Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Text with EEA relevance)

ANNEX I

LIST OF ANIMALS REFERRED TO IN ARTICLE 10

1. Mouse (*Mus musculus*)
2. Rat (*Rattus norvegicus*)
3. Guinea pig (*Cavia porcellus*)
4. Syrian (golden) hamster (*Mesocricetus auratus*)
5. Chinese hamster (*Cricetulus griseus*)
6. Mongolian gerbil (*Meriones unguiculatus*)
7. Rabbit (*Oryctolagus cuniculus*)
8. Dog (*Canis familiaris*)
9. Cat (*Felis catus*)
10. All species of non-human primates
11. Frog (*Xenopus* (*laevis*, *tropicalis*), Rana (*temporaria*, *pipiens*))
12. Zebra fish (*Danio rerio*)

ANNEX II

LIST OF NON-HUMAN PRIMATES AND DATES REFERRED TO IN THE SECOND SUBPARAGRAPH OF ARTICLE 10(1)

Species	**Dates**
Marmoset (*Callithrix jacchus*)	1 January 2013
Cynomolgus monkey (*Macaca fascicularis*)	5 years after the publication of the feasibility study referred to in Article 10(1), fourth subparagraph, provided the study does not recommend an extended period
Rhesus monkey (*Macaca mulatta*)	5 years after the publication of the feasibility study referred to in Article 10(1), fourth subparagraph, provided the study does not recommend an extended period
Other species of non-human primates	5 years after the publication of the feasibility study referred to in Article 10(1), fourth subparagraph, provided the study does not recommend an extended period

ANNEX III

REQUIREMENTS FOR ESTABLISHMENTS AND FOR THE CARE AND ACCOMMODATION OF ANIMALS

Section A:

General section

1. The physical facilities

1.1. Functions and general design

(a) All facilities shall be constructed so as to provide an environment which takes into account the physiological and ethological needs of the species kept in them. Facilities shall also be designed and managed to prevent access by unauthorised persons and the ingress or escape of animals.

(b) Establishments shall have an active maintenance programme to prevent and remedy any defect in buildings or equipment.

1.2. Holding rooms

(a) Establishments shall have a regular and efficient cleaning schedule for the rooms and shall maintain satisfactory hygienic standards.

(b) Walls and floors shall be surfaced with a material resistant to the heavy wear and tear caused by the animals and the cleaning process. The material shall not be detrimental to the health of the animals and shall be such that the animals cannot hurt themselves. Additional protection shall be given to any equipment or fixtures so that they are not damaged by the animals nor do they cause injury to the animals themselves.

(c) Species that are incompatible, for example predator and prey, or animals requiring different environmental conditions, shall not be housed in the same room nor, in the case of predator and prey, within sight, smell or sound of each other.

1.3. General and special purpose procedure rooms

(a) Establishments shall, where appropriate, have available laboratory facilities for the carrying out of simple diagnostic tests, post-mortem examinations, and/or the collection of samples that are to be subjected to more extensive laboratory investigations elsewhere. General and special purpose procedure rooms shall be available for situations where it is undesirable to carry out the procedures or observations in the holding rooms.

(b) Facilities shall be provided to enable newly-acquired animals to be isolated until their health status can be determined and the potential health risk to established animals assessed and minimised.

(c) There shall be accommodation for the separate housing of sick or injured animals.

1.4. Service rooms

(a) Store-rooms shall be designed, used and maintained to safeguard the quality of food and bedding. These rooms shall be vermin and insect-proof, as far as possible. Other

materials, which may be contaminated or present a hazard to animals or staff, shall be stored separately.

(b) The cleaning and washing areas shall be large enough to accommodate the installations necessary to decontaminate and clean used equipment. The cleaning process shall be arranged so as to separate the flow of clean and dirty equipment to prevent the contamination of newly-cleaned equipment.

(c) Establishments shall provide for the hygienic storage and safe disposal of carcasses and animal waste.

(d) Where surgical procedures under aseptic conditions are required there shall be provision for one or more than one suitably equipped room, and facilities provided for postoperative recovery.

2. The environment and control thereof

2.1. Ventilation and temperature

(a) Insulation, heating and ventilation of the holding room shall ensure that the air circulation, dust levels, and gas concentrations are kept within limits that are not harmful to the animals housed.

(b) Temperature and relative humidity in the holding rooms shall be adapted to the species and age groups housed. The temperature shall be measured and logged on a daily basis.

(c) Animals shall not be restricted to outdoor areas under climatic conditions which may cause them distress.

2.2. Lighting

(a) Where natural light does not provide an appropriate light/dark cycle, controlled lighting shall be provided to satisfy the biological requirements of the animals and to provide a satisfactory working environment.

(b) Illumination shall satisfy the needs for the performance of husbandry procedures and inspection of the animals.

(c) Regular photoperiods and intensity of light adapted to the species shall be provided.

(d) When keeping albino animals, the lighting shall be adjusted to take into account their sensitivity to light.

2.3. Noise

(a) Noise levels including ultrasound, shall not adversely affect animal welfare.

(b) Establishments shall have alarm systems that sound outside the sensitive hearing range of the animals, where this does not conflict with their audibility to human beings.

(c) Holding rooms shall where appropriate be provided with noise insulation and absorption materials.

2.4. Alarm systems

(a) Establishments relying on electrical or mechanical equipment for environmental control and protection, shall have a stand-by system to maintain essential services and emergency lighting systems as well as to ensure that alarm systems themselves do not fail to operate.

(b) Heating and ventilation systems shall be equipped with monitoring devices and alarms.

(c) Clear instructions on emergency procedures shall be prominently displayed.

3. Care of animals

3.1. Health

(a) Establishments shall have a strategy in place to ensure that a health status of the animals is maintained that safeguards animal welfare and meets scientific requirements. This strategy shall include regular health monitoring, a microbiological surveillance programme and plans for dealing with health breakdowns and shall define health parameters and procedures for the introduction of new animals.

(b) Animals shall be checked at least daily by a competent person. These checks shall ensure that all sick or injured animals are identified and appropriate action is taken.

