Recommendations for euthanasia of experimental animals: Part 2

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This document was prepared for DGXI of the European Commission to be used with Directive 86/609/EEC of 24 November 1986, *on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes* (No L 358, ISSN 0378-6978). It refers especially to Article 2(1) published by the European Commission in October 1995 which defines 'humane methods of killing' as 'the killing of an animal with a minimum of physical and mental suffering, depending on the species'.

This is the second part of the working party's report and comprises Section 3 of the report, the list of all references cited in both parts and details of training materials. The first part, comprising Sections 1 and 2 of the report together with a reading list, was published in the October 1996 issue of Laboratory Animals *(***30***: 293–316). Reprints combining both parts of the report will be available from Mrs S E Wolfensohn, Supervisor of Veterinary Services, University of Oxford, Veterinary Services, c/o University Laboratory of Physiology, Parks Road, Oxford OXI 3PT, UK (Tel: +44(0)1865-272545, Fax: +44(0)1865-272118, Email: vet@vax.ox.ac.uk).*

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3 Methods of euthanasia for each species group

Section 1 must be consulted before considering this section.

3.1 Fish

There are over 20 000 species of fish with enormously varying lifestyles which makes it very difficult to generalize on methods of euthanasia. The methods listed below are meant as a guide and the operator must assess which is the best method for the species being killed or obtain information from experts. A summary is given in Table 2. Although fish may not have the same spinothalamic pathways as mammals for pain perception, there is evidence that they do feel pain and should therefore be killed with the same care and consideration.

All fish are sensitive to changes in the physical and chemical parameters of their water (especially temperature, dissolved gas levels, salinity, pH, etc.) but some species are much more tolerant of changes in any one of these factors than are others. Therefore unless the species' response is known it is advisable to practise euthanasia in the fish's normal water. If drugs are used the water level should be lowered to ensure rapid sedation but not so much as to distress the fish before the addition of the agent. Dosing is always preferable to injection as the latter technique involves handling the fish and thus induces stress. It may be necessary to fast fish 24–48 h prior to chemical euthanasia as this will permit more rapid absorption by the gut and minimize the risk of regurgitation which could reduce the effect of the chemicals on the gill lamellae (Brown 1988). The tanks used should enable the operator to observe the fish and react quickly if there are signs of suffering. It is generally true that cooling will reduce the metabolic and locomotory processes, thus facilitating handling, but it is essential that the normal temperature of the fish and its degree of tolerance be considered. It is also vital to note that in marine fish ice crystals will form in the cells before the sea water freezes, thereby causing the fish extreme pain. In freshwater fish the water will freeze before internal ice crystals form. However, it must be remembered that cooling does not reduce the ability to feel pain.

Overexposure to an anaesthetic is first manifested by a cessation of respiratory movements, followed by spasmodic overextension or flaring of the opercula. These are spaced 15–30 s apart at first and then at longer intervals. When the interval between spasms is approximately 1 min, cardiac arrest and death follow within a few minutes (Table 1).

Recognition and confirmation of death

Death may be recognized by cessation of respiration (opercular movement) and cessation of heartbeat (palpation). Death should be confirmed by destruction of the brain where possible.

Larvae

Fish may be classed as oviparous, ovoviviparous or viviparous depending on whether they produce eggs which hatch outside the body, produce eggs which hatch inside the body, or give birth to free-living young

Table 1 Stages of loss of consciousness, leading to death in fish (after McFarland and Klontz, 1969)

Level	Designation	Parameter(s)
0	Normal	Reactive to external stimuli; equilibrium and muscle tone normal
1	Light sedation	Slight loss of reactivity to external visual and tactile stimuli; equilibrium normal
2	Deep sedation	Total loss of reactivity to external stimuli *except* strong pressure; slight decrease in opercular rate; equilibrium normal
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactive only to strong tactile and vibrational stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; rapid opercular rate; reactive only to deep pressure stimuli
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements very shallow; heart rate very slow
6	Medullary collapse	Opercular movements cease immediately after gasping, followed by cardiac arrest

(larvae). For simplicity, it is considered that all fish should be protected as soon as they have hatched and the methods of euthanasia recommended for adults are considered acceptable for larvae. Viviparous fish should be treated by immersion or injecting the parent.

Adults

Further details of methods may be found in Section 2.

Physical methods

Concussion This involves a blow to the back of the head and if carried out by experienced personnel is a humane method of euthanasia. Death must be confirmed by destruction of the brain.

Cervical dislocation This is the breaking of the backbone near the head. Small to medium fish are killed by inserting a rod or thumb into the mouth, holding the fish with the opposite hand and displacing it dorsally (Clifford 1984). It is feasible and effective in small fish, but should be confirmed by exsanguination or destruction of the brain. The stress caused by handling reduces the acceptability of this method. *It is not possible or humane in larger fish.*

Maceration Small fish of less than 2 cm in length may be humanely killed by placing down a waste disposal unit.

Chemical methods

Agents can be administered by dissolving the chemical in the tank water. Water temperatures often alter the efficacy of the drug and induction is often more rapid at higher temperatures. However, the temperature must not be raised so that it causes any stress to the fish. Drugs may also be administered by intramuscular or intraperitoneal injection. For euthanasia anaesthetic drugs are generally used at double or triple the recommended anaesthetic dose. In all cases, death must be confirmed by destruction of the brain.

Tricaine methane sulphonate (buffered MS-222) This acts by depression of the central nervous system. This is a benzocaine type drug and is the most effective way to kill most fish. It is soluble in both salt and fresh water. However, it is expensive and so may deter some users, especially if they need to kill large numbers of fish. Bicarbonate, imidazole, sodium hydrogen phospate or sodium hydroxide should be added to neutralize the water (to pH 7.5) to reduce irritation and tissue damage. It may be used in conjunction with quinaldine or quinaldine sulphate to increase effectiveness.

Benzocaine (ethyl aminobenzoate) This acts in a similar way to MS-222 but has pH-independent efficacy. However, because it lowers the pH of the water, it should be neutralized to pH 7.5. The breakdown time in water is about 4 h, thus making this drug acceptable in terms of environmental contamination. As it is insoluble in water benzocaine should first be dissolved in acetone before adding to water.

Etomidate It is a potent imidazole-based agent with no analgesic properties. It is highly soluble in water. Stress hormone measurements of fish have indicated that etomidate may give fewer problems than MS-222 (Zwart *et al.* 1989) and it is therefore considered acceptable for euthanasia of fish.

Metomidate It is an imidazole-based non-barbiturate hypnotic agent that has no analgesic properties. Used in overdose, this is considered acceptable for killing most species of fish.

Quinaldine (2-methylquinoline) This is difficult to obtain in Europe but is commonly used in the USA for humane euthanasia. The recommended dosages for euthanasia vary depending upon species, temperature and water hardness. It accumulates in lipid tissues such as the brain and depresses the sensory centres of the central nervous system. Quinaldine sulphate is also considered acceptable as an effective euthanasia agent for fish.

Halothane This may be bubbled through the tank and causes anaesthesia. Death must be ensured by destruction of the brain.

Injectable agents Barbiturates may be used but as this requires removal from water and

handling, with resultant stress, other methods are preferable. The intraperitoneal route is generally recommended.

Methods acceptable for unconscious fish

Pithing For small fish pithing can be employed in which a metal spike is pushed into the top of the head between the eyes and pushed forwards and backwards to destroy the brain and proximal end of the spinal cord. *This method should only be carried out on unconscious animals.* This method is considered acceptable when chemical methods are not appropriate for the study.

Decapitation This is possible in small fish but becomes problematic in larger fish. Decapitation of fish should only be carried out under anaesthesia or after stunning as there is some doubt about immediate loss of consciousness. Research on eels showed that the brain was still functioning for up to 35 min after decapitation (Verheijen & Flight 1995) and therefore the brain should be immediately destroyed. This method is acceptable only if other methods are not considered suitable and under the above constraints. Spinal transection (the neck cut) is also unacceptable except on insensible fish (Flight & Verheijen 1993).

Exsanguination This is not considered acceptable as a method of euthanasia as it is too slow and too difficult to locate the veins, unless the fish is insensible.

Methods not acceptable for euthanasia of fish

Removal from water This causes distress and suffering because of the length of time to unconsciousness. Cooling prolongs this period considerably. This is not an acceptable method of euthanasia of fish (Kestin 1993, Kestin *et al.* 1991).

Whole body crushing This is not considered a humane method of euthanasia.

Electrical stunning This could be dangerous for the operator, if an isolated circuit is not used. Electrical stunning does not work with all fish (e.g. eels) and used alone it is not necessarily lethal (it may only stun larger fish). Alternating current stimulates contraction of skeletal, cardiac and smooth muscle, and it induces tetany, not anaesthesia (Summerfelt & Smith 1990). Although it is used on fish farms by experts, often to catch fish, it is not considered acceptable in the laboratory situation for euthanasia.

Hypothermia Putting fish into a freezer or crushed ice prolongs the period of consciousness in fish and does not reduce the ability to feel pain; therefore it should not be used as a method of euthanasia.

Hyperthermia Fish when put into hot water will press their opercula tightly to the body and thus have a depot of oxygen which prolongs their period of consciousness. Boiling water will cause extreme pain. This method should therefore not be employed to kill fish of any kind.

2-Phenoxyethanol Its main use is as an antibiotic but is used as an anaesthetic agent as well. It requires large doses to achieve death with a long induction period. Some species exhibit hyperactivity prior to loss of consciousness. It is not considered acceptable for use for euthanasia of fish.

Carbon dioxide This is not acceptable for euthanasia of fish as it causes intense activity prior to loss of consciousness and is slow acting.

