on floor pens and environmental enrichment in sandy lop rabbits. Anim. Technology 42, 109-120, 1991.

Batchelor GR: Group housing on floor pens and environmental enrichment of Sandy Lop rabbits (II): the 24h behavioural time budget of group housed rabbits. Anim. Technology 46, 167-190, 1995.

Batchelor, GR: The Laboratory Rabbit. In: UFAW Handbook on the Care and Management of Laboratory Animals. Poole T (ed.). Blackwell Science Ltd., Oxford, vol. 1, 395-408, 1999.

Baumans V: Environmental enrichment: practical applications. In: Animal Alternatives, Welfare and Ethics. Van Zutphen L.F.M. and Balls M. (eds.), Elsevier, 187-197, 1997.

Baumans, V: The laboratory mouse. In: UFAW Handbook on the Care and Management of Laboratory Animals. T Poole (ed.). Blackwell Science Ltd., Oxford, vol. 1, 282-312, 1999.

Baumans, V, Stafleu FR, Bouw J: Testing housing systems for mice - the value of a preference test. Z. Versuchstierkunde 29, 9-14, 1987.

Bayne K: Normal and abnormal behaviors of laboratory animals: what do they mean? Lab. Anim. 25, 21-24, 1996.

Beer R, Kaiser S, Sachser N, Stanzel K: Meerschweinchen. Merkblatt zur tierschutzgerechten Haltung von Versuchstieren. Deutsche Tierärztliche Vereinigung für Tierschuz, 1995.

Beer R, Sachser N: Social structure and welfare in all-male groups of guinea-pigs. In: Proceedings of the Congress of the International Society for Applied Ethology 1993, Berlin, 254-258, 1993.

Benn DM: Innovations in research animal care. J. Am. Vet. Med. Assoc. 206, 465-468, 1995.

Berthelsen H, Hansen LT: The effect of hay on the behaviour of caged rabbits (Oryctolagus cuniculi). Animal Welfare 8, 149-158, 1999.

Bia FJ, Thornton GF, Main AJ, Fong CKY, Hsiung GD; Western equine encephalitis mimicking herpes simplex encephalitis, J. Amer. Med. Ass. 244, 367-369, 1980.

Bigler L: Mutter-Kind-Beziehung beim Hauskaninchen. Lizenziatsarbeit, University of Berne, Switzerland, 1985.

Bigler L: Vergleich des Verhaltens und gewisser veterinärmedizinischer Parameter von einzeln gehaltenen “ELCO”- und “ZIKA”-Zibben. Schlussbericht z.Hd. Bundesamt für Veterinärwesen, Bern, Schweiz, 1995.

Bigler L: Zusammenfassung der Ergebnisse radiologischer Untersuchungen an Zuchtzibben-Wirbelsäulen und Mastkaninchen-Femurknochen in der Schweiz von 1984-1995. Bericht z.Hd. Bundesamt für Veterinärwesen, Bern, Schweiz, 1998.

Bigler L, Oester H: Paarhaltung nicht reproduzierender Zibben im Käfig. Berlin. Münchn. Tierärztl. Wochenschrift 107, 202-205, 1994b.

Bigler L, Oester H: Group housing for male rabbits. 6th World Rabbit Congress, Toulouse, Vol. 2, 411-415, 1996.

Bisazza A: Social organizazion and territorial behaviour in three strains of mice. Bolletino Zoologica 48, 157-167, 1981.

Bixler GS, Booss J: Establishment of immunologic memory concurrent with suppression of the primary immune response during cytomegalovirus infection of mice, J. Immunol. 125, 893-896, 1980.

Blom H.: Evaluation of housing conditions for laboratory mice and rats. Thesis Utrecht University, 1993.

Blom HJM, van Tintelen G, Baumans V, van den Broek J, Beynen AC: Development and application of a preference test system to evaluate housing conditions for laboratory rats. Appl. Anim. Behav. Sci. 43, 279-290, 1995.

Blom HJM, van Tintelen G, Van Vorstenbosch CJAHV, Baumans V J, Beynen AC: Preferences of mice and rats for types of bedding material. Lab. Anim. 30, 234-244, 1996.

Boice J: Burrows of wild and albino rats: effects of domestication, outdoor raising, age, experience and maternal state. J. Comp. Physiol. Psychol. 91, 649-661, 1977.

Borer KT, Pryor A, Conn CA, Bonna R, Kielb M: Group housing accelerates growth and induces obesity in adult hamsters, Am. J. Physiol. 255 (1 Pt 2):R12833, 1988.

Bradshaw AL, Poling AL: Choice by rats for enriched versus standard home cages: plastic pipes, wood platforms, wood chips, and paper towels as enrichment items. J. Exp. Analysis Behav. 55, 245-250, 1991

Brain PF: What does individual housing mean to a mouse? Life Sciences 16, 187-200, 1975

Brain PF: Stress in agonistic contexts in rodents. In: Social stress in domestic animals, Zayan R and Dantzer R (eds.). Kluwer, Dordrecht, 73-85, 1990.

Brain PF: Understanding the behaviours of feral species may facilityte design of optimal living conditions for common laboratory rodents. Anim. Technol. 43, 99-105, 1992.

Brain, PF: The Laboratory Gerbil. In: UFAW Handbook on the Care and Management of Laboratory Animals. Poole T (ed.). Blackwell Science Ltd., Oxford, vol. 1, 345-355, 1999.

Brain PF, Kusumorini N, Benton D: 'Anxiety' in laboratory rodents: a brief review of some recent behavioural developments. Behav. Processes 25, 71-80, 1991.

Brain PF, Parmigiani S: Variation in aggressiveness in house mouse populations. Biol. J. Linnean Soc. 41, 257-269, 1990.

Brinton MA: Lactate dehydrogenase-elevating virus, In: The Mouse in Biomedical Research. Foster HL, Small JD, Fox JG. (eds.), Academic Press, New York, London Paris San Diego San Francisco Sao Paulo Sydney Tokyo Toronto, 1982, chapt. 10.

Brooks DL, Huls W, Leamon C, Thomson J, Parker J, Twomey S: Cage enrichment for female New Zealand White rabbits. Lab. Anim. 30-38, 1993.

Broom DM: Indicators of poor welfare. Brit. Vet. J. 142, 524-526, 1986.

Bruhin H (ed.): Planung und Struktur von Versuchstierbereichen tierexperimentell tätiger Institutionen. 4. Aufl., GV-SOLAS, Biberach, 1988.

Büttner D: Climbing on the cage lid, a regular component of locomotor activity in the mouse. J. Exp. Anim. Sci. 34, 165-169, 1991.

Büttner D: Upright standing in the laboratory rat: time expenditure and its relation to locomotor activity. J. Experim. Anim. Sci. 36, 19-26, 1993.

Canali E, Ferrante V, Todeschini R, Verga M, Carenz C: Rabbit nest construction and its relationship with litter development. Appl. Anim. Behav. Sci. 31, 259-266., 1991.