3.2. Animals taken from the wild

(a) Transport containers and means of transport adapted to the species concerned shall be available at capture sites, in case animals need to be moved for examination or treatment.

(b) Special consideration shall be given and appropriate measures taken for the acclimatisation, quarantine, housing, husbandry, care of animals taken from the wild and, as appropriate, provisions for setting them free at the end of procedures.

3.3. Housing and enrichment

(a) Housing

Animals, except those which are naturally solitary, shall be socially housed in stable groups of compatible individuals. In cases where single housing is allowed in accordance with article 33(3) the duration shall be limited to the minimum period necessary and visual, auditory, olfactory and/or tactile contact shall be maintained. The introduction or re-introduction of animals to established groups shall be carefully monitored to avoid problems of incompatibility and disrupted social relationships.

(b) Enrichment

All animals shall be provided with space of sufficient complexity to allow expression of a wide range of normal behaviour. They shall be given a degree of control and choice over their environment to reduce stress-induced behaviour. Establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species. Environmental enrichment in animal enclosures shall be adapted to the species and individual needs of the animals concerned. The enrichment strategies in establishments shall be regularly reviewed and updated.

(c) Animal enclosures

Animal enclosures shall not be made out of materials detrimental to the health of the animals. Their design and construction shall be such that no injury to the animals is caused. Unless they are disposable, they shall be made from materials that will withstand cleaning and decontamination techniques. The design of animal enclosure floors shall be adapted to the species and age of the animals and be designed to facilitate the removal of excreta.

3.4. Feeding

(a) The form, content and presentation of the diet shall meet the nutritional and behavioural needs of the animal.

(b) The animals' diet shall be palatable and non-contaminated. In the selection of raw materials, production, preparation and presentation of feed, establishments shall take measures to minimise chemical, physical and microbiological contamination.

(c) Packing, transport and storage shall be such as to avoid contamination, deterioration or destruction. All feed hoppers, troughs or other utensils used for feeding shall be regularly cleaned and, if necessary, sterilised.

(d) Each animal shall be able to access the food, with sufficient feeding space provided to limit competition.

3.5. Watering

(a) Uncontaminated drinking water shall always be available to all animals.

(b) When automatic watering systems are used, they shall be regularly checked, serviced and flushed to avoid accidents. If solid-bottomed cages are used, care shall be taken to minimise the risk of flooding.

(c) Provision shall be made to adapt the water supply for aquaria and tanks to the needs and tolerance limits of the individual fish, amphibian and reptile species.

3.6. Resting and sleeping areas

(a) Bedding materials or sleeping structures adapted to the species shall always be provided, including nesting materials or structures for breeding animals.

(b) Within the animal enclosure, as appropriate to the species, a solid, comfortable resting area for all animals shall be provided. All sleeping areas shall be kept clean and dry.

3.7. Handling

Establishments shall set up habituation and training programmes suitable for the animals, the procedures and length of the project.

Section B:

Species-specific section

1. Mice, rats, gerbils, hamsters and guinea pigs

In this and subsequent tables for mice, rats, gerbils, hamsters and guinea pigs, 'enclosure height' means the vertical distance between the enclosure floor and the top of the enclosure and this height applies over more than 50 % of the minimum enclosure floor area prior to the addition of enrichment devices.

When designing procedures, consideration shall be given to the potential growth of the animals to ensure adequate space is provided (as detailed in Tables 1.1 to 1.5) for the duration of the study.

Table 1.1. **Mice**

	Body weight(g)	**Minimum enclosure size(cm^2)**	**Floor area per animal(cm^2)**	**Minimum enclosure height(cm)**	**Date referred to in Article 33(2)**
In stock and during procedures	up to 20	330	60	12	1 January 2017
	over 20 to 25	330	70	12	
	over 25 to 30	330	80	12	
	over 30	330	100	12	
Breeding		330 For a monogamous pair (outbred/ inbred) or a trio (inbred). For each additional female plus litter 180 cm^2 shall be added.		12	
Stock at breeders[a]Enclosure size950 cm^2	less than 20	950	40	12	
Enclosure size1 500 cm^2	less than 20	1 500	30	12	

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

Table 1.2. **Rats**

	Body weight(g)	**Minimum enclosure size(cm^2)**	**Floor area per animal(cm^2)**	**Minimum enclosure height(cm)**	**Date referred to in Article 33(2)**
In stock and during procedures[a]	up to 200	800	200	18	1 January 2017
	over 200 to 300	800	250	18	

a In long-term studies, if space allowances per individual animal fall below those indicated above towards the end of such studies, priority shall be given to maintaining stable social structures.

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

	over 300 to 400	800	350	18
	over 400 to 600	800	450	18
	over 600	1 500	600	18
Breeding		800 Mother and litter. For each additional adult animal permanently added to the enclosure add 400 cm^2		18
Stock at breeders[b]Enclosure size1 500 cm^2	up to 50	1 500	100	18
	over 50 to 100	1 500	125	18
	over 100 to 150	1 500	150	18
	over 150 to 200	1 500	175	18
Stock at breeders[b]Enclosure size2 500 cm^2	up to 100	2 500	100	18
	over 100 to 150	2 500	125	18
	over 150 to 200	2 500	150	18

a In long-term studies, if space allowances per individual animal fall below those indicated above towards the end of such studies, priority shall be given to maintaining stable social structures.