Diethyl ether This should not be used because of irritation to the mucous membranes as well as danger to the operator.

Secobarbital and amobarbital Both have the disadvantage that induction is too long.

Urethane This is carcinogenic and therefore extremely dangerous to the operator.

Chloral hydrate This has a long induction period and only acts as a sedative.

Tertiary amyl alcohol This causes irritation during induction.

Tribromoethanol This is irritant and has a long induction period.

Chlorobutanol This has the disdavantage of requiring a broad range of dosages across the different species.

Methyl pentynol This causes stress by respiratory arrest.

Pyridines These are dangerous to the operator.

3.2. Amphibians

There are many species of amphibians making it difficult to generalize on methods of euthanasia. A summary is given in Table 3. The skin is thin, protected by a cuticle bearing many mucus glands. This results in it being generally more sensitive to physical and chemical insults than in other vertebrates. Because amphibians are ectothermic and thus accustomed to fluctuations in body temperature, their central nervous system (CNS) is less sensitive to hypoxia and anoxia. Even when the cranial nerves and brain are deprived of blood supply these animals are able to respond to stimuli for some time. Although decapitation, by itself, does not produce rapid unconsciousness in the severed heads of amphibians, rapid destruction of the brain does extinguish responses usually thought to indicate consciousness. There is, however, a remarkably intact set of somatic responses to stimuli — long continued body movements, foot withdrawals in response to toe pinching, etc., as well as continued heartbeat in many cases for hours following brain destruction. This continued somatic activity is attributed to:

(1) prolonged tolerance of the spinal cord, peripheral nerves and muscle (smooth, cardiac and skeletal) to hypoxic and hypotensive conditions, and
(2) a far greater degree of integration of somatic responses at the level of the spinal cord instead of the brain. (UFAW/WSPA 1989)

Table 2 Characteristics of methods for euthanasia of fish

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
MS-222	++	++	++	++	++	5	Acceptable
Benzocaine	++	++	++	++	++	5	Acceptable
Etomidate	++	++	++	++	++	5	Acceptable
Metomidate	++	++	++	++	++	5	Acceptable
Concussion	++	+	+	++	–	4	Death to be confirmed
Maceration	++	++	++	++	+	4	Only for fish less than 2 cm in length
Quinaldine	++	++	++	+	++	4	Difficult to obtain in Europe
Sodium pentobarbitone	++	++	–	+	++	3	May be useful for large fish, intraperitoneal injection
Cervical dislocation	++	++	+	++	–	3	Not in large fish. To be followed by destruction of the brain
Halothane	+	+	++	++	++	2	Other methods preferable. Death to be confirmed

The following methods may only be used on unconscious fish: pithing, decapitation and exsanguination

The following methods are not to be used for killing fish: removal from water, whole body crushing, electrical stunning, hypothermia, hyperthermia, 2-phenoxyethanol, carbon dioxide, diethyl ether, secobarbital, amobarbital, urethane, chloral hydrate, tertiary amyl alcohol, tribromoethanol, chlorobutanol, methyl pentynol, pyridines

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

Recognition and confirmation of death

Death may be recognized by cessation of heartbeat and respiration and in cases where this is not obvious, it may be confirmed by destruction of the brain.

Larvae

Tadpoles and newts can be effectively killed by placing in a dish of water with MS-222 or benzocaine (dissolved in acetone). These produce rapid anaesthesia, followed by death.

Adults

Further details of methods may be found in Section 2.

When handling these species, it is important to obtain a firm hold, for example by wearing rough textured but non-abrasive gloves or by holding them in coarse material. Cooling to 3–4°C will reduce metabolic and locomotory processes, thus facilitating handling prior to euthanasia. However, it must be remembered that cooling does not reduce the ability to feel pain (UFAW/WSPA, 1989).

Physical methods

Concussion This method, if carried out by a person who is well trained in the technique is an effective and humane way of stunning all amphibians. The hind legs should be grasped and the dorsal surface of the head struck against a hard, solid object. Alternatively, the dorsal surface of the head can be struck with a suitable instrument. Accuracy is essential to ensure immediate unconsciousness and death. Death must be ensured by destroying the brain after concussion.

Microwave This is an extremely quick method of euthanasia but must only be carried out by experienced personnel who know exactly where to direct the beam. Only specialized microwave equipment designed for this purpose is to be used. *Under no circumstances should domestic appliances be used for this purpose.* It is not considered a routine method of euthanasia.

Electrical stunning Frogs rendered insensible by electrical means may recover after 10 min but if followed immediately by destruction of the brain, it may be considered an acceptable method of euthanasia.

Chemical methods

Drugs are best administered by dissolving them in the water in which the amphibians are placed. This reduces stress from handling and injection.

Tricaine methane sulphonate (buffered MS-222) This is a quick, non-irritant and humane method of killing amphibians when dissolved in the water into which the amphibians are placed. It is recommended to neutralize the solution with bicarbonate to reduce irritation to the sensitive amphibian skin.

Benzocaine Dissolved in the water in which amphibians are placed, benzocaine is an effective drug acting on the CNS quickly and humanely. As benzocaine is not soluble in water, it must be first dissolved in acetone. The solution must be neutralized to avoid irritation as benzocaine reduces the pH.

Sodium pentobarbitone This drug, when injected intravenously or intraperitoneally acts quickly on the CNS, rendering the animal unconscious with little distress. However, this should only be carried out by experienced people to ensure the correct site for injection and to reduce handling to a minimum.

T-61 Intravenously injected, or in frogs, injected into the dorsal lymph sac, this drug is effective and humane for the euthanasia of amphibians.

Methods acceptable for unconscious amphibians

Pithing Ensures a rapid destruction of the brain resulting in immediate unconsciousness. This is a rapid and humane method of killing amphibians if carried out by well trained and experienced operators. *This method should only be carried out on unconscious animals.* In some species it is difficult to bend the head forward to expose the atlanto-occipital space and other methods are preferable in these cases.

Decapitation Is acceptable only in insensible amphibians as the time to unconsciousness is unknown because the nervous system is very tolerant of anoxia.

Methods not acceptable for the euthanasia of amphibians

Hypothermia This will make the animal torpid but does not reduce pain. Freezing is not acceptable, as the formation of ice crystals within the body tissues is likely to be extremely painful. Freezing may only be used as a method to confirm death after another method of euthanasia has been used.

Hyperthermia Amphibians should not be dropped into hot or boiling water as a method of euthanasia as this is extremely painful and inhumane.

Exsanguination Together with subsequent hypovolaemic shock and anoxia this may not render amphibians immediately unconscious, making this an unacceptable method of euthanasia.

Strangulation This is considered inhumane, and is not an acceptable method of killing amphibians.

Carbon dioxide This may cause irritation to the skin, and induction takes too long, and is therefore not considered an acceptable method.

Ether This is irritant to the mucous membranes and because of danger to the operator, it should not be used for killing amphibians.

Chloroform This is hepatoxic and carcinogenic and because of the consequent possible danger to personnel, chloroform should not be used for euthanasia.

Volatile inhalational anaesthetics These are not considered acceptable as they are slow to act and may be irritant to the skin.

Table 3 Characteristics of methods for euthanasia of amphibians

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
MS-222	++	++	++	++	++	5	Acceptable
Benzocaine	++	++	++	++	++	5	Acceptable
Sodium pentobarbitone	+	++	–	+	+	4	Involves handling and intravenous or intraperitoneal injection
Concussion	++	++	+	++	–	4	Acceptable for use by experienced personnel
T-61	+	++	–	+	+	3	Involves handling and intravenous injection
Microwave	++	++	–	+	++	3	Only for small amphibians. Not a routine procedure
Electrical stunning	+	+	+	–	–	2	To be followed immediately by destruction of the brain

The following methods are only to be used on unconscious amphibians: pithing and decapitation

The following methods are not to be used for killing amphibians: hypothermia, hyperthermia, exsanguination, strangulation, carbon dioxide, diethyl ether, chloroform, volatile inhalational anaesthetics, chloral hydrate, ketamine hydrochloride, chlorbutanol, methylpentynol, 2-phenoxyethanol, tertiary amyl alcohol, tribromoethanol and urethane

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

Other agents not considered acceptable include: *chloral hydrate*, *ketamine hydrochloride*, *chlorbutanol*, *methylpentynol*, *2-phenoxyethanol*, *tertiary amyl alcohol*, *tribromoethanol*, *urethane*.

3.3 Reptiles

Because reptiles are ectothermic and thus accustomed to fluctuations in body temperature, their central nervous system is less sensitive to a drop in oxygen tension. Even when the cranial nerves and brain are deprived of blood supply following decapitation, these animals are able to respond to stimuli for some time. Although decapitation, by itself, does not produce rapid unconsciousness in the severed heads of reptiles (Warwick 1990), rapid destruction of the brain does extinguish responses usually thought to indicate consciousness. There is, however, a remarkably intact set of somatic responses to stimuli — long continued body movements, foot withdrawals in response to toe pinching, etc., as well as continued heartbeat in many cases for hours following brain destruction. This continued somatic activity is attributed to:

(1) prolonged tolerance of the spinal cord, peripheral nerves and muscle (smooth, cardiac and skeletal) to hypoxic and hypotensive conditions, and
(2) a far greater degree of integration of somatic responses at the level of the spinal cord instead of the brain. (UFAW/WSPA 1989).

Good methods of restraint are important to ensure minimal stress prior to carrying out euthanasia.

Particular care must be taken when handling venomous species, such as many types of snake, especially when they are not used to being handled. Padded grasping implements are useful in handling lizards and snakes to ensure a firm but non-damaging restraint. Cooling of most reptiles to 3–4°C will reduce metabolic and locomotory processes (this temperature may kill some tropical species), thus facilitating handling prior to euthanasia. However it must be remembered that cooling does not reduce the ability to feel pain.