Cassell GH: The pathogenic potential of mycoplasmas: Mycoplasma pulmonis as a model, Rev. Infect Dis., 4, S18-S34, 1982.

Cassell GH, Lindsey JR, Davis JK: Respiratory and genital mycoplasmosis of laboratory rodents: Implications for biomedical research, Israel. J. Med. Sci. 17, 548-554, 1981.

Chamove AS: Environmental enrichment: a review. Animal Technology 40, 155-178, 1989.

Chmiel DJ, Noonan M: Preference of laboratory rats for potentially enriching stimulus objects. Lab. Anim. 30, 97-101, 1996.

Claassen V: Neglected factors in pharmacology and neuroscience research, Elsevier, 225-250, 1994.

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C: Recommendations for euthanasia of experimental animals Part 1. Lab. Anim. 30, 293-316, 1996.

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C: Recommendations for euthanasia of experimental animals Part 2. Lab. Anim. 31, 1-32, 1997.

Clough G: Environmental effects on animals used in biomedical research. Biol. Rev. 57, 487-523, 1982.

Clough G, Fasham JAL: A 'silent' fire alarm. Lab. Anim. 9, 193-196, 1975.

Clough G: Housing and Welfare of Laboratory Rodents, In Beynen AC & Solleveld HA: New Developments in Biosciences. Their Implications for Laboratory Animal Science, pp 239-244, Martin Nijhoff Publishers, 1988.

CornwellJones CA, Palfai T, Krasenbaum D, Byer E, Clark R, Kinnard K: Housing Influences Exploration and Social-interaction of Control and DSP-4-treated Rats. Physiol. Behav., 52:(2) 271-276, 1992.

Coulon J: Etude de la hierarchie sociale chez le cobaye domestique. Behaviour 53, 183-199, 1975.

Council of Europe: European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123), Strasbourg, 1986.

Council of Europe: Resolution on the Accommodation and Care of Laboratory Animals, adopted by the multilateral consultation on 30 May 1997, Strasbourg, 1997.

Coureaud G, Schaal B, Orgeur P, Coudert P: Le contrôle de laccès au nid chez la lapine: conséquences sur la mortalité des laperaux. J. Recherche Cunicol. France 7, 245-148, 1998.

Coureaud G, Benoist S, Coudert P, Hudson R, Rideaud P, Orgeur P: Mimicking natural nursing conditions promotes early pup survival in domestic rabbits. Ethology 106, 207-227, 2000a.

Coureaud G, Schaal B, Coudert P, Rideaud P, Fortunelamothe L, Hudson R, Orgeur P: Immediate postnatal sucking in the rabbit: its influence on pup survival and growth. Reprod. Nutr. Develop. 40, 19-21, 2000b.

Crabbe JC, Wahlsten D, Dudek BC: Genetics of Mouse Behavior: Interactions with Laboratory Environment. Science 284, 1670-1672, 1999.

Cunliffe-Beamer TL, Freeman LC, Myers DD: Barbiturate Sleeptime in Mice Exposed to Autoclaved or Un-autoclaved Wood Beddings. Lab. Anim. Sci. 31, 672-675, 1981.

Dean SW: Environmental enrichment of laboratory animals used in regulatory toxicology studies. Lab. Anim. 33, 309-327, 1999.

Delaveau A: La mortalité des laperaux sous la mère effets de la qualité du nid. Cuniculture 43, 21-27, 1982.

Denenberg VH, Wyly MV, Burns JK, Zarrow, MX: Behavioural Effects of Handling Rabbits in Infancy. Physiol. Behav. 10, 1001-1004, 1973.

Drescher B: The effects of housing systems for rabbits with special reference to ulcerative pododermatitis. Tierärztl. Umschau 48, 72-78, 1993a.

Drescher B: Zusammenfassende Betrachtung über den Einfluss unterschiedlicher Haltungsverfahren auf die Fitness von Versuchs- und Fleischkaninchen. Tierärztl. Umschau 48, 72-78, 1993b.

Drescher B, Loeffler K: Einfluss unterschiedlicher Haltungsverfahren und Bewegungsmöglichkeiten auf die Kompakta der Röhrenknochen von Versuchs- und Fleischkaninchen. Tierärztl. Umschau 46, 736-741, 1991.

Drescher B, Loefflrer K: Skoliosen, Lordosen und Kyphosen bei Zuchtkaninchen. Tierärztl. Praxis 24, 292-300, 1996.

Drescher B, Schlender-Bobbis L: Etude pathologique de la pododermatite chez les lapins reproducteurs de souche lourde sur grillage. World Rabbit Sci. 4, 143-148, 1996.

Ellis DH Follett BK: Gonadotrophin secretion and testicular function in golden hamsters exposed to skeleton photoperiods with ultrashort light pulses. Biol. Repro. 29, 805-818, 1983.

Ernst C: Vergleichende Untersuchungen an Laborratten. PhD Thesis Freie Universität Berlin, Germany, 1994

Eskola S, Kaliste-Korhonen E: Effects of cage type and gnawing blocks on weight gain, organ weights and open-field behaviour in Wistar rats. Scand. J. Lab. Anim. Sci. 25, 180-193, 1998.

Eskola S, Lauhikari M, Voipio HM, Nevalainen T: The use of aspen blocks and tubes to enrich the cage environment of laboratory rats. Scand. J. Lab. Anim. Sci. 26, 1-10, 1999a.

Eskola S, Lauhikari M, Voipio HM, Laitinen M, Nevalainen T: Environmental enrichment may alter the number of rats needed to achieve statistical significance. Scand. J. Lab. Anim. Sci. 26, 134- 144, 1999b.

Fleischner A: Ethologische Untersuchungen an Mastkaninchen zur Präferenz unterschiedlicher Bodenarten sowie von Licht und Dunkelheit anhand von Wahlversuchen. PhD Thesis, Hohenheim University, Germany, 1998

Fletcher JL: Influence of noise on animals. In: Control of the animal House Environment, T. McSheehy (ed.), 51-62, Laboratory Animals Ltd., London, 1976.

Fortmeyer HP: The influence of exogenous factors such as maintenance and nutrition on the courdse and results of animal experiments. Animals in Tox. Research, 13-32, 1982.

Fox GJ, Cohen BJ & Loew FM: Laboratory Animal Medicine, Academic Press, 1984.

Fox JG, Dewhirst FE, Tully JG, Paster BJ, Yan L, Taylor NS, Collins Jr. MJ Gorelick PL, Ward JM: Helicobacter hepaticus sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. J. Clin. Microbiol. 32, 12381245, 1994a.

Fox JG, Yan L, Shames B, Hayward A, Murphy JC, Dewhirst FE, Paster BJ: Helicobacter bilisassociated active, chronic hepatitis in aged inbred strains of mice. Contemporary Topics 33:A2, 1994b.

Fox JG, Yan LL, Dewhirst FE, Paster BJ, Shames B, Murphy JC, Hayward A, Belcher JC, Mendes EN: Helicobacter bilis sp. nov., a novel Helicobacter species isolated form the bile, liver, and intestines of aged, inbred mice. J. Clin. Microbiol. 33, 445454, 1995.