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

Table 1.3. **Gerbils**

	Body weight(g)	**Minimum enclosure size(cm^2)**	**Floor area per animal(cm^2)**	**Minimum enclosure height(cm)**	**Date referred to in Article 33(2)**
In stock and during procedures	up to 40	1 200	150	18	1 January 2017
	over 40	1 200	250	18	
Breeding		1 200		18	

		Monogamous pair or trio with offspring			

Table 1.4. **Hamsters**

	Body weight(g)	**Minimum enclosure size(cm^2)**	**Floor area per animal(cm^2)**	**Minimum enclosure height(cm)**	**Date referred to in Article 33(2)**
In stock and during procedures	up to 60	800	150	14	1 January 2017
	over 60 to 100	800	200	14	
	over 100	800	250	14	
Breeding		800 Mother or monogamous pair with litter		14	
Stock at breeders[a]	less than 60	1 500	100	14	

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

Table 1.5. **Guinea pigs**

	Body weight(g)	**Minimum enclosure size(cm^2)**	**Floor area per animal(cm^2)**	**Minimum enclosure height(cm)**	**Date referred to in Article 33(2)**
In stock and during procedures	up to 200	1 800	200	23	1 January 2017
	over 200 to 300	1 800	350	23	
	over 300 to 450	1 800	500	23	
	over 450 to 700	2 500	700	23	
	over 700	2 500	900	23	
Breeding		2 500 Pair with litter. For each		23	

		additional breeding female add 1 000 cm^2			

2. Rabbits

During agricultural research, when the aim of the project requires that the animals are kept under similar conditions to those under which commercial farm animals are kept, the keeping of the animals shall at least follow the standards laid down in Directive 98/58/EC[(1)].

A raised area shall be provided within the enclosure. This raised area must allow the animal to lie and sit and easily move underneath, and shall not cover more than 40 % of the floor space. When for scientific or veterinary reasons a raised area cannot be used, the enclosure shall be 33 % larger for a single rabbit and 60 % larger for two rabbits. Where a raised area is provided for rabbits of less than 10 weeks of age, the size of the raised area shall be at least of 55 cm by 25 cm and the height above the floor shall be such that the animals can make use of it.

Table 2.1. **Rabbits over 10 weeks of age**

Table 2.1 is to be used for both cages and pens. The additional floor area is as a minimum 3 000 cm^2 per rabbit for the third, the fourth, the fifth and the sixth rabbit, while 2 500 cm^2 as a minimum shall be added for each additional rabbit above a number of six.

Final body weight(kg)	**Minimum floor area for one or two socially harmonious animals(cm^2)**	**Minimum height(cm)**	**Date referred to in Article 33(2)**
less than 3	3 500	45	1 January 2017
from 3 to 5	4 200	45	
over 5	5 400	60	

Table 2.2. **Doe plus litter**

Doe weight(kg)	**Minimum enclosure size(cm^2)**	**Addition for nest boxes(cm^2)**	**Minimum height(cm)**	**Date referred to in Article 33(2)**
less than 3	3 500	1 000	45	1 January 2017
from 3 to 5	4 200	1 200	45	
over 5	5 400	1 400	60	

Table 2.3. **Rabbits less than 10 weeks of age**

Table 2.3 is to be used for both cages and pens.

Age	Minimum enclosure size(cm^2)	Minimum floor area per animal(cm^2)	Minimum height(cm)	Date referred to in Article 33(2)
Weaning to 7 weeks	4 000	800	40	1 January 2017
From 7 to 10 weeks	4 000	1 200	40	

Table 2.4. **Rabbits: Optimal dimensions for raised areas for enclosures having the dimensions indicated in Table 2.1.**

Age in weeks	Final body weight(kg)	Optimum size(cm x cm)	Optimum height from the enclosure floor(cm)	Date referred to in Article 33(2)
over 10	less than 3	55 × 25	25	1 January 2017
	from 3 to 5	55 × 30	25	
	over 5	60 × 35	30	

3. Cats

Cats shall not be single-housed for more than 24 hours at a time. Cats that are repeatedly aggressive towards other cats shall be housed singly only if a compatible companion cannot be found. Social stress in all pair- or group-housed individuals shall be monitored at least weekly. Females with kittens under four weeks of age or in the last two weeks of pregnancy may be housed singly.

Table 3. **Cats**

The minimum space in which a queen and litter may be held is the space for a single cat, which shall be gradually increased so that by 4 months of age litters have been rehoused following the space requirements for adults.

Areas for feeding and for litter trays shall not be less than 0,5 metres apart and shall not be interchanged.

	Floor[a](m^2)	Shelves(m^2)	Height(m)	Date referred to in Article 33(2)
Minimum for one adult animal	1,5	0,5	2	1 January 2017
For each additional animal add	0,75	0,25	—	

a Floor area excluding shelves.

4. Dogs

Dogs shall where possible be provided with outside runs. Dogs shall not be single-housed for more than 4 hours at a time.

The internal enclosure shall represent at least 50 % of the minimum space to be made available to the dogs, as detailed in Table 4.1.

The space allowances detailed below are based on the requirements of beagles, but giant breeds such as St Bernards or Irish wolfhounds shall be provided with allowances significantly in excess of those detailed in Table 4.1. For breeds other than the laboratory beagle, space allowances shall be determined in consultation with veterinary staff.

Table 4.1. **Dogs**

Dogs that are pair or group housed may each be constrained to half the total space provided (2 m^2 for a dog under 20 kg, 4 m^2 for a dog over 20 kg) while they are undergoing procedures as defined in this Directive, if this separation is essential for scientific purposes. The period for which a dog is so constrained shall not exceed 4 hours at a time.

A nursing bitch and litter shall have the same space allowance as a single bitch of equivalent weight. The whelping pen shall be designed so that the bitch can move to an additional compartment or raised area away from the puppies.

Weight(kg)	**Minimum enclosure size(m^2)**	**Minimum floor area for one or two animals(m^2)**	**For each additional animal add a minimum of(m^2)**	**Minimum height(m)**	**Date referred to in Article 33(2)**
up to 20	4	4	2	2	1 January 2017
over 20	8	8	4	2	

Table 4.2. **Dogs — post-weaned stock**

Weight of dog(kg)	**Minimum enclosure size(m^2)**	**Minimum floor area/ animal(m^2)**	**Minimum height(m)**	**Date referred to in Article 33(2)**
up to 5	4	0,5	2	1 January 2017
over 5 to 10	4	1,0	2	
over 10 to 15	4	1,5	2	
over 15 to 20	4	2	2	
over 20	8	4	2	

5. Ferrets

Table 5. **Ferrets**

	Minimum enclosure size(cm^2)	**Minimum floor area per animal(cm^2)**	**Minimum height(cm)**	**Date referred to in Article 33(2)**
Animals up to 600 g	4 500	1 500	50	1 January 2017
Animals over 600 g	4 500	3 000	50	
Adult males	6 000	6 000	50	
Jill and litter	5 400	5 400	50	

6. Non-human primates

Young non-human primates shall not be separated from their mothers until they are, depending on the species, 6 to 12 months old.

