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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)

8<sup>th</sup> 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

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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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### **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* 

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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).

<u>Part A</u>. 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].

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The revision of the proposals proceeded preferably via e-mail communication in the Expert Group on Amphibians and Reptiles. On September 10<sup>th</sup> and 11<sup>th</sup> 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 19<sup>th</sup> and November 6<sup>th</sup>, 2003.

At the 6<sup>th</sup> 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 7<sup>th</sup> and 8<sup>th</sup> 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 8<sup>th</sup> 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. <u>Examining results already available and practical experience acquired which could answer these questions:</u>

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. <u>Providing information in particular to the ethological and physiological needs of reptiles:</u>

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



Cladogram

modified after Hennig and other authors



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.

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

Table J.1. Two habitat categories and examples of reptile species of each habitat frequently used

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 <i>Testudo graeca</i>	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:

-	Amphibians	Reptiles
Research Discipline	Publications 1995-2000 %	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. <u>Temperature</u>

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 speciestypical environment. Therefore, the design of their artificial habitats demands special care, since researchbiasing 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, energyefficient 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. <u>Noise</u>

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. <u>Alarm systems (See also paragraph 2.6. of the General Section)</u>

# 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. <u>Reproduction</u>

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, *XVIII<sup>th</sup> (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 Ychromosome 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. <u>Social housing</u> (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.

Body length <sup>1)</sup>	Minimum water surface area	Minimum water surface area for each additional animal in	Minimum water depth
(cm)	(am <sup>2</sup> )	(cm²)	(cm)
_	(CM <sup>-</sup> )	(00	10
= 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

Table J.2. Aquatic chelonians, e.g., Trachemys spp.: Minimum enclosure dimensions and space allowances

#### 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.

Body length <sup>1)</sup>	Minimum floor area	Minimum area for each additional animal in group holding	Minimum cage height
(cm)	(cm <sup>2</sup> )	(cm <sup>2</sup> )	(cm)
$\leq 10$	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

Minimum space requirements for semi-terrestrial and terrestrial chelonians

<sup>1)</sup> measured in a straight line from the front edge to the back edge of the shell

Minimum space requirements for terrestrial lizards

Body length <sup>1)</sup>	Minimum floor	Minimum	Minimum	Minimum cage
	area	area for each immature <sup>2)</sup>	area for each mature <sup>3)</sup>	height <sup>4)</sup>
		additional animal in group	additional animal in group	
		holding	holding	
		$(cm^2)$	$(cm^2)$	
(cm)	$(cm^2)$			(cm)
$\leq 10$	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

<sup>1)</sup> measured from snout to tail; if tail is broken (autotomy) or regenerated, the original length of the tail will be approximated

<sup>2)</sup> a lizard is considered to be immature until it reaches half the length of the adult animal, and before it shows signs of sexrelated rivalry and aggressiveness against conspecifics

<sup>3)</sup> 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

<sup>4)</sup> 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

Body length <sup>1)</sup>	Minimum floor area	Minimum area for each additional animal in group-bolding	Minimum enclosure height <sup>2)</sup>
(cm)	$(cm^2)$	(cm <sup>2</sup> )	(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

Table J.3. Terrestrial snakes, e.g. Thamnophis spp: Minimum enclosure dimensions and space allowances

<sup>1)</sup> measured from snout to tail <sup>2)</sup> measured from the surface

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 <sup>1)</sup>	Minimum floor area	Minimum area for each immature <sup>2)</sup> additional animal in group	Minimum area for each mature <sup>3)</sup> animal in group holding	Minimum cage height <sup>4)</sup>
	(cm <sup>2</sup> )	holding (cm <sup>2</sup> )	(cm <sup>2</sup> )	
(cm)				(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

<sup>1)</sup> measured from snout to tail; if tail is broken (autotomy) or regenerated, the original length of the tail will be approximated

<sup>2)</sup> 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

<sup>3)</sup> 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

<sup>4)</sup> 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	Food items	Calcium	Phosphorus
Crickets	1	4	bananas	1	4
Mealworms	1	8	grapes	1	2
Waxworms	1	13	mushrooms	1	7
Meat	1	16	peas	1	4
beef heart	1	38	collard greens	3	1
Adult mice	1.5	1	kale	3	1
Pinkies	1.1	1	mustard greens	3.5	1
Fuzies	1	2	turnip greens	7	1
Apples	1	2			

A calcium supplement can be used to correct this imbalance.

#### 4.4.1. <u>Turtles</u>

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 = $\frac{1}{2}$ 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	Green vegetables	Other vegetables	Fruits
Kale	frozen mixed vegs.	apples	dandelions	beans	strawberries
mustard greens Turnip greens collard greens	broccoli carrots peas	peaches grapes melons	parsley spinach romaine lettuce	zucchini mushrooms cauliflower	bananas blueberries 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. <u>Snakes</u>

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*).

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. <u>Watering</u>

#### 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. <u>Cleaning</u> (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., intermale, 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. <u>Humane killing (See also paragraph 4.11. of the General section)</u>

# 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. <u>Records</u> (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<sup>1</sup>)

<sup>1</sup>) Source: Swedish Board of Agriculture, Department of Animal Production and Health, Animal Welfare Division

Sn	akes	
Α,	Biotope	1 - in trees 2 - on ground
Β,	Flooring: sand and water	<ol> <li>1 - sand as floor covering</li> <li>2 - water (pool)</li> <li>3 - drinking water, dropping or spraying</li> </ol>
<b>C</b> ,	General temperature during the day	<b>1</b> - cool (+20 to 24°C) <b>2</b> - middle warm (+24 to 27°C) <b>3</b> - warm (+27 to 30°C)
D,	Atmospheric humidity	<b>1</b> - low (< 50% RH) <b>2</b> - middle (50 to 70% RH) <b>3</b> - 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	Α	В	С	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	2	3	2-3	2-3
purpureomaculatus				
Trimeresurus stejnegeri	1	3	2-3	2-3
Trimeresurus trigonocephalus	1	2	2-3	2-3
Trimeresurus wagleri	1	3	3	3

Liz	Lizards						
<b>A</b> ,	Biotope	1 - in trees 2 - on rock 3 - on ground					
Β,	Flooring: sand and water	<ol> <li>1 - sand as floor covering</li> <li>2 - water (pool)</li> <li>3 - drinking water, dropping or spraying</li> </ol>					
<b>C</b> ,	General temperature during the day	<b>1</b> - cool (+20 to 24°C) <b>2</b> - middle warm (+24 to 27°C) <b>3</b> - warm (+27 to 31°C)					
D,	Atmospheric humidity	<b>1</b> - low (< 50% RH) <b>2</b> - middle (50 to 70% RH) <b>3</b> - 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	Α	В	С	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	3		2	2
algeriensis				
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 quentheri	2		3	2
	3		5	5

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)

Α,	Biotope	1 - land 2 - swamp 3 - water
Β,	Facility for turtles	1 - possibility to dig in the soft ground

Species	Α	В
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

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