Friis AS, Ladefoged O: The influence of Bacillus piliformis (Tyzzer) infections on the reliability of pharmacokinetic experiments in mice. Lab. Anim. 13, 257-261, 1979.

Garlinghouse L, van Hoosier G: Studies on adjuvant-induced arthritis, tumor transplantability, and serologic response to bovine serum albumin in Sendai virus-infected rats, Amer. J. Vet. Res., 39, 297-300, 1978.

Gärtner K: Physiologische Psychologie der Gruppen- und Einzelhaltung. Deutsche Tierärztliche Wochenschrift 4, 97-100, 1968.

Gärtner K: Cage enrichment may enhance variance of experimental data and number of animals needed. Abstract 36th GV SOLAS meeting, Hamburg, 1998.

Gärtner K: Reduction or refinement: standard or enriched cages for mice in experiments? Influence of cage enrichment on deviation. Abstracts of Scientific Papers, ICLAS-FELASA Joint Meeting, Palma de Mallorca, 120, 1999.

Gärtner K, Küpper W, Maess J: Zum artgemässen Bewegungsbedürfnis der Versuchstiere (Maus, Ratte, Meerschweinchen, Kaninchen, Hund, Katze). Fortschritte der Veterinärmedizin 25, 130-138, 1976

Geber WF, Anderson TA, Van Dyne, B: Physiologic responses of the albino rat to chronic noise stress. Arch. Environ. Health, 12, 751-754, 1966.

Gray S, Hurst JL: The effects of cage cleaning on aggression within groups of male laboratory mice. Anim Behav. , 49:821-826, 1995.

Griffith BP, Askenase PW, Hsiung GD: Serum and cell-mediated viral-specific delayed cutaneous basophil reactions during cytomegalovirus infection of guinea pigs. Cell. Immunol. 69, 138-149, 1982.

Griffith BP, Lavallee J T, Booss J, Hsiung GD: Asynchrous depression of responses to T- and B- cell mitogens during acute infection with cytomegalovirus in the guinea pig. Cell. Immunol. 87, 727-733, 1984.

Guignard R, Potworowski EF, Lussier G: Mouse thymic-virus-mediated immunosuppression; association with decreased helper T cells and increased suppressor T cells. Viral Immunol. 2, 215-220, 1989.

Gunn D: Evaluation on welfare in the husbandry of laboratory rabbits. PhD Thesis, University of Birmingham, United Kingdom, 1994.

Hackbarth HJ, Bohnert W, Tsai PH: Allometric comparison of recommendations of minimum floor area for laboratory animals. Lab. Anim. 33, 351-355, 1999.

Haemisch A, Gärtner K: The cage design affects intermale aggression in small groups of male laboratory mice: strain specific consequences on social organization, and endocrine activations in two inbred strains. J. Exp. Anim. Sci. 36, 101-116, 1994.

Haemisch A, Voss T, Gärtner K: Effects of environmental enrichment on aggressive behaviour, dominance hierarchies and endocrine states in male DBA/2J mice. Physiol. Behav. 56, 1041-1048, 1994.

Haemisch A, Weisweiler H: Der Einfluss permanent wechselnder Käfigpartner auf die Körpergewichtsentwicklung und renale Ausscheidung von Kortikosteron und Katecholaminen bei verschiedenen Rattenstämmen. XXX. Wissenschaftliche Tagung GV-SOLAS, Salzburg, 112 (abstract), 1992.

Hamilton HH, Lukefahr SD, McNitt JI: Maternal nest quality and its influence on litter survival an weaning performance in commercial rabbits. J. Anim. Sci. 75, 926-933,1997.

Hamilton JD, Fitzwilliam JF, Cheung KS, Lang DJ: Effect of murine cytomegalovirus infection on the immune response to a tumor allograft. Rev. Infect. Dis. 1, 976-987, 1979.

Hansen AK: Zucht und Haltung des Mongolischen Gerbils im Hinblick auf die Verwendung als Modell in Einem Biomizinischen Versuch, In Fortmeyer, HP, Madry M & Schumacher J (eds): Hamster und Gerbil, Zucht, Haltung, Ernährung, Versuchsmodelle, Altomin Tier-Labor Service/Akademie für Tierärtzliche Fortbildung, 1990.

Hansen AK: Health status and the effect of microbial organisms on animal experiments, In: Handbook of Laboratory Animal Science, Vol. I, Animal Selection and Handling in Biomedical Research, Svendsen P, Hau J (Eds.), CRC Press, Boca Raton, 1994, Chapt. 11.

Hansen AK, Dagnæs-Hansen F, Møllegaard-Hansen KE: Correlation between Megaloileitis and Antibodies to Bacillus piliformis in Laboratory Rat Colonies. Lab. Anim. Sci., 42(5):449-453, 1992.

Hansen AK, Andersen HV, Svendsen O: Studies on the Diagnosis of Tyzzer's Disease in Laboratory Rat Colonies with Antibodies against Bacillus piliformis (Clostridium piliforme).Lab. Anim. Sci., 44(5):424-429, 1994.

Hansen LT, Berthelsen, H: The effect of environmental enrichment on the behaviour of cages rabbits (Oryctolagus cuniculus). Appl. Anim. Behav. Sci. 68, 163-178, 2000.

Harri M, Lindblom J, Malinen H, Hyttinen M, Lapvetelainen T, Eskola S, Helminen HJ: Effect of access to a running wheel on behaviour of C57BL/6J mice. Lab. Anim. Sci. 49, 401-405, 1999.

Haseman JK, Bourbina J, Eustis SL: Effect of individual housing and other experimental design factors on tumor incidence in B6C3F1 mice. Fundamental and Applied Toxicology 23, 44-52, 1994.

Hatch AM, Wiberg GS, Zawidzka Z, Cann M, Airth JM, Grice HC: Isolation syndrome in the rat. Toxicology and Applied Pharmacology 7, 737-745, 1965.

Havenaar R, Meijer J, Morton DB, Ritskes-Hoitinga J, Zwart P: Biology and husbandry of laboratory animals. In: Principles of Laboratory Animal Science. Zutphen LFM, Baumans V, Beynen AC (eds.). Elsevier, Amsterdam, 17-74, 1993.

Heath E: Sexual and related territorial behaviuour in the laboratory rabbit. Lab. Anim. Sci. 22, 684-691, 1972.

Heath M, Stott E: Housing rabbits the unconventional way. Anim. Techn. 41, 13-25, 1990.

Hebb DO: The effects of early experience on problem-solving at maturity. Am. Psychol. 2, 306-307, 1947.

Held S: Group-housing of female laboratory rabbits - studies on behaviour and immunocompetence. PhD Thesis, University of Wales, 1995.

Held SDE, Turner RJ, Wootton RJ: Choices of laboratory rabbits for individual or group-housing. Appl. Anim. Behav. Sci. 46, 81-91, 1995.