The environment shall enable non-human primates to carry out a complex daily programme of activity. The enclosure shall allow non-human primates to adopt as wide a behavioural repertoire as possible, provide it with a sense of security, and a suitably complex environment to allow the animal to run, walk, climb and jump.

Table 6.1. **Marmosets and tamarins**

	Minimum floor area of enclosures for 1[a] or 2 animals plus offspring up to 5 months old(m^2)	**Minimum volume per additional animal over 5 months(m^3)**	**Minimum enclosure height(m)[b]**	**Date referred to in Article 33(2)**
Marmosets	0,5	0,2	1,5	1 January 2017
Tamarins	1,5	0,2	1,5	

a Animals shall be kept singly only in exceptional circumstances.

b The top of the enclosure shall be at least 1,8 m from the floor.

For marmosets and tamarins, separation from the mother shall not take place before 8 months of age.

Table 6.2. **Squirrel monkeys**

Minimum floor area for 1[a] or 2 animals(m^2)	**Minimum volume per additional animal over 6 months of age(m^3)**	**Minimum enclosure height(m)**	**Date referred to in Article 33(2)**
2,0	0,5	1,8	1 January 2017

a Animals shall be kept singly only in exceptional circumstances.

For squirrel monkeys, separation from the mother shall not take place before 6 months of age.

TABLE 6.3.

Macaques and vervets[0]

	Minimum enclosure size(m^2)	**Minimum enclosure volume(m^3)**	**Minimum volume per animal(m^3)**	**Minimum enclosure height(m)**	**Date referred to in Article 33(2)**
Animals less than 3 yrs of age[b]	2,0	3,6	1,0	1,8	1 January 2017
Animals from 3 yrs of age[c]	2,0	3,6	1,8	1,8	
Animals held for breeding purposes[d]			3,5	2,0	

a	Animals shall be kept singly only in exceptional circumstances.
b	An enclosure of minimum dimensions may hold up to three animals.
c	An enclosure of minimum dimensions may hold up to two animals.
d	In breeding colonies no additional space/volume allowance is required for young animals up to 2 years of age housed with their mother.

For macaques and vervets, separation from the mother shall not take place before 8 months of age.

TABLE 6.4.

Baboons[0]

	Minimum enclosure size(m^2)	**Minimum enclosure volume(m^3)**	**Minimum volume per animal(m^3)**	**Minimum enclosure height(m)**	**Date referred to in Article 33(2)**
Animals less than 4 yrs of age[b]	4,0	7,2	3,0	1,8	1 January 2017
Animals from 4 yrs of age[b]	7,0	12,6	6,0	1,8	
Animals held for breeding purposes[c]			12,0	2,0	

a	Animals shall be kept singly only in exceptional circumstances.
b	An enclosure of minimum dimensions may hold up to 2 animals.
c	In breeding colonies no additional space/volume allowance is required for young animals up to 2 years of age housed with their mothers.

For baboons, separation from the mother shall not take place before 8 months of age.

7. Farm animals

During agricultural research, when the aim of the project requires that the animals are kept under similar conditions to those under which commercial farm animals are kept, the keeping of the animals shall comply at least with the standards laid down in Directives 98/58/EC, 91/629/EEC[(2)] and 91/630/EEC[(3)].

Table 7.1. **Cattle**

Body weight(kg)	**Minimum enclosure size(m^2)**	**Minimum floor area/ animal(m^2/ animal)**	**Trough space for ad-libitum feeding of polled cattle(m/ animal)**	**Trough space for restricted feeding of polled cattle(m/ animal)**	**Date referred to in Article 33(2)**
up to 100	2,5	2,3	0,1	0,3	1 January 2017
over 100 to 200	4,25	3,4	0,15	0,5	
over 200 to 400	6,0	4,8	0,18	0,6	
over 400 to 600	9,0	7,5	0,21	0,7	
over 600 to 800	11,0	8,75	0,24	0,8	
over 800	16,0	10,0	0,3	1,0	

Table 7.2. **Sheep and goats**

Body weight(kg)	**Minimum enclosure size(m^2)**	**Minimum floor area/ animal(m^2/ animal)**	**Minimum partition height(m)**	**Trough space for ad-libitum feeding(m/ animal)**	**Trough space for restricted feeding(m/ animal)**	**Date referred to in Article 33(2)**
less than 20	1,0	0,7	1,0	0,1	0,25	1 January 2017
over 20 to 35	1,5	1,0	1,2	0,1	0,3	
over 35 to 60	2,0	1,5	1,2	0,12	0,4	
over 60	3,0	1,8	1,5	0,12	0,5	

Table 7.3. **Pigs and minipigs**

Live weight(kg)	Minimum enclosure size[a](m^2)	Minimum floor area per animal(m^2/animal)	Minimum lying space per animal (in, thermoneutral conditions) (m^2/animal)	Date referred to in Article 33(2)
Up to 5	2,0	0,2	0,1	1 January 2017
over 5 to 10	2,0	0,25	0,11	
over 10 to 20	2,0	0,35	0,18	
over 20 to 30	2,0	0,5	0,24	
over 30 to 50	2,0	0,7	0,33	
over 50 to 70	3,0	0,8	0,41	
over 70 to 100	3,0	1,0	0,53	
over 100 to 150	4,0	1,35	0,7	
over 150	5,0	2,5	0,95	
Adult (conventional) boars	7,5		1,3	

a Pigs may be confined in smaller enclosures for short periods of time, for example by partitioning the main enclosure using dividers, when justified on veterinary or experimental grounds, for example where individual food consumption is required.

Table 7.4. **Equines**

The shortest side shall be a minimum of 1,5 times the wither height of the animal. The height of indoor enclosures shall allow animals to rear to their full height.