In tortoises, turtles and terrapins, retraction of the head and protection by the carapace can cause difficulty for euthanasia. To assist in exposing the head, land tortoises can be placed in shallow, tepid water; large marine species may be put on a frame at 45° head up, inducing neck extension; and soft-shelled species can be placed on their backs to induce neck extension. Rough textured but non-abrasive gloves may be worn when handling aquatic species to facilitate handling.

Effective restraint of the jaws and tail is the key factor to operator safety for restraining crocodilians and this should only be done by experts (UFAW/WSPA 1989).

A summary of recommendations is given in Table 4.

Recognition and confirmation of death

As it is difficult to determine whether reptiles are unconscious or dead, it is recommended that death be confirmed by destruction of the brain. Usually, but by no means always, a lack of pupillary–blink–nictitating membrane responses, except in snakes which do not possess movable eyelids, implies a lack of consciousness. Rigor mortis is a reliable indicator of death as is the prolonged absence of a heartbeat and/or circulation.

Embryos

In the case of reptiles two distinct stages have to be considered: the eggs and the newly-hatched individuals. For all practical purposes all newly hatched reptiles can be treated in the same way as adults. As reptiles are born as fully developed individuals (with the exception of not being able to reproduce), killing of the embryos at the egg stage must be carried out in a humane way to account for potentially advanced development. In general eggs may succumb to high and low temperatures but some may withstand freezing. Hypothermia and hyperthermia cannot be considered acceptable because humane death cannot be guaranteed. Drowning is not considered humane as this results in death by anoxia and is slow. Eggs with no embryo may be frozen. Recom-

mended methods would include disruption of the egg and killing of the embryos by injection of sodium pentobarbitone, anaesthetic overdose or an appropriate physical method to destroy the brain or whole egg or early life form.

Adults

As the class Reptilia is varied, it is best to consider three main groups: the snakes and lizards (Squamata); tortoises, turtles and terrapins (Testudines); and crocodiles and alligators (Crocodilia). Larger reptiles may need to be sedated before being killed.

Further details on methods may be obtained in Section 2.

Physical methods

Captive bolt This method may be used within the laboratory situation with relative safety. It is considered an acceptable method for large reptiles but should only be carried out by experts who know exactly where to position the pistol. Care must be taken to ensure that the pistol is well maintained and is of the correct calibre and cartridge length for the species being killed. Good restraint is necessary to ensure humane killing. If the bolt goes through the brain, it should kill the reptile, otherwise it may just stun the animal. Death must be ensured by destruction of the brain.

Concussion Small reptiles and those with fine bone structures such as some snakes and lizards may be rendered unconscious by stunning. This involves striking the back of the head of the animal with some hard implement or object, the principle being either to kill the animal outright or to render it unconscious. Ideally, the blow should be given with such force as to cause cessation of brain activity. This must only be performed by people trained and experienced in handling and killing reptiles. Concussion must always be followed by destruction of the brain.

Shooting This is an effective method of killing most large reptiles, causing rapid and substantial destruction of the brain. A high level of skill is required in order to hit the brain through the two brain cases found in many reptiles. This method could also be dangerous to the operator and should therefore only be used in field conditions. A heavy calibre rifle or shotgun of a suitable calibre is necessary for large animals such as adult crocodiles. It is necessary to ensure that the animal does not move its head prior to shooting. The Testudines must have their head exposed and held thus for accurate positioning of the gun.

Chemical methods

Sodium pentobarbitone Sodium pentobarbitone is an effective and humane method of euthanasia in reptiles. The intravenous route can be used by well trained personnel. Where intravenous injection is difficult the intraperitoneal route may be used but it is slower-acting. Injections should not be made intracardially or into the lungs as this is regarded as painful and irritant.

Methods acceptable for unconscious reptiles

Pithing This may only be carried out on unconscious animals and by experienced personnel.

Decapitation This may only be used if the reptile has been made unconscious by other methods, such as concussion, as long periods of post-decapitation consciousness have been recorded (Warwick 1990).

Methods not acceptable for euthanasia of reptiles

Spinal cord severance Because of the ability of reptiles to withstand anoxia and cerebral hypoxaemia, spinal cord severance is not an acceptable method of euthanasia. It has been shown that crocodiles may remain conscious for up to 1 h 50 min after spinal cord severance and other reptiles may remain conscious for similar lengths of time.

Hypothermia This will make the animal torpid but will not raise the pain threshold. The formation of ice crystals within the body tissues is likely to be extremely painful. Hypothermia is not acceptable for euthanasia.

Hyperthermia This is not considered acceptable as the time to unconciousness is unknown. Boiling water must never be used to kill reptiles.

Exsanguination This is not considered humane due to the animal's tolerance of hypoxia.

Chloroform This has been used to kill tortoises by injection into the peritoneal cavity with apparently no undesirable effects. Because of the potential trauma to the injected animal and danger to the operator, other methods are considered more acceptable. Chloroform is hepatotoxic and carcinogenic to the operator.

Tricaine methane sulphonate (MS-222) This has been injected intramuscularly in snakes and alligators. There is little information on the humaneness of this method and it is therefore not considered acceptable.

Reptiles are capable of holding their breath for a relatively long period of time and therefore inhalational methods such as *ether*, *halothane*, *enflurane*, *isoflurane* and *methyoxyflurane* cannot be considered as practicable or humane due to slow induction. Other agents not to be used for killing reptiles include CO_2, *neuromuscular blocking agents*, *ketamine hydrochloride* (takes too long to induction), *chloral hydrate* and *procaine*.

3.4 Birds

Birds have a complex respiratory system comprising the lungs and numerous air sacs with a one-way flow of air. This may influence the rate of absorption of inhalational agents and thus increase their efficiency.

A summary of recommendations is given in Table 5.

Recognition and confirmation of death

Death may be recognized by the absence of signs of breathing, cardiac arrest and absence of reflexes in the head (i.e cranial nerve reflexes rather than spinal cord reflexes). Reflexes to be checked would include pinching of wattles or blink reflexes. Death can be ensured by destruction of the brain or ensuring cessation of the heartbeat.

Embryos

Bird embryos from the stage at which the neural tube has developed into a functional brain (>50% gestation) must be destroyed humanely as they may be capable of perceiving pain from that stage. The most commonly used method of destroying eggs is cooling or freezing. The recommended temperature is <4°C for 4 h. Death must be

Table 4 Characteristics of methods for euthanasia of reptiles

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
Sodium pentobarbitone	++	++	++	+	++	5	Acceptable, but involves handling
Captive bolt	++	++	++	+	+	5	Acceptable for large reptiles
Concussion	+	+	+	++	+	4	To be followed by destruction of the brain
Shooting	++	++	++	–	+	4	Acceptable only in field conditions

The following methods are to be used on unconscious reptiles only: pithing and decapitation

The following methods are not to be used for killing reptiles: spinal cord severance, hypothermia, hyperthermia, exsanguination, chloroform, MS-222, ether, halothane, methoxyflurane, isoflurane, enflurane, carbon dioxide, neuromuscular blocking agents, ketamine hydrochloride, chloral hydrate and procaine

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

confirmed by decapitation or some other suitable method. In cases where the embryo has been exposed for studies, decapitation is considered an acceptable method of euthanasia as is an overdose of anaesthetic. Disruption of the membranes and maceration (in a macerator designed for the purpose) are also considered as humane methods of killing bird embryos (Bandow 1987).

Adults

Further details on methods may be found in Section 2.

Physical methods

Cervical dislocation Cervical dislocation, if carried out near to the head, causes damage to the lower brain region, resulting in rapid and painless loss of consciousness. This must always be followed immediately by destruction of the brain or section of the major blood vessels in the neck. However, research has shown that visual evoked potentials may remain up to 30 s after dislocation which may indicate lack of insensibility (Gregory & Wotton 1990). Therefore, other methods are considered preferable. This method is not aesthetically pleasant as reflexes remain present for some time. This should not be used for birds of over 3 kg or some older birds where the neck is difficult to pull quickly. This method may be used for day-old chicks as long as the numbers are kept low to avoid human error due to tiredness (Jaksch 1981). The wings of the birds should be secured to avoid involuntary flapping (Clifford 1984).

Maceration This method may be used for chicks up to 72 h old using specialist apparatus which contains rapidly rotating mechanically operated killing blades (Commission of the European Communities 1993). Only equipment designed for this purpose and complying to the standards of Council Directive 93/119/EC should be used. The blades must turn at >5000 revs/min. Operators must be trained in the use of such equipment and also have training in the maintenance of the equipment to ensure correct functioning at all times. The capacity and design of the apparatus must be sufficient to ensure that all animals are killed immediately with no possibility of animals being thrown out by the revolving blades. Chicks must be dropped one at a time down a special chute to reduce the chances of being thrown out by the blades in smaller apparatuses, but larger ones have been designed to take larger number of chicks at a time, without this risk. This method may be aesthetically unpleasant for some operators. *Under no circumstances should domestic appliances be used.*

Concussion This is carried out by a hard blow to the head and in small birds (<250 g) this may be done by striking the head on the sharp edge of a table. Although not aesthetically pleasant it is quick and humane if carried out correctly by a person trained and experienced in the technique. This method may also be acceptable for small numbers of day-old chicks. Unless sufficient brain damage has been caused resulting in immediate death, this must be followed by destruction of the brain.