Hill AC, Stalley GC: Mycoplasma pulmonis infection with regard to embryo freezing and hysterectomy derivation, Lab. Anim. Sci. 41, 563-566, 1991.

Holson RR, Scallet AC, Ali SF, Turner BB: "Isolation stress" revisited: Isolation-rearing effects depend on animal care methods. Physiol. Behav. 49, 1107-1119, 1991.

Home Office UK: Code of Practice for the housing and care of animals used in scientific procedures. London: HMSO, 1989

Home Office UK: Code of Practice for the housing and care of animals in designated breeding and supplying establishments. London: HMSO, 1995

Hudson R, Distel H: The pattern of behaviour of rabbit pups in the nest. Behaviour 79, 255-71, 1982.

Hudson R, Distel H: The temporal pattern of suckling in rabbit pups: a model of circadian synchrony between mother and young. In: Development of circadian Rhythmicity and Photoperiodism in Mammals. Perinatology Press, Boston, 83-102, 1989.

Hudson R, Schaal B, Bilko, A, Altbäcker V: Just three minutes a day: the behaviour of young rabbits viewed in the context of limited maternal care. Proceedings 6th Rabbit World Congress, Toulouse, 1996, Vol. 2, 395-403, 1996

Huldt S, Gard S, Olovson SG: Effect of Toxoplasma gondii on the thymus, Nature 244, 301-303, 1973.

Huls WL, Brooks DL, Bean-Knudson D: Response of adult New Zealand White Rabbits to enrichment objects and paired housing. Lab. Anim. Sci. 41, 609-612, 1991

Hurst JL, Barnard CJ, Tolladay U, Nevison CM, West CD: Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. Anim. Behav. 56, 563-586, 1999.

Hurst JL., Barnard CJ., West CD: Welfare by design: the natura/selection of welfare criteria in laboratory rats. In: Animal Alternatives, Welfare and Ethics, Van Zutphen LFM, Balls M (eds.), Elsevier, 209-214, 1997a.

Hurst JL, Barnard CJ, Nevison CM, West CD: Housing and welfare in laboratory rats: welfare implications of isolation and social contact among caged males. Animal Welfare 6, 329-247, 1997b.

Hurst JL, Fang J, Barnard CJ: The role of substrate odours in maintaining social tolerance in male house mice (Mus musculus domesticus). Anim. Behav. 45, 997-1006, 1993

Ikemoto S, Panksepp J: The effect of early social isolation on the motivation for social play in juvenile rats. Developmental Psychobiology 25, 261-274, 1992.

Isakov N, Feldman M, Segal, S: Acute infections of mice with lactic dehydrogenase virus (LDV) impairs the antigen-presenting capacity of their macrophages, Cell. Immun. 66, 317-332, 1982.

Iturrian WB: The effect of noise on immature rodents. Carworth letter no. 92, Carworth, 1973.

Jahkel M, Rilke O, Koch R, Oehler J:. Open field locomotion and neurotransmission in mice evaluated by principal component factor analysis-effects of housing condition, individual activity disposition and psychotropic drugs. Prog. Neuro-Psychopath. 24: (1) 61-84, 2000.

Jennings M, Batchelor GR, Brain PF, Dick A, Elliott H, Francis RJ, Hubrecht RC, Hurst JL, Morton DB, Peters AG, Raymond R, Sales GD, Sherwin CM, West C: Refining rodent husbandry: the mouse. Report of the Rodent Refinement Working Party. Lab. Anim. 32, 233-259, 1998.

Jilge B: The rabbit: a diurnal or a nocturnal animal? J. Exp. Anim. Sci. 34, 170-183, 1991

Jilge B: The ontogeny of circadian rhythms in the rabbit. J. Biol. Rhythms 8, 247-260, 1993.

Jones RB: Varied cages and aggression. Appl. Anim. Behav. Sci. 27, 295-296, 1991

Juhr NC, Ratsch H: Modifikation der Teratogenese von Actinomycin-D und Cyclophosphamid durch eine Mycoplasma pulmonis-Infektion bei der Ratte, Z. Versuchstierkunde 33, 265-270, 1990.

Kaliste-Korhonen E, Eskola S, Rekilä T, Nevalainen T: Effects of gnawing material, group size and cage level in rack on Wistar rats. Scand. J. Lab. Anim. Sci. 22, 291-299, 1995.

Keely PJ, Millican KG, Organ PJ, (eds): The Principles of Animal Technology I, ppK1 : K5. The Institute of Animal Technology, 1988.

Kimbrough R, Gaines TB: Toxicity of hexylmethylphosphoramide in rats. Nature 211, 146-147, 1966.

King JA: Social relations of the domestic guinea-pig living under semi-natural conditions. Ecology 37, 221-228, 1956.

Koehl PF: RENALP 1997. Cuniculture 138, 247-252, 1999.

Korotzer TL, Weiss HS, Hamparian VV, Somerson NL: Oxygen uptake and lung function in mice infected with Streptococcus pneumoniae, influenza virus, or Mycoplasma pulmonis. J. Lab. Clin. Med. 91, 280-294, 1978.

Kraft R: Vergleichende Verhaltensstudien an Wild- und Hauskaninchen. I. Das Verhaltensinventar von Wild- und Hauskaninchen. Tierzüchtung Züchtungsbiologie 95, 140-162, 1979.

Kraft V, Deeney AA, Blanchet HM, Boot R, Hansen AK, Hem A, van Herck H, Kunstyr I, Milite G, Needham JR, Nicklas W, Perrot A, Rehbinder C, Richard Y, de Vroy G: Recommendations relatives au controle sanitaier des elevages de souris, rats, hamsters, cobayes et lapins. Translation of the report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management November 1992, Lab. Anim. 28, 1-12, 1994.

Krinke GJ: The Laboratory Rat, Academic Press, 2000.

Krohn TC, Ritskes-Hoitinga J, Svendsen P: The effect of feeding and housing on the behaviour of the laboratory rabbit. Lab. Anim. 33, 101-107, 1999.

Kuhnen G: The effect of cage size and environmental enrichment on the generation of fever in golden hamster. Ann.N.Y.Acad.Sci. 813, 398-400, 1997.

Kuhnen G: Reduction of fever by housing in small cages. Lab. Anim. 32, 4245, 1998.

Kuhnen G: The effect of cage size and enrichment on core temperature and febrile response of the golden hamster. Lab. Anim. 33, 221-227, 1999a.

Kuhnen G: Housing-induced changes in the febrile response of juvenile and adult golden hamsters, J. Exp. Anim. Sci. 39:151-155, 1999b.

Laber-Laird K, Swindle M & Flecknell: Rodent and Rabbit Medicine, Pergamon, 1996.

Laubach HE, Kocan AA, Sartain KE: Lung lysophospholidase activity in specific-pathogen-free rats infected with Pasteurella pneumotropica or Mycoplasma pulmonis. Infect. Immun. 22, 295-297, 1978.

Lawlor M: Behavioural approaches to rodent management. In: Standards in laboratory animal management UFAW (ed.), UFAW, Potters Bar, 40-49, 1983.