Wither height(m)	Minimum floor area/animal(m^2/animal)			Minimum enclosure height(m)	Date referred to in Article 33(2)
	For each animal held singly or in groups of up to 3 animals	For each animal held in groups of 4 or more animals	Foaling box/mare with foal		
1,00 to 1,40	9,0	6,0	16	3,0	1 January 2017
over 1,40 to 1,60	12,0	9,0	20	3,0	
over 1,60	16,0	$(2 \times WH)^{2a}$	20	3,0	

a To ensure adequate space is provided, space allowances for each individual animal shall be based on height to withers (WH).

8. Birds

During agricultural research, when the aim of the project requires that the animals are kept under similar conditions to those under which commercial farm animals are kept, the keeping of the animals shall comply at least with the standards laid down in Directives 98/58/EC, 1999/74/EC[(4)] and 2007/43/EC[(5)].

Table 8.1. **Domestic fowl**

Where these minimum enclosure sizes cannot be provided for scientific reasons, the duration of the confinement shall be justified by the experimenter in consultation with veterinary staff. In such circumstances, birds can be housed in smaller enclosures containing appropriate enrichment and with a minimum floor area of 0,75 m^2.

Body mass(g)	**Minimum enclosure size(m^2)**	**Minimum area per bird(m^2)**	**Minimum height(cm)**	**Minimum length of feed trough per bird(cm)**	**Date referred to in Article 33(2)**
Up to 200	1,0	0,025	30	3	1 January 2017
over 200 to 300	1,0	0,03	30	3	
over 300 to 600	1,0	0,05	40	7	
over 600 to 1 200	2,0	0,09	50	15	
over 1 200 to 1 800	2,0	0,11	75	15	
over 1 800 to 2 400	2,0	0,13	75	15	
over 2 400	2,0	0,21	75	15	

Table 8.2. **Domestic turkeys**

All enclosure sides shall be at least 1,5 m long. Where these minimum enclosures sizes cannot be provided for scientific reasons, the duration of the confinement shall be justified by the experimenter in consultation with veterinary staff. In such circumstances, birds can be housed in smaller enclosures containing appropriate enrichment and with a minimum floor area of 0,75 m^2 and a minimum height of 50 cm for birds below 0,6 kg, 75 cm for birds below 4 kg, and 100 cm for birds over 4 kg. These can be used to house small groups of birds in accordance with the space allowances given in table 8.2.

Body mass(kg)	**Minimum enclosure size(m^2)**	**Minimum area per bird(m^2)**	**Minimum height(cm)**	**Minimum length of feed**	**Date referred to in Article 33(2)**

				trough per bird(cm)	
Up to 0,3	2,0	0,13	50	3	1 January 2017
over 0,3 to 0,6	2,0	0,17	50	7	
over 0,6 to 1	2,0	0,3	100	15	
over 1 to 4	2,0	0,35	100	15	
over 4 to 8	2,0	0,4	100	15	
over 8 to 12	2,0	0,5	150	20	
over 12 to 16	2,0	0,55	150	20	
over 16 to 20	2,0	0,6	150	20	
over 20	3,0	1,0	150	20	

Table 8.3. **Quails**

Body mass(g)	**Minimum enclosure size(m^2)**	**Area per bird pair-housed(m^2)**	**Area per additional bird group-housed(m^2)**	**Minimum height(cm)**	**Minimum length of trough per bird(cm)**	**Date referred to in Article 33(2)**
Up to 150	1,0	0,5	0,1	20	4	1 January 2017
Over 150	1,0	0,6	0,15	30	4	

Table 8.4. **Ducks and geese**

Where these minimum enclosures sizes cannot be provided for scientific reasons, the duration of the confinement shall be justified by the experimenter in consultation with veterinary staff. In such circumstances, birds can be housed in smaller enclosures containing appropriate enrichment and with a minimum floor area of 0,75 m^2. These can be used to house small groups of birds in accordance with the space allowances given in table 8.4.

Body mass(g)	**Minimum enclosure size(m^2)**	**Area per bird(m^2)[a]**	**Minimum height(cm)**	**Minimum length of feed trough per bird(cm)**	**Date referred to in Article 33(2)**
Ducks					1 January 2017
Up to 300	2,0	0,1	50	10	

a This shall include a pond of minimum area 0,5 m^2 per 2 m^2 enclosure with a minimum depth of 30 cm. The pond may contribute up to 50 % of the minimum enclosure size.

b Pre-fledged birds may be held in enclosures with a minimum height of 75 cm.

Over 300 to 1 200[b]	2,0	0,2	200	10
Over 1 200 to 3 500	2,0	0,25	200	15
Over 3 500	2,0	0,5	200	15
Geese				
Up to 500	2,0	0,2	200	10
Over 500 to 2 000	2,0	0,33	200	15
Over 2 000	2,0	0,5	200	15

a This shall include a pond of minimum area 0,5 m^2 per 2 m^2 enclosure with a minimum depth of 30 cm. The pond may contribute up to 50 % of the minimum enclosure size.

b Pre-fledged birds may be held in enclosures with a minimum height of 75 cm.

TABLE 8.5.

Ducks and geese: Minimum pond sizes[0]

	Area(m^2)	**Depth(cm)**
Ducks	0,5	30
Geese	0,5	from 10 to 30

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

Table 8.6. **Pigeons**

Enclosures shall be long and narrow (for example 2 m by 1 m) rather than square to allow birds to perform short flights.

Group size	**Minimum enclosure size(m^2)**	**Minimum height(cm)**	**Minimum length of food trough per bird(cm)**	**Minimum length of perch per bird(cm)**	**Date referred to in Article 33(2)**
Up to 6	2	200	5	30	1 January 2017
from 7 to 12	3	200	5	30	
for each additional bird above 12	0,15		5	30	

Table 8.7. **Zebra finches**

Enclosures shall be long and narrow (for example 2 m by 1 m) to enable birds to perform short flights. For breeding studies, pairs may be housed in smaller enclosures containing appropriate enrichment with a minimum floor area of 0,5 m^2 and a minimum height of 40 cm. The duration of the confinement shall be justified by the experimenter in consultation with veterinary staff.