Microwave Small birds may be killed rapidly and humanely by microwave delivered by specialist apparatus (Zeller *et al.* 1989). *Under no circumstances should domestic appliances be used.* Operators must receive specialist training in this technique to ensure accurate direction of the microwave beam and thus humane death. *This method should not be considered as a routine method of euthanasia.*

Electrical stunning Electrical stunning is commonly used in slaughterhouses but is generally *not considered acceptable for use in the laboratory* unless specialized equipment, safe for personnel, under legal controls, is used. The bird must be stunned before cardiac arrest occurs (i.e. the electrodes are placed in such a position that the brain is first affected).

Chemical methods

Inhalational agents

Carbon dioxide This method is used on a large scale for chicks up to 72-h old (Clifford 1984). These chicks are young and relatively

insensitive to CO_2 so higher doses may be needed than for adult birds. The chicks should be placed in non-porous bags or containers filled with 100% CO_2 for at least 10 min using a vapour feed system. A closed system is preferable and quicker in induction than open systems. Care should be taken to avoid overcrowding and the levels of CO_2 must be constantly monitored and maintained. Carbon dioxide may also be used in conjunction with argon and oxygen for poultry (Raj & Gregory 1994). The CO_2 causes loss of consciousness and the argon causes death by hypoxia. When killing larger birds with CO_2, care must be taken to ensure that the chamber is completely filled prior to putting the birds into it to ensure uniform levels of CO_2 throughout the chamber. Older birds may flap their wings after losing consciousness which may not be acceptable to some operators.

Volatile inhalational anaesthetics Air or oxygen must be provided during the induction period. Appropriate gas scavenging equipment needs to be used with all these agents.

Halothane, enflurane, isoflurane These agents are considered acceptable for the euthanasia of most birds. They are safe for personnel if used with gas scavenging apparatus and they are effective in causing anaesthesia and euthanasia.

Carbon monoxide Carbon monoxide causes rapid death as it combines with the red blood cells in preference to oxygen, thus causing hypoxia. However, it is extremely dangerous to personnel because it is not easily detectable and should only be used by people trained in the technique and with appropriate gas scavenging apparatus. Only the use of commercially compressed CO should be used for euthanasia. Death must be confirmed by physical means.

Injectable agents

Sodium pentobarbitone This is an acceptable method of euthanasia for birds of all ages. Sodium pentobarbitone causes rapid and relatively stress-free death if carried out by experienced personnel. It should be injected intraperitoneally. Some experienced operators may inject it into the foramen magnum at the base of the skull (intracephalic) which is quick.

T-61 Injected intramuscularly into the pectoral muscles of small birds, T-61 is very effective. It should not be used for larger birds or poultry because it takes some time to act and causes convulsions.

Methods acceptable for unconscious birds

Decapitation This reduces the blood pressure very quickly which may result in unconsciousness as well as massive trauma across the cord at the level of the brain stem with an ascending and descending effect on neural activity. However, work carried out by Gregory and Wotton (1986, 1990) on chickens shows that there are visually evoked responses for up to 30 s after decapitation. Other methods are considered preferable until further research can show that the birds are made immediately insensible.

Pithing This is not acceptable unless the bird is fully anaesthetized.

Nitrogen This kills birds by anoxia. Day-old chicks should not be killed with nitrogen because of their ability to withstand low concentrations of oxygen. The responses by the unconscious bird may be objectionable to personnel. In general the use of nitrogen for the euthanasia of birds is not considered acceptable unless unconscious.

Potassium chloride This is cardiotoxic and causes muscle spasms and convulsive seizures, thus making it unpleasant for the operator. It may only be used once the birds are fully anaesthetized.

Methods not acceptable for euthanasia of birds

Neck crushing The neck of the small bird is pressed against a bar, or a specialized set of pliers, designed for the purpose, is used. It is not known whether neck crushing produces immediate unconsciousness (Gregory &

Wotton 1990). This method is not considered acceptable for euthanasia as there are other more humane methods.

Exsanguination Is not an acceptable method of killing birds because the blood clots easily, possibly resulting in incomplete exsanguination.

Decompression (creating a vacuum) This induces dypsnoea. After 20 s at 60 mmHg the birds collapse. Because the time to unconsciousness is not known and decompression causes rapid expansion of gases in the air sacs and pneumatic bones which may cause pain, this method is not acceptable in the laboratory situation.

Nitrous oxide Hypoxic concentrations of up to 100% are required to be effective and it is slow-acting. The bird will convulse after losing consciousness which may deter some operators. It is not acceptable for euthanasia of birds.

Ether and chloroform These are not to be used for the killing of birds due to extreme operator danger and irritation to the respiratory passages of the bird.

Table 5 Characteristics of methods for euthanasia of birds

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
T-61	++	++	+	+	++	4	Requires expertise: acceptable for small birds only (< 250 g)
Carbon dioxide	++	++	++	++	+	4	Acceptable method, especially for chicks
Halothane, enflurane, isoflurane	++	++	++	+	++	4	Acceptable
Maceration	++	++	++	++	–	4	Acceptable for chicks up to 72 h
Cervical dislocation	++	++	–	++	–	4	Acceptable for small and young birds (< 250 g) if followed by destruction of the brain
Microwave	++	++	–	++	+	3	To be used by experienced personnel only. Not a routine procedure
Concussion	++	++	–	++	–	3	Acceptable for birds under 250 g
Carbon monoxide	+	+	++	–	+	2	Danger to operator
Electrical stunning	+	+	+	–	–	1	Danger to operator. Other methods preferable

The following methods may only be used on unconscious birds: decapitation, pithing, nitrogen, potassium chloride

The following methods are not to be used for killing birds: neck crushing, decompression, exsanguination, nitrous oxide, diethyl ether, chloroform, cyclopropane, hydrogen cyanide gas, trichlorethylene, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

Cyclopropane This is humane and causes rapid anaesthesia, but is flammable and explosive in air. Because of the danger to the operator, cyclopropane is not acceptable for euthanasia of birds.

Hydrogen cyanide gas This causes rapid and irreversible death by cytotoxic hypoxia but it also causes excitability and distress before death which makes it totally unacceptable.

Other agents not to be used include *methoxyflurane*, *trichlorethylene*, *chloral hydrate*, *strychnine*, *nicotine*, *magnesium sulphate*, *ketamine alone* and *neuromuscular blocking agents*.

3.5 Rodents

Rodents are the most commonly used animals for experimental purposes and include mice, rats, hamsters, guineapigs, gerbils, shrews, and dormice. A summary of recommendations is given in Table 6.

Recognition and confirmation of death

Cessation of respiration and heartbeat, and absence of reflexes are good indicators of irreversible death in rodents. Death may be confirmed by exsanguination or extraction of the heart, evisceration, deep-freezing or decapitation. Personnel must be trained to recognize and ensure death when killing rodents.

Embryos

The time at which the neural tube develops into a functional brain (about 60% gestation) must be taken as the time at which the foetus may perceive pain and should therefore be killed humanely. There is a large variation in degree of development at birth of various rodents. Mice and rats are totally dependent on the nest and have very few layers of neuronal development in the cerebral cortex, whereas guineapigs are fully developed and independent at birth.

If a foetus is removed from an anaesthetized mother, and is also insensible, then it may be killed by decapitation or removal of the heart. However, when the foetus is to be removed an increased amount of anaesthetic must be administered to the dam and maintained for longer to ensure that the anaesthetic has crossed the placenta. In many cases inhalational anaesthetics will not anaesthetize a foetus. Foetuses under 4 g not anaesthetized prior to removal from the dam may be killed by rapid cooling in liquid nitrogen.

Neonates

These are newborn rodents up to 10 days old. They may react more like embryos than adults to painful stimuli. They may be killed by decapitation or concussion. Hypothermia may be considered (Phifer & Terry 1986). Carbon dioxide is not recommended as neonates are more resistant to it, increasing the time to unconsciousness. Further research is necessary to assess which methods are the most humane.

Adults

Further details on the methods may be found in Section 2.

Physical methods

When carrying out physical methods of killing rodents, consideration must be given to the careful handling and restraint of the animal prior to killing. Minimal handling and restraint is preferable. Fear and anxiety to the animal may be reduced by prior sedation or handling by familiar people.

Concussion This is a quick and humane method of stunning rodents as long as it is performed by experienced and confident operators. This should only be used on rodents of less than 1 kg, above which considerable skill and sometimes a great deal of strength are required to perform it efficiently. Death must always be confirmed.

Cervical dislocation This is a commonly used and humane method of killing most small rodents (under 150 g (Marshall *et al.* 1994)) as it causes extensive damage to the brainstem resulting in immediate unconsciousness and death. It is more difficult on hamsters and guineapigs due to their short necks, stronger neck muscles and loose skin over neck and shoulders. In mice and rats the thumb and index finger are placed on either

side of the neck at the base of the skull, or, alternatively, a rod is pressed at the base of the skull and then with the other hand, the base of the tail or hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. Larger rodents and older rats should be sedated or stunned prior to dislocation. Death must be confirmed as indicated in the section, *recognition and confirmation of death*.

Decapitation The immediate lack of circulation of blood to the brain and subsequent anoxia is thought to render the head rapidly insensible (Derr 1991). Prior anaesthetization is not generally recommended as this involves further handling and consequent stress to the animal. Other methods are preferable until further research can show immediate unconsciousness. Specialized apparatus should always be used. Care must be taken to ensure that the apparatus is kept clean and the blades are sharp.

Microwave This involves the use of specialized apparatus and should only be carried out by personnel specially trained in this technique to ensure correct positioning of the beam. When carried out correctly, it is an extremely quick method of killing rodents and therefore very humane. *Under no circumstances should domestic appliances be used for this purpose.* This is not a routine method of euthanasia.

Rapid freezing This is carried out by putting the animal into liquid nitrogen. This may only be used for foetuses and small neonates (<4 g) with no fur. Larger or furred animals will not die immediately as it takes some time for the core to become frozen.