Lawlor M: The size of rodent cages. In: Guidelines for the well-being of rodents in research, Guttman HN (ed.). Scientists Centre for Animal Welfare, Bethesda, 19-27, 1990.

Lawrence AB, Rushen J: Stereotypic animal behaviour: fundamentals and applications to animal welfare. CAB-International, Wallingford, 1993.

Lehmann M: Interference of a restricted environment as found in battery cages with normal behaviour of young fattening rabbits. In: Rabbit production systems including welfare. Auxilia T (ed.). Commission of the European Communities, ECSC-EED-EAEC, Luxembourg, 257-268, 1987.

Lehmann M: Das Verhalten junger Hauskaninchen unter verschiedenen Umgebungsbedingungen. Beurteilung von Haltungssystemen sowie Entwicklung eines Haltungskonzeptes für Mastgruppen. PhD Thesis, University of Berne, Switzerland, 1989.

Lehmann M: Beschäftigungsbedürfnis junger Hauskaninchen: Rohfaseraufnahme und Tiergerechtheit. Schweiz. Archiv Tierheilkunde 132, 375-381, 1990.

Lehmann M: Social behaviour in young domestic rabbits under semi-natural conditions. Appl. Anim. Behav. Sci. 32, 269-292, 1992.

Lehmann M, Wieser R: Indikatoren für mangelnde Tiergerechtheit sowie Verhaltensstörungen bei Hauskaninchen. Zeeb K (ed.). Aktuelle Arbeiten zur artgemässen Tierhaltung 1984, KTBL-Schrift 307, Darmstadt, Gemany, 96-107, 1985.

Lidfors L: Behavioural effects of environmental enrichment for individually caged rabbits. Appl. Anim. Behav. Sci. 52, 157-169, 1997

Love JA: Group housing: meeting the physical and social needs of the laboratory rabbit. Lab. Anim. Sci. 44, 5-11, 1994.

Love JA, Hammond K: Group housing rabbits. Lab Animal 20, 37-43,1991.

Mahmoud AA, Warren KD, Strickland GT: Acquired resistance to infection with Schistosoma mansoni induced by Toxoplasma gondii, Nature 263, 56-57, 1976.

Maier J: Verhaltensbiologische Untersuchungen zur Boden-Gruppenhaltung von Zucht- und Mastkaninchen. PhD Thesis, University of Hohenheim, Germany, 1992.

Manser CE: The assessment of stress in laboratory animals. RSPCA Publ., Horseham, West Sussex, 208 p., 1992.

Manser CE, Morris TH, Broom DM: An investigation into the effects of solid or grid cage flooring on the welfare of laboratory rats. Lab. Anim. 29, 353-363, 1995.

Manser CE, Elliott H, Morris TH, Broom DM: The use of a novel operant test to determine the strength of preference for flooring in laboratory rats. Lab. Anim. 30, 1-6, 1996.

Manser CE, Broom DM, Overend P, Morris TM: Investigations into the preferences of laboratory rats for nest-boxes and nesting materials. Lab. Anim. 32, 23-35, 1998.

Marcanto PS, Rosmini R: Ulcerative pododermatitis. In: Pathology of the Rabbit and Hare. Esculapio (ed.), Bologna, 11, 1996.

Martrenchar A., Morrisse P: Wire-floor pens as an alternative to metallic cages in fattening rabbits: incidence on some welfare traits. Animal Welfare, in press.

McGregor PK, Barnard C, Hurst JL: Reply to Jones RB (1991), Appl. Anim. Behav. Sci. 27, 297-299, 1991

Mench JA: Environmental enrichment and the importance of exploratory behaviour. In: Second nature, environmental enrichment for captive animals, Shepherdson DJ, Mellen JD, Hutchins M (eds.). Smithsonian Institution Press, 30-46, 1998.

Mering S, Kaliste-Korhonen E, Nevalainen T: Estimates of appropriate numberr of rats: interaction with housing environment. Lab. Anim., in press.

Merkenschlager M, Wilk W: Gutachten über tierschutzgerechte Haltung von Versuchstieren / Recommendations for the keeping of laboratory animals in accordance with animal protection principles (ordered by the German Ministry of Food, Agriculture and Forestry). Schriftenreihe Versuchstierkunde 6, Paul Parey Verlag, Berlin and Hamburg, 1979.

Metz J: Behavioural problems of rabbits in cages. In: Rabbit production systems including welfare. Auxilia T (ed.). Commission of the European Communities, ECSC-EED-EAEC, Luxembourg, 221-230, 1987.

Milligan SR, Sales GD, Khirnykh K: Sound levels in rooms housing laboratory animals: An uncontrolled daily variable. Physiol. Behav. 53, 1067-1076, 1993.

Mims C: Virus-Related Immunomodulation, In: Viral and Mycoplasmal Infections of Laboratory Rodents, Effects on biomedical research, Bhatt PN, Jacoby RO, Morse III HC, New AE (eds.): Academic Press Inc., Orlando, 1986, chap. 28.

Mohammed AK, Maehlen J, Magnusson O, Fonnum F, Kristensson K Persistent changes in behaviour and brain serotonin during ageing in rats subjected to infant nasal virus infection. Neurobiol. Aging., 13(1):83-87, 1992.

Morisse JP, Boilletot E, Martrenchar A: Preference testing in intensively kept meat production rabbits for straw on wire grid floors. Appl. Anim. Behav. Sci. 64, 71-80,1999.

Morisse JP, Maurice R: Influence of stocking density or group size on behaviour of fattening rabbits kept under intensive conditions. Appl. Anim. Behav. Sci. 54, 351-357, 1997.

Morton DB, Jennings M, Batchelor GR, Bell D, Birke L, Davies K, Eveleigh JR, Gunn D, Heath M, Howard B, Koder P, Phillips J, Poole T, Sainsbury AW, Sales GD, Smith DJA, Stauffacher M, Turner RJ: Refinements in rabbit husbandry. Second report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Lab. Anim. 27, 301-329, 1993

Nagel R, Stauffacher M: Ethologische Grundlagen zur Beurteilung der Tiergerechtheit von Laborratten in Vollgitterkäfigen. Tierlaboratorium 17, 119-32

National Research Council: Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996.

Nayfield KC, Besch EL: Comparative responses of rabbits and rats to elevated noise. Lab. Anim. Sci. 31, 386-390, 1981.

Nettesheim PH, Schreiber H, Cresia DA, Richter CB, Respiratory infections in the pathogenesis of lung cancer, Rec. Res. Cancer Res. 44, 138, 1974.

Nevison CM, Hurst JL, Barnard CJ: Strain-specific effects of cage enrichment in male laboratory mice (Mus musculus). Anim. Welfare 8, 361-379, 1999.

Newberry RC: Environmental enrichment: increasing the biological relevance of captive environment. Appl. Anim. Behav. Sci. 44, 229-243, 1995.