Group size	**Minimum enclosure size(m^2)**	**Minimum height(cm)**	**Minimum number of feeders**	**Date referred to in Article 33(2)**
Up to 6	1,0	100	2	1 January 2017
7 to 12	1,5	200	2	
13 to 20	2,0	200	3	
for each additional bird above 20	0,05		1 per 6 birds	

9. Amphibians

Table 9.1. **Aquatic urodeles**

Body length[a](cm)	**Minimum water surface area(cm^2)**	**Minimum water surface area for each additional animal in group-holding(cm^2)**	**Minimum water depth(cm)**	**Date referred to in Article 33(2)**
Up to 10	262,5	50	13	1 January 2017
over 10 to 15	525	110	13	
over 15 to 20	875	200	15	
over 20 to 30	1 837,5	440	15	
Over 30	3 150	800	20	

a Measured from snout to vent.

TABLE 9.2.

Aquatic anurans[0]

Body length[b](cm)	**Minimum water surface area(cm^2)**	**Minimum water surface area for each**	**Minimum water depth(cm)**	**Date referred to in Article 33(2)**

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

b Measured from snout to vent.

		additional animal in group-holding(cm^2)		
Less than 6	160	40	6	1 January 2017
from 6 to 9	300	75	8	
over 9 to 12	600	150	10	
over 12	920	230	12,5	

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

b Measured from snout to vent.

Table 9.3. **Semi-aquatic anurans**

Body length[a](cm)	**Minimum enclosure size[b](cm^2)**	**Minimum area for each additional animal in group holding(cm^2)**	**Minimum enclosure height[c](cm)**	**Minimum water depth(cm)**	**Date referred to in Article 33(2)**
up to 5,0	1 500	200	20	10	1 January 2017
over 5,0 to 7,5	3 500	500	30	10	
Over 7,5	4 000	700	30	15	

a Measured from snout to vent.

b One-third land division, two-thirds water division sufficient for animals to submerge.

c Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.

Table 9.4. **Semi-terrestrial anurans**

Body length[a](cm)	**Minimum enclosure size[b](cm^2)**	**Minimum area for each additional animal**	**Minimum enclosure height[c](cm)**	**Minimum water depth(cm)**	**Date referred to in Article 33(2)**

a Measured from snout to vent.

b Two-thirds land division, one-third water division sufficient for animals to submerge.

c Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.

		in group-holding(cm^2)			
Up to 5,0	1 500	200	20	10	1 January 2017
over 5,0 to 7,5	3 500	500	30	10	
over 7,5	4 000	700	30	15	

a Measured from snout to vent.

b Two-thirds land division, one-third water division sufficient for animals to submerge.

c Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.

Table 9.5. **Arboreal anurans**

Body length[a](cm)	**Minimum enclosure size[b](cm^2)**	**Minimum area for each additional animal in group-holding(cm^2)**	**Minimum enclosure height[c](cm)**	**Date referred to in Article 33(2)**
up to 3,0	900	100	30	1 January 2017
Over 3,0	1 500	200	30	

a Measured from snout to vent.

b Two-thirds land division, one-third pool division sufficient for animals to submerge.

c Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.

10. Reptiles

Table 10.1. **Aquatic chelonians**

Body length[a](cm)	**Minimum water surface area(cm^2)**	**Minimum water surface area for each additional animal in group holding(cm^2)**	**Minimum water depth(cm)**	**Date referred to in Article 33(2)**
up to 5	600	100	10	1 January 2017
Over 5 to 10	1 600	300	15	
Over 10 to 15	3 500	600	20	
Over 15 to 20	6 000	1 200	30	

a Measured in a straight line from the front edge to the back edge of the shell.

Over 20 to 30	10 000	2 000	35
Over 30	20 000	5 000	40

a Measured in a straight line from the front edge to the back edge of the shell.

Table 10.2. **Terrestrial snakes**

Body length[a](cm)	**Minimum floor area(cm^2)**	**Minimum area for each additional animal in group-holding(cm^2)**	**Minimum enclosure height[b](cm)**	**Date referred to in Article 33(2)**
up to 30	300	150	10	1 January 2017
Over 30 to 40	400	200	12	
Over 40 to 50	600	300	15	
Over 50 to 75	1 200	600	20	
Over 75	2 500	1 200	28	

a Measured from snout to tail.

b 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 shall be adapted to the interior design.

11. Fish

11.1. Water supply and quality

Adequate water supply of suitable quality shall be provided at all times. Water flow in re-circulatory systems or filtration within tanks shall be sufficient to ensure that water quality parameters are maintained within acceptable levels. Water supply shall be filtered or treated to remove substances harmful to fish, where necessary. Water-quality parameters shall at all times be within the acceptable range that sustains normal activity and physiology for a given species and stage of development. The water flow shall be appropriate to enable fish to swim correctly and to maintain normal behaviour. Fish shall be given an appropriate time for acclimatisation and adaptation to changes in water-quality conditions.

11.2. Oxygen, nitrogen compounds, pH, and salinity

Oxygen concentration shall be appropriate to the species and to the context in which the fish are held. Where necessary, supplementary aeration of tank water shall be provided. The concentrations of nitrogen compounds shall be kept low.

The pH level shall be adapted to the species and kept as stable as possible. The salinity shall be adapted to the requirements of the fish species and to the life stage of the fish. Changes in salinity shall take place gradually.

11.3. Temperature, lighting, noise

Temperature shall be maintained within the optimal range for the fish species concerned and kept as stable as possible. Changes in temperature shall take place gradually. Fish shall be maintained

on an appropriate photoperiod. Noise levels shall be kept to a minimum and, where possible, equipment causing noise or vibration, such as power generators or filtration systems, shall be separate from the fish-holding tanks.