Chemical methods

When using chemical methods for euthanasia, death must be confirmed by one of the methods listed above.

Inhalational agents

Volatile inhalational anaesthetics With these agents the rodent is put into an anaesthetic chamber or an appropriate receptacle with gauze or cotton wool soaked in the anaesthetic. Because the liquid state of these anaesthetics are irritant, care must be taken to ensure that the rodent cannot come into contact with the chemical. Air or oxygen must be provided during the induction period.

Halothane, enflurane, isoflurane These agents act by depression of the cardiovascular and respiratory systems. They induce anaesthesia and subsequent death. These are all acceptable agents when used with appropriate gas scavenging apparatus.

Carbon dioxide It is recommended that a minimum of 70% CO_2 in oxygen or air be used for quick loss of consciousness without hypoxia. This results in rapid anaesthesia followed by death with reduced effects from irritation of the respiratory passages. One hundred per cent CO_2 is recommended for guineapigs (Noonan 1994). Only CO_2 from commercially available gas cylinders should be used for euthanasia of rodents. See Section 2 for further details.

Carbon monoxide Although this is a relatively quick and humane method of killing rodents, because of the danger to the operator it must be used with extreme caution. If chosen, it should be used with appropriate gas scavenging appparatus and only commercially available gas in cylinders is recommended. The rodents must be placed into a container prefilled with at least 6% CO by volume.

Injectable agents

In larger rodents, where venepuncture is possible without due stress to the animal, intravenous injection is recommended as this results in rapid anaesthesia and death. If venepuncture is not easily performed, intraperitoneal injection is preferred, although it takes longer to act and irritation of the peritoneum may occur. Under no circumstances should intrapulmonary or intracardial injection be given unless the animal is fully anaesthetized.

Sodium pentobarbitone Injected intravenously or intraperitoneally sodium pento-

barbitone acts quickly and humanely to kill all rodents. This is the most acceptable agent for euthanasia. All personnel must be trained in the method of injection. Sodium pentobarbitone may cause irritation of the peritoneum, which can be avoided by dilution. Three times the anaesthetic dose is usually recommended (Marshall *et al.* 1994, Noonan 1994).

T-61 T-61 acts quickly but must be injected intravenously very slowly, which is not always easy in rodents. It should never be injected by any other route in these species. Prior sedation may be required to assist with restraint during injection. Personnel must be well trained in intravenous injection techniques.

Methods acceptable for unconscious rodents

Rapid freezing This may only be used once the rodent (>4 g) is fully unconscious.

Exsanguination This may be used once the rodent is unconscious.

Air embolism This may only be used on unconscious rodents as it can be painful.

Potassium chloride This is cardiotoxic and causes gasping, vocalizations, muscle spasms and convulsive seizures which make it

Table 6 Characteristics of methods for euthanasia of rodents

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
Halothane, enflurane, isoflurane	++	++	++	+	++	5	Acceptable
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
Concussion	++	++	+	++	–	4	Acceptable for rodents under 1 kg. Death to be confirmed by cessation of circulation
Cervical dislocation	++	++	+	++	–	4	Acceptable for rodents under 150 g. Death to be confirmed by cessation of circulation
T-61	++	++	–	+	++	4	Only to be injected intravenously
Carbon dioxide	+	++	++	++	++	4	Acceptable method at >70%
Microwave	++	++	–	++	+	3	To be used by experienced personnel only. Not a routine procedure
Decapitation	+	+	+	++	–	2	Other methods preferable
Carbon monoxide	+	+	+	–	++	2	Danger to operator
Rapid freezing	–	+	++	++	+	1	Only in small neonates (<4 kg)

The following methods may only be used on unconscious rodents: rapid freezing, exsanguination, air embolism, potassium chloride, ethanol

The following methods are not to be used for killing rodents: hypothermia, decompression, asphyxia, drowning, nitrogen, nitrous oxide, cyclopropane, diethyl ether, chloroform, methoxyflurane, hydrogen cyanide gas, trichlorethylene, strychnine, nicotine, chloral hydrate, magnesium sulphate and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

unacceptable for many operators. It may only be used once the animal is fully anaesthetized.

Ethanol This has been used injected intraperitoneally at 70% (Lord 1989). However, Wallgren & Barry III (1970) state that it is irritant at above 10%, making this unacceptable for euthanasia unless the rodent is unconscious.

Methods not acceptable for euthanasia of rodents

Hypothermia *Under no circumstances should any rodent be killed by placing in a freezer.* Freezing may only be used as a method of ensuring death.

Nitrogen This kills rodents by hypoxia making it unacceptable as it takes longer than other agents to achieve unconsciousness. Rats exhibit signs of panic and distress prior to unconsciousness (Hornett & Haynes 1984).

Nitrous oxide This kills by anoxia, is slow, and the rodents show signs of increased activity prior to death, indicating a degree of anxiety making it unacceptable as a method for euthanasia.

Cyclopropane This is a humane and quick agent for killing rodents but it is extremely dangerous to the operator and is therefore not considered acceptable.

Ether and chloroform These are not to be used under any circumstances for euthanasia of rodents. They are both extremely dangerous to the operator and ether causes irritation to the respiratory passages upon inhalation.

The following agents are not be used for killing rodents: *decompression*, *asphyxia*, *drowning*, *trichlorethylene*, *methoxyflurane*, *hydrogen cyanide gas*, *strychnine*, *nicotine*, *chloral hydrate*, *magnesium sulphate*, *curariform drugs* and *other neuromuscular blocking agents*.

3.6 Rabbits

A summary of recommendations is given in Table 7.

Recognition and confirmation of death

Cessation of respiration and heartbeat, and absence of reflexes are good indicators of irreversible death in rabbits. Death may be confirmed by exsanguination or removal of the heart, evisceration, or decapitation. Personnel must be trained to recognize and ensure death when killing rabbits.

Embryos

The time at which the neural tube develops into a functional brain (about 60% gestation) must be taken as the time at which the foetus may perceive pain and should therefore be killed humanely. If an insensible foetus is removed from an anaesthetized mother it may be killed by decapitation or removal of the heart. However, when the foetus is to be removed an increased amount of anaesthetic must be administered to the dam and maintained for longer to ensure that the anaesthetic has crossed the placenta. In many cases inhalational anaesthetics will not anaesthetize a foetus. Those foetuses that are not removed from the dam will die of anoxia when the dam is killed and no further method is necessary to ensure death of the foetus.

Neonates

These are newborn rabbits up to 10 days old. They may react to painful stimuli more like an embryo than an adult. They may be killed by decapitation and concussion. Further research is necessary to assess which methods are the most humane.

Adults

Further details on methods may be obtained in Section 2.

Physical methods

When carrying out physical methods of killing rabbits, consideration must be given to the careful handling and restraint of the animal prior to killing. Minimal handling and restraint is preferable. Fear and anxiety to the animal may be reduced by prior sedation or handling by familiar people.

Concussion This is a quick and humane method of stunning rabbits as long as it is performed by experienced and confident operators. This involves striking the base of the head at the top of the neck in the occipital region. Death must always be confirmed by cessation of circulation.

Cervical dislocation This is a humane method of killing rabbits of under 1 kg as it causes extensive damage to the brainstem resulting in immediate unconsciousness and death. It should only be carried out by experienced personnel. Death must always be confirmed by cessation of circulation. Sedation may be necessary prior to dislocation.

Captive bolt This method may be useful for large rabbits (over 4 kg) in limited situations (Holtzmann 1991). Only captive bolts designed specifically for use on rabbits may be used. Personnel must be well trained in order to ensure correct positioning of the weapon. The bolt must penetrate about 3 cm into the brain (Holtzmann 1991). Death must be confirmed by ensuring cessation of circulation.

Decapitation Decapitation may be considered a humane method of killing small or young rabbits (under 1 kg) as loss of blood supply ensures rapid loss of consciousness. However, this is not possible in larger and older rabbits where the neck is too thick and strong for quick decapitation.

Electrical stunning Only electric tongs designed for this purpose may be used. Care must be taken to ensure that the correct level of current passes directly through the brain to ensure immediate unconsciousness. Death must be confirmed by cessation of circulation.

Microwave This involves the use of specialized apparatus and should only be carried out by personnel specially trained in this technique to ensure correct positioning of the beam. It is a quick method of killing small rabbits of under 300 g. *Under no circumstances should domestic appliances be used for this purpose.* This is not a routine method of euthanasia.

Rapid freezing Foetuses of under 4 g may be killed by putting into liquid nitrogen. Larger or furred animals will not die immediately as it takes some time for the core to become frozen.

Chemical methods

When using chemical methods of euthanasia, death must be confirmed by one of the methods listed above.

Inhalational methods

Volatile inhalational anaesthetics Rabbits react adversely to all gases (Green 1979) and other methods are preferable if possible.

With these agents the rabbit is put into an anaesthetic chamber or an appropriate receptacle with gauze or cotton wool soaked in the anaesthetic. Vapours are inhaled until respiration ceases and death ensues. Because the liquid state of these anaesthetics are irritant, care must be taken to ensure that the rabbit cannot come into contact with the chemical. Air or oxygen must be provided during the induction period. Appropriate gas scavenging equipment needs to be used with all these agents.

Halothane, isoflurane, enflurane At high concentrations these agents cause rapid anaesthesia and consequent death. These are all acceptable agents when used with appropriate gas scavenging apparatus.

Carbon dioxide Large rabbits may become distressed initially whilst still conscious and therefore other methods are considered preferable if possible. One hundred per cent CO_2 has been recommended by Von Cranach *et al.* (1991a) but may cause distress.