Nicklas W, Kraft V, Meyer B: Contamination of transplantable tumors, cell lines, and monoclonal antibodies with rodent viruses, Lab. Anim. Sci. 43, 296300, 1993.

Nicklas W, Homberger FR, Illgen-Wilcke B, Jacobi K, Kraft V, Kustyr I, Mählrer M, Meyer H, Pohlmeyer-Esch G: Implications of infectious agents on results of animal experiments. Lab. Anim. 33, suppl. 1, 1999.

Niethammer J: Wühler. In: Säugetiere, Bd. 3. Grzimek Enzyklopädie, Kindler Verlag, München, 206-265, 1988.

North D: The Guinea Pig. In: UFAW Handbook on the Care and Management of Laboratory Animals. Poole T (ed.). Blackwell Science Ltd., Oxford, vol. 1, 367-388, 1999.

Notkins AL: Enzymatic and immunologic alterations in mice infected with lactic dehydrogenase virus, Am. J. Pathol 64, 733-746, 1971.

Oester H, Lehmann M: Die Mindestanforderungen der schweizerischen Tierschutzgesetzgebung zur Haltung von Kaninchen im Vergleich zu den Haltungsempfehlungen der deutschen WRSA. Bericht 8. Arbeitstagung Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere, Celle, Germany, 33-41, 1993.

Olivier B, Molewijk E, van Oorschot R, van der Poel G, Zethof T, van der Heyden J, Mos J.: New animal models of anxiety. Eur. Neuropsychopharmacol. 4, 93-102, 1994

Orok-Edem E, Key D: Response of rats to enrichment objects. Anim, Technol. 45, 25-30, 1994.

Osborn J E: Cytomegalovirus and other herpesviruses of mice and rats, In: Viral and Mycoplasmal Infections of Laboratory Rodents, Effects on biomedical research, Bhatt PN, Jacoby RO, Morse III HC, New AE (Eds.), Academic Press Inc., Orlando, 1986, chap. 19.

O'Steen WK, Shear CR and Anderson KV: Retinal damage after prolonged exposure to visible light. Am. J. Anat. 134, 5-22, 1972.

Peng X, Lang CM, Drozdowicz FK, Ohlsson-Wilhelm BM: Effect of cage population density on plasma corticosterone and peripheral lymphocyte populations of laboratory mice. Lab. Anim. 23, 302-306, 1989.

Pfeuffer C: Wahlversuche zur Haltung von Laborratten. Dissertation. Veterinärmedizinische Fakultät, Freie Universität Berlin, 1996.

Podberscek AL, Blackshaw JK, Beattie AW: The behaviour of group penned and individually caged laboratory rabbits. Appl. Anim. Behav. Sci. 28, 352-363, 1991.

Pollack JD, Weiss HS, Somerson NL: Lecitin changes in murine Mycoplasma pulmonis respiratory infection. Infect. Immun. 24, 94-101, 1979.

Prior H, Sachser N: Effects of enriched housing environment on the behaviour of young male and female mice in four exploratory tasks. J. Exp. Anim. Sci. 37(2), 57-68,1995.

Rehbinder C, Baneux P, Forbes D, van Herck H, Nicklas W, Rugaya Z, Winkler G: FELASA recommendations for the health monitoring of mouse, rat, hamster, gerbil, guinea pig and rabbit experimental units. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management, Lab. Anim. 30, 193-208 1996.

Renner MJ, Hackett Renner C: Expert and novice intuitive judgments about animal behaviour. Bull. Psychon. Soc. 31, 551-552, 1993.

Rilke O, Jahkel M, Oehler J: Dopaminergic parameters during social isolation in low- and high-active mice, Pharmacol. Biochem. 60:(2), 499-505, 1998.

Rommers J, Meijerhof R: The effect of different floor types on foot pad injuries of rabbit does. In: Proceedings of the 6th World Rabbit Science Congress 1996, Toulouse, 431-436, 1996.

Rommers J, Meijerhof R: Effect of group size on performance, bone strengthz and skin lesions of meat rabbits housed under commercial conditions. World Rabbit Science 6, 299-302, 1998.

Rothfritz P, Drescher B, Loeffler K: Einfluss unterschiedlicher Haltungsverfahren und Bewegungsmöglichkeiten auf die Spongiosastruktur der Rippen sowie Brust- und Lendenwirbel von Versuchs- und Fleischkaninchen. Tierärztl. Umschau 47, 758-768, 1992.

Rose MA: Environmental factors likely to impact on an animal's well-being - an overview. In: Improving the well-being of animals in the research environment, Baker RM et al (eds). ANZCCART Conference, 99-116, 1994.

Ruskin J, Remington JS: Immunity and intracellular infection: resistance to bacteria in mice infected with a protozoan, Science 160, 72-74, 1968.

Sachser N: Different forms of social organization at high and low population densities in guinea pigs. Behaviour 97, 253-272, 1986a.

Sachser N: The effect of long-term isolation on physiology and behavior in male guinea pigs. Physiol. Behav. 38, 31-39, 1986b.

Sachser N: Social organization, social status, behavioural strategies and endocrine respondses in male guinea pigs. In: Hormones Brain and Behaviour in Vertebrates. 2. Behavioural Activation in Males and Females - Social Interaction and Reproductive Endocrinology. Balthazart J (ed.), Comparative Physiology, Karger, Basel, 176-187, 1990.

Sachser N: Sozialphysiologische Untersuchungen an Hausmeerschweinchen: Gruppen strukturen, soziale Situation und Endokrinium, Wohlergehen. Schriftenreihe Versuchstierkunde, Heft 16, Paul Parey, 1994.

Sachser N: Of Domestic and Wild Guinea Pigs: Studies in Sociophysiology, Domestication, and Social Evolution, Naturwissenschaften 85:219-228, 1998

Sachser N, Beer R: Long-term influences of social situation and socialization on adaptability in behaviour. In: Research and Captive Propagation. Gansloser U, Hodges JK, Kaumanns W W (eds.). Filander Verlag, Furth, 1995.

Sachser N, Kaiser S: Prenatal social stress masculinizes the females' behaviour in guinea pigs. Physiol. Behav. 60, 589-594, 1996.

Sachser N, Lick C, Stanzel K: The environment, hormones, and aggressive behaviour: a five-year study in guinea pigs. Psychoneuroendocrinology 19, 697-707, 1994.

Sales GD, Wilson KJ, Spencer KEV, Milligan SR. Environmental ultrasound in laboratories and animal housed: a possible cause for concern in the welfare and use of laboratory animals. Lab. Anim. 22, 369-375, 1988.

Sales GD, Milligan SR, Khirnykh K: Sources of sound in the laboratory animal environment: a survey of the sounds produced by procedures and equipment. Animal Welfare 8, 97-115, 1999

Scharmann W: Improved housing of mice, rats and guinea pigs: a contribution to the refinement of animal experiments. ATLA 19, 108-114, 1991.

Schlingmann F, Pereboom W, Remie R: The sensitivity of albino and pigmented rats to light. Anim. Tech. 44, 71-85, 1993a.