11.4. Stocking density and environmental complexity

The stocking density of fish shall be based on the total needs of the fish in respect of environmental conditions, health and welfare. Fish shall have sufficient water volume for normal swimming, taking account of their size, age, health and feeding method. Fish shall be provided with an appropriate environmental enrichment, such as hiding places or bottom substrate, unless behavioural traits suggest none is required.

11.5. Feeding and handling

Fish shall be fed a diet suitable for the fish at an appropriate feeding rate and frequency. Particular attention shall be given to feeding of larval fish during any transition from live to artificial diets. Handling of fish shall be kept to a minimum.

ANNEX IV

METHODS OF KILLING ANIMALS

1. In the process of killing animals, methods listed in the table below shall be used.

Methods other than those listed in the table may be used:

(a) on unconscious animals, providing the animal does not regain consciousness before death;

(b) on animals used in agricultural research, when the aim of the project requires that the animals are kept under similar conditions to those under which commercial farm animals are kept; these animals may be killed in accordance with the requirements laid down in Annex I to Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing[6].

2. The killing of animals shall be completed by one of the following methods:

(a) confirmation of permanent cessation of the circulation;

(b) destruction of the brain;

(c) dislocation of the neck;

(d) exsanguination; or

(e) confirmation of the onset of *rigor mortis*.

3. Table

Animals-Fish	Amphibians	Reptiles	Birds	Rodents	Rabbits	Dogs, cats, ferrets and foxes	Large mammals	Non-human primates	remarks/ methods

Anaesthetic overdose	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Captive bolt	X	X	(2)	X	X		X		X
Carbon dioxide	X	X	X		(3)	X	X	X	X
Cervical dislocation	X	X	X	(4)	(5)	(6)	X	X	X
Concussion/ percussive blow to the head				(7)	(8)	(9)	(10)	X	X
Decapitation	X	X	X	(11)	(12)	X	X	X	X
Electrical stunning	(13)	(13)	X	(13)	X	(13)	(13)	(13)	X
Inert gases (Ar, N_2)	X	X	X			X	X	(14)	X
Shooting with a free bullet with appropriate rifles, guns and ammunition	X	X	(15)	X	X	X	(16)	(15)	X

Requirements

1. Shall, where appropriate, be used with prior sedation.
2. Only to be used on large reptiles.
3. Only to be used in gradual fill. Not to be used for foetal and neonate rodents.
4. Only to be used for birds under 1 kg. Birds over 250 g shall be sedated.
5. Only to be used for rodents under 1 kg. Rodents over 150 g shall be sedated.
6. Only to be used for rabbits under 1 kg. Rabbits over 150 g shall be sedated.
7. Only to be used for birds under 5 kg.
8. Only to be used for rodents under 1 kg.
9. Only to be used for rabbits under 5 kg.

10. Only to be used on neonates.

11. Only to be used for birds under 250 g.

12. Only to be used if other methods are not possible.

13. Specialised equipment required.

14. Only to be used on pigs.

15. Only to be used in field conditions by experienced marksmen.

16. Only to be used in field conditions by experienced marksmen when other methods are not possible.

ANNEX V

LIST OF ELEMENTS REFERRED TO IN ARTICLE 23(3)

1. National legislation in force relevant to the acquisition, husbandry, care and use of animals for scientific purposes.

2. Ethics in relation to human-animal relationship, intrinsic value of life and arguments for and against the use of animals for scientific purposes.

3. Basic and appropriate species-specific biology in relation to anatomy, physiological features, breeding, genetics and genetic alteration.

4. Animal behaviour, husbandry and enrichment.

5. Species-specific methods of handling and procedures, where appropriate.

6. Animal health management and hygiene.

7. Recognition of species-specific distress, pain and suffering of most common laboratory species.

8. Anaesthesia, pain relieving methods and killing.

9. Use of humane end-points.

10. Requirement of replacement, reduction and refinement.

11. Design of procedures and projects, where appropriate.

ANNEX VI

LIST OF ELEMENTS REFERRED TO IN ARTICLE 37(1)(c)

1. Relevance and justification of the following:

   (a) use of animals including their origin, estimated numbers, species and life stages;

   (b) procedures.

2. Application of methods to replace, reduce and refine the use of animals in procedures.

3. The planned use of anaesthesia, analgesia and other pain relieving methods.

4. Reduction, avoidance and alleviation of any form of animal suffering, from birth to death where appropriate.

5. Use of humane end-points.

6. Experimental or observational strategy and statistical design to minimise animal numbers, pain, suffering, distress and environmental impact where appropriate.

7. Reuse of animals and the accumulative effect thereof on the animals.

8. The proposed severity classification of procedures.

9. Avoidance of unjustified duplication of procedures where appropriate.

10. Housing, husbandry and care conditions for the animals.

11. Methods of killing.

12. Competence of persons involved in the project.

ANNEX VII

DUTIES AND TASKS OF THE UNION REFERENCE LABORATORY

1. The Union Reference Laboratory referred to in Article 48 is the Commission's Joint Research Centre.

2. The Union Reference Laboratory shall be responsible, in particular, for:

(a) coordinating and promoting the development and use of alternatives to procedures including in the areas of basic and applied research and regulatory testing;

(b) coordinating the validation of alternative approaches at Union level;

(c) acting as a focal point for the exchange of information on the development of alternative approaches;

(d) setting up, maintaining and managing public databases and information systems on alternative approaches and their state of development;

(e) promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry, biomedical scientists, consumer organisations and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.

3. The Union Reference Laboratory shall participate in the validation of alternative approaches.

ANNEX VIII

SEVERITY CLASSIFICATION OF PROCEDURES

The severity of a procedure shall be determined by the degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the course of the procedure.

Section I:

Severity categories

Non-recovery:

Procedures which are performed entirely under general anaesthesia from which the animal shall not recover consciousness shall be classified as ‘non-recovery’.
Mild:

Procedures on animals as a result of which the animals are likely to experience short-term mild pain, suffering or distress, as well as procedures with no significant impairment of the well-being or general condition of the animals shall be classified as ‘mild’.
Moderate:

Procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress as well as procedures that are likely to cause moderate impairment of the well-being or general condition of the animals shall be classified as ‘moderate’.
Severe:

Procedures on animals as a result of which the animals are likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress as well as procedures, that are likely to cause severe impairment of the well-being or general condition of the animals shall be classified as ‘severe’.