Carbon monoxide Although this is a relatively quick and humane method of killing rabbits, because of the danger to the operator it is less acceptable for routine use. If used, it should be used with appropriate gas scavenging apparatus and only commercially available gas in cylinders should be used as fumes

from combustion engines are likely to be irritant.

Injectable agents

In rabbits, where venepuncture is possible via the ear vein (unless damaged) intravenous injection is recommended as this results in rapid anaesthesia and death. Personnel must be trained in the techniques of intravenous and intraperitoneal injection. If venepuncture is not easily performed, intraperitoneal injection is acceptable, although it takes longer to act. Under no circumstances should intrapulmonary or intracardial injection be given unless the animal is fully anaesthetized.

Sodium pentobarbitone Injected intravenously, sodium pentobarbitone acts quickly and humanely to kill rabbits. This is the most acceptable agent for euthanasia. Sodium pentobarbitone may cause irritation to the peritoneum which may be avoided by dilution.

Table 7 Characteristics of methods for euthanasia of rabbits

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
T-61	++	++	–	+	++	4	Acceptable. Intravenous injection only
Cervical dislocation	++	++	–	++	–	4	Acceptable for rabbits under 1 kg. Sedation prior to dislocation. Death to be confirmed by cessation of circulation
Captive bolt	++	++	–	+	+	4	Requires skill. Death to be confirmed by another method
Concussion	++	+	–	++	–	3	Expertise required. Death to be ensured by another method
Electrical stunning	++	+	++	–	+	3	Death to be confirmed by another method
Microwave	++	++	–	++	+	3	To be used by experienced personnel only on small rabbits. Not a routine procedure
Decapitation	+	+	+	++	–	2	Acceptable for rabbits under 1 kg if other methods not possible
Halothane, enflurane, isoflurane	++	++	++	+	–	2	Rabbits show signs of distress
Carbon dioxide	+	+	++	++	+	1	Large rabbits show distress
Carbon monoxide	+	+	++	–	++	1	Danger to operator
Rapid freezing	+	+	++	++	+	1	Only in foetuses under 4 kg. Other methods preferred

The following methods are only to be used on unconscious rabbits: exsanguination, nitrogen, potassium chloride and air embolism

The following methods are not to be used for killing rabbits: hypothermia, decompression, asphyxia, drowning, nitrous oxide, cyclopropane, diethyl ether, chloroform, trichlorethylene, hydrogen cyanide gas, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, hydrocyanic acid, ketamine hydrochloride and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

T-61 T-61 acts quickly and humanely but may only be injected very slowly intravenously. It should never be injected by any other route. Prior sedation may be considered necessary to assist with restraint during injection. Personnel must be well trained in intravenous injection techniques. Personnel must take extreme care when handling this agent.

Methods acceptable for unconscious rabbits

Exsanguination This may be used to kill rabbits once they are fully unconscious.

Nitrogen This kills rabbits by hypoxia and is therefore not considered acceptable for euthanasia of rabbits unless they are unconscious.

Potassium chloride This is cardiotoxic and causes gasping, vocalizations, muscle spasms and convulsive seizures which makes it unacceptable for many operators. It may only be used once the animal is fully anaesthetized.

Air embolism Intravenous injection of air at 5–50 ml/kg causes rapid death but as this is accompanied by convulsions, opisthotonos, pupillary dilation and vocalizations, it is not an acceptable method unless the rabbit is fully unconscious.

Methods not acceptable for euthanasia of rabbits

Hypothermia Under no circumstances should any rabbits be killed by placing in a freezer.

Nitrous oxide This kills by anoxia, is slow acting, and the rabbits show signs of increased activity prior to death, indicating a degree of anxiety. It is therefore not considered an acceptable method.

Methoxyflurane This takes too long to act with a large chance of recovery.

Cyclopropane This may be a humane and quick method of euthanasia, but is very dangerous to the operator and is therefore not considered acceptable for general use.

Ether and chloroform These are not to be used under any circumstances for euthanasia of rabbits. They are both dangerous to the operator and ether causes irritation to the respiratory passages upon inhalation.

Ketamine hydrochloride Intravenous injection causes tonic contractions, accompanied by vocalizations, thus making this unacceptable for euthanasia of rabbits.

Other agents not to be used for killing rabbits include *decompression*, *asphyxia*, *drowning*, *trichlorethylene*, *hydrogen cyanide gas*, *hydrocyanic acid*, *strychnine*, *nicotine*, *chloral hydrate*, *magnesium sulphate* and *neuromuscular blocking agents*.

3.7 Carnivores — dogs, cats, ferrets

A summary of recommendations is given in Table 8.

Recognition and confirmation of death

Cessation of respiration and heartbeat, and loss of reflexes are good indicators of irreversible death in carnivores. Death should be confirmed by exsanguination. Personnel should be trained to recognize and ensure death when killing dogs, cats, ferrets or other carnivores.

Embryos

The time at which the neural tube develops into a functional brain must be taken as the time at which the foetus may perceive pain (30% gestation) and therefore should be killed humanely. If an insensible foetus is removed from an anaesthetized mother then it may be killed by decapitation or removal of the heart. However, when the foetus is to be removed an increased amount of anaesthetic must be administered to the dam and maintained for longer to ensure that the anaesthetic has crossed the placenta. In many cases inhalational anaesthetics will not anaesthetize a foetus.

Neonates

Neonate carnivores should generally be treated as adults. Sodium pentobarbitone is

the preferred method, but CO_2, cervical dislocation and concussion may be considered (Hall 1972) (see Section 2). Operators must be well trained in the physical techniques to ensure that they are correctly and humanely carried out. The animals must be exsanguinated immediately after concussion or cervical dislocation.

Adults

Further details on methods are given in Section 2.

Physical methods

In general it is not recommended to use physical methods of euthanasia on carnivores. However, where chemical agents may interfere with the aims of the experiment, the following methods may be used. Restraint of cats for physical methods may be difficult and it is recommended that all animals be sedated prior to euthanasia.

Captive bolt Captive bolts designed specifically for the purpose of killing animals of this size may be used. Personnel must be trained in these techniques to ensure correct positioning of the pistol and immediate death. Death must be confirmed by cessation of circulation by exsanguination.

Shooting Shooting of carnivores using a free bullet is only acceptable under field conditions when no other methods can be used. Only specialized marksmen should be used.

Electrocution Ear clips are attached to ensure that the current flows directly through the brain and death is confirmed by passing the current through the heart. There are two phases: stunning with 500 V shock between the ears, followed by a lethal shock at 1 kV passing from the ear to hindleg. Cats should not be killed by electrocution due to the
high conductivity of their coats. Only specially designed apparatus should be used for this purpose and personnel must be well trained in this technique. Equipment must be regularly checked and maintained to ensure correct voltage. Death must be confirmed by one of the methods in the section *recognition and confirmation of death*.

Chemical methods

In general, chemical methods of euthanasia are preferred for all dogs, cats, ferrets and foxes. It may be preferable to sedate the animal prior to euthanasia to reduce stress and anxiety.

Inhalational methods

Volatile inhalational anaesthetics These include halothane, isoflurane and enflurane. These are all acceptable methods of euthanasia for carnivores. Appropriate gas scavenging apparatus should be used to prevent operator exposure.

Injectable agents

If possible injection should be given intravenously in order to achieve rapid anaesthesia and euthanasia with minimal stress.

Sodium pentobarbitone Injected intravenously, this agent provides rapid and humane euthanasia. Intracardiac and intrapulmonary routes of injection should not be used as they are extremely painful, unless under full anaesthesia. All personnel must be trained in these techniques.

Secobarbital/dibucaine Secobarbital is a short-acting analogue of thiamylol sodium. Dibucaine is a highly toxic local anaesthetic causing rapid loss of consciousness, loss of respiration and cardiac arrest (Herschler *et al.* 1981, Wallach *et al.* 1981).

T-61 This agent is very effective but must only be injected very slowly intravenously. Animals must be sedated prior to administration. It may cause convulsions in the unconscious animal, which may be aesthetically unpleasant.

Methods acceptable for unconscious carnivores

Exsanguination This may be used to kill carnivores once they are unconscious.

Dislocation of neck This may be used on small animals under anaesthetic. Death must always be confirmed by one of the methods listed above.

Potassium chloride This may be used to kill unconscious carnivores.

Methods not acceptable for euthanasia of carnivores

Striking of chest of cats Has been suggested as a method of euthanasia but this is not considered as humane and is not to be used under any circumstances.

Decompression Has been used as a method of euthanasia in the USA and Japan. It probably causes much anxiety and stress to the animals and they may experience pain due to the expansion of air in the sinuses and other body cavities. It is not considered acceptable for euthanasia of carnivores.

Although *carbon dioxide* causes cats to become unconscious within 1 min, they move about the cage, licking, sneezing and trying to climb out, indicating that it may be stressful. The animals also convulse which makes this method aesthetically unpleasant for the operator. This is not considered acceptable as a method of euthanasia for carnivores except for neonates.