Schlingmann F, De Rijk SHLM, Pereboom WJ, Remie R: Avoidance as a behavioural parameter in the determination of distress amongst albino and pigmented rats at various light intensities. Anim. Techn. 44, 87-107, 1993b.

Schmitter J: Optimierung der Käfige für Gruppenhaltung von Meerschweinchen. Dissertation, Universität Tübingen, 1989.

Schwentker P: Care and Maintenance of the Mongolian Gerbil, Tumblebrook Farms, 1968.

Seitz K, Hoy S, Lange K: Untersuchungen zum Einfluss verschiedener Faktoren auf das Säugeverhalten bei Hauskaninchen. Berl. Münch. Tierärztl. Wochenschrift 111, 48-52, 1998.

Semple-Rowland SL, Dawson WW: Cyclic light intensity threshold for retinal damage on albino rats raised under 6LX. Exp. Eye Res. 44, 643-661, 1987a.

Semple-Rowland SL, Dawson WW: Retinal cyclic light damage threshold for albino rats. Lab. Anim. Sci. 37(3):289-98, 1987b.

Sharp PE, La Regina MC: The Laboratory Rat. CRC Press LLC, Boca Raton, Florida, USA, 1998.

Shepherdson DJ: Tracing the path of environmental enrichment in zoos. In: Second Nature: environmental enrichment for captive animals, Shepherdson DJ, Mellen JD, Hutchins M (eds.), Smithsonian Institution Press, 1-12, 1998.

Sherwin C M, Nicol CJ: Behavioural demand functions of caged laboratory mice for additional space. Anim. Behav. 53, 67-74, 1997.

Sherwin C.M. Observations on the prevalence of nestbuilding in non-breeding TO strain mice and their use of two nesting materials. Lab. Anim. 31, 125-132, 1997.

Shoji R, Murakhami U, Shimizu T: Influence of low intensity ultrasonic irradiation on prenatal development of two inbred mouse strains. Teratology 12, 227-232, 1975.

Simberkoff MS, Thorbecke GJ, Thomas L: Studies on PPLO infection. Inhibition of lymphocyte mitosis and antibody formation by mycoplasmal extracts. J. Exp. Med. 129, 1163-1181, 1969.

SOAP (Swiss Ordinance on Animal Protection (Schweiz Tierschutzverordnung vom 27. Mai 1981, Änderung vom 23. Oktober 1991, in Kraft seit 1. Dezember 1991). Eidgenössische Daten- und Materialzentrale, Bern, 1991.

Specter SC, Bendinelli M, Ceglowski WS, Friedman, H: Macrophage-induced reversal of immunosuppression by leukemia viruses. Fed. Proc. 37, 97-101, 1978.

Spiegel A, Gönnert R: Neue Käfige für Mäuse und Ratten. Z. Versuchstierkunde 1, 38-46, 1961.

Spinelli JS, Markowitz H: Prevention of cage associated distress. Lab Animal 14, 19-24, 1985.

Stanzel K, Sachser N: The ontogeny of male guinea pigs living under different housing conditions. In: Proceedings of the Congress of the International Society for Applied Ethology 1993, Berlin, 92-96, 1993.

Stauffacher M: Social contacts and relationships in domestic rabbits kept in a restrictive artificial environment. In: Ethology of Domestic Animals (ed. M. Nichelmann). Proc. XIXth Int. Ethol. Congr., IX, Toulouse, Privat, 100-106, 1986a.

Stauffacher M: Steuerung des agonistischen Verhaltens bei der Entwicklung einer tiergerechten Bodenhaltung für Hauskaninchen-Zuchtgruppen. In: Aktuelle Arbeiten zur artgemässen Tierhaltung 1985. KTBL-Schrift 311, Darmstadt, 153-167, 1986b.

Stauffacher M: Maternal behaviour in domestic rabbits and advantages of rearing young rabbits in breeding groups. PhD Thesis, Part IV, University of Berne, Switzerland, 1988.

Stauffacher M: Group housing and enrichment cages for breeding, fattening and laboratory rabbits. Animal Welfare 1, 105-125, 1992.

Stauffacher M: Refinement bei der Haltung von Laborkaninchen. Ein Beitrag zur Umsetzung von Tierschutzforderungen in die Praxis. Der Tierschutzbeauftragte 2/3, 18-33, 1993.

Stauffacher M: Improved husbandry systems - an ethological concept. In: Welfare and science. Bunyan J (ed.). Proceedings of the 5th FELASA Symposium, Brighton 1993, Royal Soc. Med., London, 68-73, 1994.

Stauffacher M: Environmental enrichment, fact and fiction. Scand. J. Lab. Anim. Sci. 22, 39-42, 1995.

Stauffacher M: Housing requirements: What ethology can tell us. In: Animal Alternatives, Welfare and Ethics, van Zutphen LFM, Balls M (eds.). Elsevier Science B.V. Publ., Amsterdam, 179-186, 1997a

Stauffacher M: Comparative studies on housing conditions. In: Harmonization of Laboratory Animal Husbandry, O'Donoghue PN (ed.). Royal Soc. Med. Press., London, 5-9, 1997b

Stauffacher M: 15 Thesen zur Haltungsoptimierung im Zoo. Zoolog. Garten N.F. 68, 201-218, 1998.

Stauffacher M: Refinement in rabbit housing and husbandry. In: Progress in the Reduction, Refinement and Replacement of Animal Experimentation, Balls M, van Zeller AM, Halder M (eds). Developments in Animal and Veterinary Sciences 31, Elsevier Science B.V. Publ., Amsterdam, 1269-1277, 2000.

Stodardt EJ, Myers K: A comparison of behaviour, reproduction and mortality of wild and domestic rabbits in confined populations. CSIRO Wildlife Research 9, 144-159, 1964.

Swartzberg JE, Krahenbuhl JL, Remington JS: Dichotomy between macrophage activation and degree of protection against Listeria monocytogenes and Toxoplasma gondii in mice stimulated with Corynebacterium parvum. Infect. Immun.12, 1037-1043, 1975.

Tattersall P, Cotmore SF: The Rodent Parvoviruses, In: Viral and Mycoplasmal Infections of Laboratory Rodents, Effects on biomedical research, Bhatt PN, Jacoby RO, Morse III HC, New AE (Eds.), Academic Press Inc., Orlando, 1986, chapt. 16.

Tiensiwakul P, Husain SS: Effect of mouse hepatitis virus infection on iron retention in the mouse liver. Br. J. Exp. Pathol. 60, 161-166, 1979.

Toolan HW: Lack of oncogenic effect of the H-viruses. Nature 214, 1036, 1967.

Toolan HW, Ledinko N: Inhibition by H1 virus of the incidence of tumors produced by adenovirus 12 in hamsters.Virology 35, 475-478, 1968.

Toolan HW, Rhode III SL, Gierthy JF: Inhibition of 7,12-Dimethylbenz(a)anthracene-induced tumors in Syrian hamsters by prior infection with H1 parvovirus. Cancer Res. 142, 2552-2555, 1982.