Section II:

Assignment criteria

The assignment of the severity category shall take into account any intervention or manipulation of an animal within a defined procedure. It shall be based on the most severe effects likely to be experienced by an individual animal after applying all appropriate refinement techniques.

When assigning a procedure to a particular category, the type of procedure and a number of other factors shall be taken into account. All these factors shall be considered on a case-by-case basis.

The factors related to the procedure shall include:

— type of manipulation, handling,
— nature of pain, suffering, distress or lasting harm caused by (all elements of) the procedure, and its intensity, the duration, frequency and multiplicity of techniques employed,
— cumulative suffering within a procedure,
— prevention from expressing natural behaviour including restrictions on the housing, husbandry and care standards.

Examples are given in Section III of procedures assigned to each of the severity categories on the basis of factors related to the type of the procedure alone. They shall provide the first indication as to what classification would be the most appropriate for a certain type of procedure.

However, for the purposes of the final severity classification of the procedure, the following additional factors, assessed on a case-by-case basis, shall also be taken into account:

— type of species and genotype,
— maturity, age and gender of the animal,
— training experience of the animal with respect to the procedure,
— if the animal is to be reused, the actual severity of the previous procedures,
— the methods used to reduce or eliminate pain, suffering and distress, including refinement of housing, husbandry and care conditions,
— humane end-points.

Section III: Examples of different types of procedure assigned to each of the severity categories on the basis of factors related to the type of the procedure

1. Mild:

(a) administration of anaesthesia except for the sole purpose of killing;

(b) pharmacokinetic study where a single dose is administered and a limited number of blood samples are taken (totalling < 10 % of circulating volume) and the substance is not expected to cause any detectable adverse effect;

(c) non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anaesthesia;

(d) superficial procedures, e.g. ear and tail biopsies, non-surgical subcutaneous implantation of mini-pumps and transponders;

(e) application of external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behaviour;

(f) administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal;

(g) induction of tumours, or spontaneous tumours, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, non-invasive nodules);

(h) breeding of genetically altered animals, which is expected to result in a phenotype with mild effects;

(i) feeding of modified diets, that do not meet all of the animals' nutritional needs and are expected to cause mild clinical abnormality within the time-scale of the study;

(j) short-term (< 24h) restraint in metabolic cages;

(k) studies involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains;

(l) models which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid;

(m) a combination or accumulation of the following examples may result in classification as 'mild':

(i) assessing body composition by non-invasive measures and with minimal restraint;

(ii) monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals;

(iii) application of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behaviour;

(iv) breeding genetically altered animals which are expected to have no clinically detectable adverse phenotype;

(v) adding inert markers in the diet to follow passage of digesta;

(vi) withdrawal of food for < 24h in adult rats;

(vii) open field testing.

2. Moderate:

(a) frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (> 10 % of circulating volume) in a conscious animal within a few days without volume replacement;

(b) acute dose-range finding studies, chronic toxicity/carcinogenicity tests, with non-lethal end-points;

(c) surgery under general anaesthesia and appropriate analgesia, associated with post surgical pain, suffering or impairment of general condition. Examples include: thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy, thyroidectomy, orthopaedic surgery with effective stabilisation and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, minipumps etc.);

(d) models of induction of tumours, or spontaneous tumours, that are expected to cause moderate pain or distress or moderate interference with normal behaviour;

(e) irradiation or chemotherapy with a sublethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects would be expected to be mild or moderate and would be short-lived (< 5 days);

(f) breeding of genetically altered animals which are expected to result in a phenotype with moderate effects;

(g) creation of genetically altered animals through surgical procedures;

(h) use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days);

(i) studies with modified diets that do not meet all of the animals' nutritional needs and are expected to cause moderate clinical abnormality within the time-scale of the study;

(j) withdrawal of food for 48 hours in adult rats;

(k) evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus, and are expected to result in moderate distress.

3. Severe:

(a) toxicity testing where death is the end-point, or fatalities are to be expected and severe pathophysiological states are induced. For example, single dose acute toxicity testing (see OECD testing guidelines);

(b) testing of device where failure may cause severe pain, distress or death of the animal (e.g. cardiac assist devices);

(c) vaccine potency testing characterised by persistent impairment of the animal's condition, progressive disease leading to death, associated with long-lasting moderate pain, distress or suffering;

(d) irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease;

(e) models with induction of tumours, or with spontaneous tumours, that are expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering. For example tumours causing cachexia, invasive bone tumours, tumours resulting in metastatic spread, and tumours that are allowed to ulcerate;

(f) surgical and other interventions in animals under general anaesthesia which are expected to result in severe or persistent moderate postoperative pain, suffering or distress or severe and persistent impairment of the general condition of the animals. Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure;

(g) organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (e.g. xenotransplantation);

(h) breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example Huntington's disease, Muscular dystrophy, chronic relapsing neuritis models;

(i) use of metabolic cages involving severe restriction of movement over a prolonged period;

(j) inescapable electric shock (e.g. to produce learned helplessness);

(k) complete isolation for prolonged periods of social species e.g. dogs and non-human primates;

(l) immobilisation stress to induce gastric ulcers or cardiac failure in rats;

(m) forced swim or exercise tests with exhaustion as the end-point.

(**1**) Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes (OJ L 221, 8.8.1998, p. 23).

(**2**) Council Directive 91/629/EEC of 19 November 1991 laying down minimum standards for the protection of calves (OJ L 340, 11.12.1991, p. 28).

(**3**) Council Directive 91/630/EEC of 19 November 1991 laying down minimum standards for the protection of pigs (OJ L 340, 11.12.1991, p. 33).

(**4**) Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens (OJ L 203, 3.8.1999, p. 53).

(**5**) Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production (OJ L 182, 12.7.2007, p. 19).

(**6**) OJ L 303, 18.11.2009, p. 1.