Carbon monoxide At concentrations above 6% it is a relatively quick method of euthanasia and is recommended for the killing of mustelids (Commission of the European Communities 1993). However, it causes convulsions and vocalizations which may still be in the conscious phase (Chalifoux & Dallaire 1983). Because of this and the

Table 8 Characteristics of methods for euthanasia of dogs, cats, ferrets, foxes

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
Sodium pentobarbitone	++	++	–	+	++	5	Acceptable. Intravenous injection
T-61	++	++	–	+	+	4	Acceptable but only by slow intravenous injection under sedation
Secobarbital/dibucaine	++	++	–	+	++	4	Acceptable. Intravenous injection
Halothane, isoflurane, enflurane	++	++	+	+	++	4	Acceptable
Captive bolt	++	++	–	++	+	3	To be followed by exsanguination
Electrocution	++	++	–	–	–	3	Use only special equipment. To be followed by exsanguination
Concussion	++	++	+	++	–	2	Only to be used on neonates. To be followed by exsanguination
Shooting	++	++	–	–	–	1	Acceptable only in field conditions by specialized marksmen when other methods not possible

The following methods are acceptable for unconscious carnivores: exsanguination, neck dislocation and potassium chloride

The following methods are not to be used for killing carnivores: decompression, decapitation, drowning, strangulation, asphyxiation, air embolism, striking chest of cats, carbon monoxide, carbon dioxide, methoxyflurane, nitrogen, nitrous oxide, trichlorethylene, hydrocyanic acid, diethyl ether, chloroform, hydrogen cyanide gas, cyclopropane, chloral hydrate, strychnine, nicotine, magnesium sulphate and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

danger to the operator, it is not considered acceptable for experimental animals.

Nitrogen This causes unconsciousness in dogs and cats in 1–2 min with hyperpnoea for about 10 s before collapse. After collapse there are vocalizations, opisthonos, convulsions and gasping. Kittens and puppies are resistant to anoxia; they fall unconscious but fail to die. This is not an acceptable method.

Ether and chloroform These are not acceptable methods of euthanasia due to irritation of the respiratory passages and danger to the operator.

The following agents are also not to be used for killing carnivores: *drowning*, *concussion* (adults), *decapitation*, *asphyxia*, *strangulation*, *nitrous oxide*, *hydrogen cyanide gas*, *cyclopropane*, *methoxyflurane*, *trichlorethylene*, *air embolism*, *hydrocyanic acid*, *chloral hydrate*, *strychnine*, *nicotine*, *magnesium sulphate*, and *neuromuscular blocking agents*.

3.8 Large mammals — pigs, sheep, goats, cattle, horses

Personnel using and having to kill any large mammal must receive specialized training in the handling, restraint and techniques of euthanasia of these animals. It is important to avoid actions which may increase the animals' awareness of the unusual situation. The animal is best killed in a familiar environment.

All operators are recommended to obtain and read EC (Council Directive 93/119/EC) (Commission of the European Communities 1993) and national regulations on slaughter methods which cover most of these animals. It may be necessary to take the animals to approved slaughterhouses where specialized equipment is available for humane euthanasia of these animals. Euthanasia may have to be carried out by a person who has been trained and holds a certificate under national slaughter legislation or by a veterinarian with appropriate training.

A summary of recommendations is given in Table 9.

Recognition and confirmation of death

Cessation of respiration and heartbeat, and loss of reflexes are good indicators of irreversible death. Death should be confirmed by exsanguination. Personnel should be trained to recognize and ensure death when killing these animals.

Embryos

The foetuses of these large mammals are well developed at birth and therefore considerable care must be taken to ensure that they are killed humanely if removed from the uterus. The time at which euthanasia should be considered should be from the time at which the neural tube develops into a functional brain and they may thus be able to feel pain (>30% gestation). Foetuses may also be large and in general any method used on an adult is considered acceptable.

Neonates

Because large mammals are born in an advanced stage of development, they should be treated as adults.

Adults

Further details on methods are given in Section 2.

Physical methods

The animals must be suitably restrained in adequate devices to ensure that the animal remains still and calm so that the method of euthanasia is accurate and quick. Personnel should be quiet and handle the animals with care so as to reduce stress and anxiety in the animals.

Captive bolt The use of captive bolt is the most acceptable physical method for large mammals and is preferable to free bullets because of operator safety. Penetrative captive bolts are preferred. Personnel must be well trained in the use of captive bolt pistols to ensure correct positioning for the species being killed (Universities Federation for Animal Welfare 1989). Care must be taken to ensure that the correct size bolt and cartridge are used and that the weapon is kept clean

and maintained in good working order. It is not recommended to use captive bolt pistols on adult pigs and mature bulls because of their thick skulls. Death must be confirmed immediately by exsanguination or pithing through the hole made by the bolt.

Free bullet humane killers A humane killer firing a free bullet is an efficient method of killing horses, mules, donkeys and old or hard-headed animals (Blackmore 1985, Dodd 1985, Oliver 1979). Extra care must be taken as it is not as safe as the captive bolt and it is therefore considered acceptable only under field conditions. All personnel must be trained in these methods to ensure correct positioning of the weapon and that the correct calibre of weapon is used. It must be noted that the position of the weapon differs with each species and whether the animal is horned or not. The animals tend to slump forwards when shot and therefore the operator must take care to avoid personal damage. Operators must ensure that the weapon is well maintained so that the chance of misfiring is minimized. Death must be confirmed immediately by exsanguination or pithing through the hole made by the bullet.

Shooting Shooting using a free bullet should only be done in field conditions when no other method can be used. Only specialized marksmen should carry this out.

Concussion This should be done using a mechanically operated instrument which administers a blow to the skull without fracture of the skull. It must be noted that the position for the application of percussion stunners differs from that for the captive bolt pistols. Death must be ensured by immediate exsanguination (within 20 s of stunning) (Blackmore 1979).

Electrical stunning This method should only be carried out in slaughterhouses where specialized equipment is available for the animals being killed. It is commonly used for stunning pigs, sheep, calves and goats. Tongs should be applied on each side of the head between the eye and ear to span the brain. Moist sponge rubber pads fitted to the tongs are considered undesirable when dealing with high voltages. Sheep and goats should be shaved in the region of the tongs to ensure good electrical contact. It should not be used where horns make it difficult to position the tongs correctly. Care must be taken to ensure that the animals cannot receive electrical shocks from contact with other animals, from wet surfaces, or from accidental contact with the tongs. Head only or head to back stunning across the head are both acceptable methods as they ensure immediate unconsciousness. Animals must be exsanguinated immediately after stunning to ensure death. Personnel must ensure that the correct voltage and current is used for the species of animal being killed.

Chemical methods

Inhalational methods

Volatile inhalational anaesthetics Halothane, isoflurane and enflurane may be used with an anaesthetic mask for lambs and kids.

Carbon dioxide Carbon dioxide has been used to kill pigs. The pigs are placed into large chambers that are previously filled with CO_2 gas to above 70%. Specialized equipment only must be used. Death must be confirmed by exsanguination. Because pigs tend to show signs of stress other methods are considered preferable. Carbon dioxide must not be used on any other large animal.

Injectable methods

Personnel must be trained in intravenous injection techniques and handling and restraint of the animals. Animals should be suitably restrained and/or sedated prior to euthanasia.

Sodium pentobarbitone Injected intravenously, sodium pentobarbitone provides rapid euthanasia. Large volumes may be required for larger mammals and this may be made easier by the insertion of a catheter into the jugular vein (Andrews *et al.* 1993). Alternatively lower volumes of highly concentrated solutions may be used, but it must be recognized that this poses a greater danger to the operator. Excitable or nervous animals

Table 9 Characteristics of methods for euthanasia of large mammals

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1–5)	Remarks
Sodium pentobarbitone	++	++	–	+	++	5	Acceptable by intravenous injection
Quinalbarbitone/ Nupercaine	++	++	–	+	++	5	Effective for horses intravenously
Captive bolt	++	++	+	+	+	5	To be followed by exsanguination
Free bullet humane killer	++	++	+	–	+	5	To be followed by exsanguination. In field conditions only
T-61	++	++	–	+	++	4	Acceptable by intravenous injection
Electrical stunning	++	++	+	–	–	4	Use only specialized equipment. To be followed immediately by exsanguination
Shooting	++	++	–	–	–	2	Only in field conditions by a specialized marksman
Concussion	++	+	–	+	+	2	To be followed immediately by exsanguination
Halothane, isoflurane, enflurane	+	+	+	+	+	2	Recommended for lambs and kids
Carbon dioxide	+	+	++	++	+	1	Only for use with pigs at $>70\%$

The following methods are acceptable only on unconscious large mammals: exsanguination, chloral hydrate and potassium chloride

The following methods are not to be used for killing large mammals: carbon monoxide, methoxyflurane, trichlorethylene, strychnine, nicotine, magnesium sulphate, thiopentone sodium, ketamine hydrochloride, neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, – slow. **Efficacy**: ++ very effective, + effective, – not effective. **Ease of use**: ++ easy to use, + requires expertise, – requires specialist training. **Operator safety**: ++ no danger, + little danger, – dangerous. **Aesthetic value**: ++ good aesthetically, + acceptable for most people, – unacceptable for many people. **Rating**: 1–5 with 5 as highly recommended

must be sedated prior to injection with sodium pentobarbitone.

Quinalbarbitone/Nupercaine This proprietary mixture causes rapid and humane death in horses. This should be injected intravenously over a period of 5–8 s. It is not available in some countries.

T-61 T-61 is also an effective agent for euthanasia of large mammals by slow intravenous injection only. It may be necessary to sedate excitable or nervous animals initially.

Methods acceptable for unconscious large mammals

Exsanguination This may be used to kill unconscious mammals.

Chloral hydrate This may be used intravenously on unconscious mammals, or in conjunction with magnesium sulphate and sodium pentobarbitone.

Potassium chloride This may be used to kill unconscious mammals.

Methods not acceptable for euthanasia of large mammals

Carbon monoxide Some animals, including pigs, show signs of severe excitation and vocalizations, sometimes before unconsciousness, at high levels of carbon monoxide. It is not an acceptable agent for euthanasia.

The following agents are also not acceptable for euthanasia of large mammals: *methoxyflurane*, *trichlorethylene*, *strychnine*, *nico-*

tine, *magnesium sulphate*, *thiopentone sodium*, *ketamine hydrochloride*, *curariform drugs* and other *neuromuscular blocking agents*.