Townsend P. Use of in-cage shelters by laboratory rats. Anim. Welf. 6, 95-103, 1997.

Tsai PP, Hackbarth H: Environmental enrichment in mice: is it suitable for every experiment? Abstracts of Scientific Papers, ICLAS-FELASA Joint Meeting, Palma de Mallorca, 112, 1999.

Turnbull GJ.: The needs of the toxicologist, In Microbiological Standardisation of Laboratory Animals, Roe FJC (ed.)., Ellis Horwood Ltd., Chicester, 1983, chapt. 1.

Turner RJ, Held SD, Hirst JE, Billinghurst G, Wootton RJ: An immunological assessment of group housed rabbits. Lab. Anim. 31, 362-372, 1997.

Van de Weerd HA: Environmental enrichment for laboratory mice: preferences and consequenvces. PhD Thesis, Utrecht University, The Netherlands, 1996.

Van de Weerd HA, Baumans V, Koolhaas JM, van Zutphen LFM: Strainspecific behavioural response to environmental enrichment in the mouse. J. Exp. Anim. Sci. 36, 117-127, 1994.

Van de Weerd HA, Baumans V.: Environmental enrichment in rodents. In: Environmental enrichment information resources for laboratory animals 1965-1995 AWIC Resource series no. 2, AWIC/UFAW, 1995.

Van de Weerd HA, van Loo PLP, van Zutphen LFM, Koolhaas JM, Baumans V: Preferences for nesting material as environmental enrichment for laboratory mice. Lab. Anim. 31, 133-143, 1997a.

Van de Weerd HA, van Loo PLP, van Zutphen LFM, Koolhaas JM, Baumans V: Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol. Behav. 62 1019-1028, 1997b.

Van PLP, Kruitwagen CLJJ, van Zutphen LFM, Koolhaas JM, Baumans V: Modulation of aggression in male Loo mice: influence of cage cleaning regime and scent marks. Animal Welfare 9, 281-295, 2000.

Van Loo PLP, Baumans V: Preference of subordinate male mice for their dominant cage mate. Aktuelle Arbeiten zur Artgemässen Tierhaltung. KTBL-Schrift 380, 45-52,1998.

Van Zutphen LFM, Baumans V, Beynen AC: Principles of Laboratory Animal Science. Elsevier Science Publ., Amsterdam, 1993.

Ventura J, Domaradzki M.: Role of mycoplasma infection in the development of experimental bronchiectasis in the rat. J. Pathol. Bacteriol. 93, 342-348, 1967.

Vesell ES, Lang CM, Passananti GT, Tripp SL: Hepatic Drug Metabolism in Rats: Impairment in a Dirty Environment, Science 179, 896-897, 1973.

Vesell ES, Lang CM, White WJ, Passananti GT, Hill RN, Clemens TL, Liu DK, Johnson WD: Environmental and Genetic Factors Affecting the Response of Laboratory Animals to Drugs, Fed. Proc. 35, 1125-1132, 1976.

Vonderfecht SL, Huber AC, Eiden J, Mader LC, Yolken RH: Infectious diarrhea of infant rats produced by a rotavirus-like agent. J.Virol. 52, 94-98, 1984.

Wainwright PE, Huang YS, BulmanFleming B, Levesque S, Mccutcheon D: The Effects of Dietary Fatty-acid Composition Combined with Environmental Enrichment on Brain and Behavior In Mice. Behav. Brain Res., 60: (2) 125-136, 1994.

Ward GE, Fiat R, De Mille D: Environmental enrichment for laboratory mice. Anim. Techn. 42, 149-156, 1991.

Weihe WH: The effect of light on animals. In McSheehy T (ed). Control of the animal House Environment, 63-76, Laboratory Animals Ltd., London, 1976.

Weihe WH: Richtwerte der Belegungsdichte von Standardkäfigen. Z. Versuchstierkunde 20, 305-309, 1978.

Weiss J, Ernst A, Schick L: Wahlverhalten als Beurteilungskriterim für die Haltungsbedingungen von Laborratten. Z. Versuchstierkunde 24, 193-201, 1982.

Weiss, J, Taylor GT: Einfluss der Käfigstruktur auf das Wahlverhalten und die Spontanmotilität von Laborratten. Z. Versuchstierkunde 27, 175-184, 1985.

Wemelsfelder F: Life in captivity: its lack of opportunities for variable behaviour. Appl. Anim. Behav. Sci. 54, 67-70, 1997

Whary M, Peper R, Borkowski G, Lawrence W, Ferguson F: The effect of group housing on the research use of the laboratory rabbit. Lab. Anim. 27, 330-341, 1993.

Whittaker D: Hamsters. In: UFAW Handbook on the Care and Management of Laboratory Animals. Poole T (ed.). Blackwell Science Ltd., Oxford, vol. 1, 356-366, 1999.

Widman DR, Abrahamson GC, Rosellini RA: Environmental enrichment: the influence of restricted daily exposure and subsequent exposure to uncontrollable stress. Physiol. Behav. 51, 309-318, 1992.

Wieser RV: Funktionale Analyse des Verhaltens als Grundlage zur Beurteilung der Tiergerechtheit. Eine Untersuchung zum Normalverhalten und Verhaltensstörungen von Hauskaninchen-Zibben. PhD Thesis, University of Berne, Switzerland, 1986.

Wiedenmayer C: Effect of cage size on the ontogeny of stereotyped behaviour in gerbils. Appl. Anim. Behav. Sci. 47, 225-233, 1996.

Wiedenmayer C: Causation of the ontogenetic development of stereotypic digging in gerbils. Anim. Behav. 53, 461-470, 1997.

Würbel H: Behaviour and the standardization fallacy. Nature Genetics 26, 263, 2000.

Würbel H, Stauffacher M: Coping with stress is not a general aspect of cage-induced stereotypies: evidence from laboratory mice. Physiol. Behav. 59, 1163-1170, 1996.

Würbel H, Stauffacher M, von Holst D: Stereotypies in laboratory mice - quantitative and qualitative description of the ontogeny of wire-gnawing and jumping in ICR and ICR nu-mice. Ethology 102, 371-385, 1996.

Wullschleger M: Nestbeschäftigung bei säugenden Hauskaninchenzibben. Revue Suisse Zoologie 94, 553-562, 1987.

Wyly MV, Denenberg VH, de Santis D, Burns JK, Zarrow, MX: Handling Rabbits in Infancy: In Search of a Critical Period. Developmental Psychol. 8, 179-186, 1975.

Yirmiya R, Rosen H, Donchin O, Ovadia H: Behavioral effects of lipopolysaccharide in rats: involvement of endogenous opioids. Brain Res., 648(1):80-86, 1994.

Zarrow MX, Denenberg H, Anderson CO: Rabbit: frequency of suckling in the pup. Science 150, 1835-1836, 1965.

Zimmermann A: Tierschutzorientierte Untersuchungen zur Standardhaltung von Laborratten. PhD Thesis, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland, 1999.