3.9 Non-human primates

Personnel handling primates should be specially trained for these purposes. It is preferable that if primates have to be killed, that this be carried out by someone familiar to them in order to reduce stress and anxiety. For all larger primates, sedation (e.g. ketamine) should be administered prior to euthanasia.

Cessation of heartbeat and respiration, and absence of reflexes may be considered as good indicators of death.

Embryos

All foetuses in which the neural tube has developed into a functional brain must be killed humanely. Foetuses are sometimes required for experimental purposes but the mother is rarely killed to allow the removal of the foetus from the uterus. These foetuses may be killed by overdose of anaesthetic or physical methods after anaesthetization.

Adults

The only recommended method for killing primates is by overdose of anaesthetic. Sodium pentobarbitone injected intravenously is the most acceptable agent. Exsanguination under inhalation anaesthesia is also considered acceptable, but this must be followed by perfusion.

Infants of some small species such as marmosets may be difficult to inject and this requires specialist expertise.

3.10 Other animals not commonly used for experiments

As vertebrate animals vary so much in size and physiology, the method chosen to kill any animal not included above should be chosen from those methods for animals that are most similar biologically. Advice should be obtained from a veterinarian. In general, an overdose of sodium pentobarbitone injected intravenously may be considered as a humane method of killing most animals. It is advisable in most cases to sedate the animal prior to euthanasia.

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Euthanasia training materials

Inter-act animal care training programmes

This is a specially designed series of programmes produced by the Association of the British Pharmaceutical Industry (ABPI) as a resource for the training of laboratory staff involved in the care and use of animals under the Animals (Scientific Procedures) Act 1986. Topic 7 covers euthanasia. Using interactive video technology, the full series of 11 programmes (held on 10 laser discs) is available from Mr M. Connelly, Redway Interactive Video, 34 Redway, Kerridge, Macclesfield, Cheshire SK10 5BA, UK.

Principles of proper laboratory animal use in research

This program is a basic program for research investigators, technicians and support staff. This program establishes the appropriate foundation for use of animals in biomedical research. It is a modular, flexible and easy to use software template. The program consists of a template divided into a series of modules containing information on the following topics: regulations, ethics, euthanasia, anaesthesia and analgesia, pre- and post-operative care, safety, disease, nutrition, alternatives and models, species information, USDA Animal Welfare Act. The program is designed with many applications in mind. Runs on Macintosh and IBM PC computers and requires a hard disk and 640K RAM. MTM Associates Inc., PO Box 1606 Manassas, Virginia 2110, USA. Item no. 4211WP.

Using animals in research: guidelines for investigators (1986)

This film records in its entirety a course sponsored by the USDA/Agricultural Research Service and presented to investigators on 25 March 1986. Speakers for the Animal and Plant Health Service, National Institutes of Health (NIH), Public Health, and Agricultural Research Service present information on laws, policies and practices that affect the use of research laboratory; and provide references for those who want to learn more about specific procedures. Tape 1: regulatory issues from an aphis perspective. Tape 2: film 'Unnecessary Fuss', assembled by PETA from University of Pennsylvania research documentation. Tape 3: principles and policies for animal use in NIH extramural programmes. Tape 4: principles and policies for animal use in the Beltsville area. Tape 5: technical information and training opportunities for animal users. Tape 6: panel discussion of audience questions. Videocassette, U-matic, 3/4'', NTSC, English, 240 min (6 tapes). National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 186.

Euthanasia interactive video programme

To provide the trainee with an understanding of the legal requirements of Schedule 1 (UK) and the Alderley Park site regulations to be found in the 'Humane Policy for Euthanasia of Protected Species'. Demonstrates the correct procedure for euthanasia when using the following methods: overdose of anaesthetic, physical methods, carbon dioxide. It provides a means by which the trainees' understanding of the above can be assessed.

Approximately 1¼ h. A printout of the trainees' responses to questions posed during the programme is available. It may be viewed without the interactive component — this lasts about 25 min. English language only. Contact Mr Bob Kemp, Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK. Tel: +44-1625-512726; Fax +44-1625-583074/586278.

Common procedures and techniques and survival surgery: tape II (1988)

Handling laboratory animals in blood collection, gavage, injection and euthanasia: emphasizes minimization of animal discomfort. Videocassette, NTSC/U-matic/VHS, English, 21 min. MDA-TV, University of Texas Cancer Centre, 151 Holcombe Houston, Texas 77030, USA. Order no. 861188.

Practical methodology: reptiles part III special laboratory (1988)

The second of a two part series, this programme covers laboratory techniques such as sexing, blood sampling, catheter placement, injection sites, oral gavaging, intubation, restraint, anaesthesia and euthanasia for reptiles. A manual accompanies the videocassette. Videocassette, 1/2'' VHS, NTSC, English, 18 min. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 414 Part II.

Biomethodology of the mouse

This video shows a close-up, detailed footage demonstrating proper techniques of handling

and restraint, identification, injections and blood sampling. The techniques presented are the most common, reproducible, safe and least stressful to the animal. Video, VHS (1/2'') U-matic (3/4'') standard tape formats. MTM Associates Inc., PO Box 1606, Manassas, Virginia 2110, USA. Item no. 3211V.

The mouse: handling, restraint, and other techniques (1975)

This programme demonstrates basic technical skills required for the proper care of mice and their use in biomedical research. It includes handling and restraint, injections and oral administration of medicine, blood and urine collection, sexing, identification, anaesthesia and euthanasia. A manual accompanies the slide set. Slides, 48 slides, audiocassette, 12 min, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no. 227, Part 2.

Biomethodology of the laboratory mouse (1987)

A demonstration of basic techniques involving laboratory mice including identification, restraint, injection, blood withdrawal and euthanasia. Videocassette 1/2'', VHS (NTSC), colour, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 200.

The mouse, rat and hamster (1988)

This training film provides information on the humane care and use of laboratory rodents for scientists, laboratory technicians and students. The recommendations are consistent with the Public Health Services 'guide for the care and use of laboratory animals' and regulations set by the USDA. Topics include housing, nutrition, environment, record keeping, animal health care, occupational safety, handling and restraint, experimental techniques, and euthanasia. A manual that includes a script, test and answer key accompanies the videocassette. Videocassette 1/2'', VHS (NTSC), English, 34 min. National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no. 338, Vol. 2.

The laboratory rat, biology, husbandry, and research methodology (1977)

This slide set describes and illustrates basic anatomy and physiology of laboratory rats, discusses standard procedures for housing rats in the laboratory, and familiarizes the viewer with basic methodology employed for manipulating rats in research. Research methodology includes handling, restraint, blood collection, anaesthesia and euthanasia. Biological values, listed in the accompanying guide. Slides, 59 slides, audiocassette, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no. 221.

Biomethodology of the rat (1987)

Demonstration of basic techniques involving laboratory animals including identification, restraint, injection, blood withdrawal, and euthanasia. Video 1/2'' VHS, English, 16 min. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 200.

Biomethodology of the guineapig (1987)

Demonstration of basic techniques involving laboratory animals including identification, restraint, injection, blood withdrawal, and euthanasia. Videocassette 1/2'' VHS, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 200.

Biomethodology of the rabbit (1987)

Demonstration of basic techniques involving laboratory animals including identification, restraint, injection, blood withdrawal, and euthanasia. Video 1/2'' VHS, English, 15 min. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 200.

Biomethodology of the cat (1987)

This film demonstrates to animal technicians basic techniques for handling, restraining, and manipulating cats for research.

Covers removal from caging, injection routes, blood collection, and euthanasia. Videocassette 1/2'' VHS (NTSC), colour, 15 min, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 337.

The dog and cat (1988)

This programme provides information on the humane care and use of laboratory dogs and cats for scientists, laboratory technicians, and students. The recommendations are consistent with the Public Health Service 'guide for the care and use of laboratory animals' and regulations set by the USDA. Topics include housing, nutrition, environment, record keeping, animal health care, occupational safety, handling and restraint, experimental techniques and euthanasia. A script, test, and answer key accompany the videocassette. Videocassette 1/2'' VHS (NTSC), colour, English, 35 min. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 338, Vol 4.

Biomethodology of the dog (1987)

This film demonstrates to animal technicians safe and humane techniques for manipulating dogs in research including removal from caging, injection routes, blood collection, and euthanasia. Videocassette 1/2'' VHS (NTSC), colour, 15 min, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 335.

Biomethodology of the primate (1987)

This film demonstrates techniques for manipulating primates for research. It includes manual and chemical restraint, identification, injection routes, blood collection, and euthanasia. Videocassette 1/2'' VHS (NTSC), colour, English. National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no. 336.

The non-human primates (1988)

This programme provides information on the humane care and use of laboratory primates for scientists, laboratory technicians, and students. The recommendations are consistent with the Public Health Services 'guide for the care and use of laboratory animals' and regulations set by the USDA. Topics include housing, nutrition, environment, record keeping, animal health care, occupational safety, handling and restraint, experimental techniques, and euthanasia. A manual that includes a script, test and answer key accompanies the videocassette. Videocassette 1/2'' VHS (NTSC), English, 29 min. National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no. 338, Vol 5.

Further information on training materials

Information on training materials may be obtained from:

(1) Dept of Laboratory Animal Science (Dr Jan Nab, Ing T.P. Rooymans) Utrecht University, Postbus 80.166, 3508 TD Utrecht, The Netherlands.
(2) NORINA database. English language database of audiovisuals for use in the biological sciences. Contact Karina and Adrian Smith, Laboratory Animal Unit Norwegian College of Veterinary Medicine, PO Box 8146 Dep., 00033 Oslo 1, Norway. Fax +47 22 96 45 35, Telephone +47 22 96 45 74, email: adrian.smith@veths.no.