Principles in laboratory animal research for experimental purposes

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SUMMARY

The present work contains information about proper husbandry and care of laboratory animals, microbiological monitoring of their health and protecting them against suffering and distress. The author also gives some advice on the improvement and unification of experimental research results through the standardisation of laboratory animals used for the experiments as well as imposing proper conditions for animal husbandry.

INTRODUCTION

Laboratory animal research for experimental purposes is practised in many fields of natural sciences, including primarily biology, medicine and veterinary medicine.

The majority of laboratory animals taking part in various experiments are used as a model replacing human body in the studies on the safety of pharmaceutical and biologically-derived products, active dermatological preparations used in cosmetology as well as in the studies of neoplasia, toxicological, genetic research and experimental surgery. Therefore, ethical considerations suggesting the withdrawal of animals from experimental studies and replacing them by tissue cultures fall through the fact that no tissue culture can replace a living organism [1,2].

The term ‘laboratory animal’ denotes a creature which is to be used in laboratory research. The animal is born, bred and reproduced in certain conditions, it has a close characteristics determining its suitability for appropriate tests and it remains in certain conditions required for the experiment all the time [3].

The largest group of laboratory animals (80% all vertebrates) are rodents. This is related to the fact that these animals adapt easily to new conditions, multiply quickly and are characterised by low nutritional-environmental requirements. Scientific experiments are also frequently conducted on rabbits. Other animals such as dogs, cats, pigs, sheep, goats and apes are not often used, but their role is considered important due to considerable resemblance between their bodies and human organism. Dogs, cats, sheep and goats are employed as a model in experimental surgery, pigs are used to test food products, while apes serve as a model for the diseases found in humans as well as in the studies of behaviourism [1].

Although the principles of humanitarian procedures are similar for all animal species, nevertheless, considering the popularity of rodents and rabbits as laboratory animals, further discussion will be focused chiefly on these species.

HUSBANDRY

Good husbandry programme should provide animals with warm, clean, dry environment, sufficient space to move around, an access to chow and water, i.e. the conditions allowing for their growth, reproduction and maintaining good health status. The latter is one of the factors which guarantee repeatable and reliable results of the experiment.
The criteria which are helpful in order to assess good condition of laboratory animals include:

- animal behaviour;
- weight loss or weight gain;
- general appearance of animals.

One of the factors responsible for the comfort of laboratory animals during husbandry are favourable environmental conditions discussed below:

- Cages big enough to enable the animals move around freely. The cages should be made of non-toxic, easily cleaned material (plastic, macrolon) and they must be strong enough to withstand escape attempts and be fit for sterilisation; experience shows that solid floor covered with litter is better than an inconvenient grate. While grouping animals in the cages one should remember that excessive density causes stress reactions and may lead to death of single individuals.

- The bedding material should be non-toxic, non-irritating, hygienic and absorptive (sawdust is recommended). The bedding should be replaced every week; to reduce aggression related to cleaning the cages of aromatic substances with which rodents recognise their territory, it is advisable to leave around 10% old litter in the cage. The cages with young animals should not be cleaned during the first week after their birth as stress related to the cleaning may push animals to cannibalism (when mother eats her offspring). It is recommended to supply rodent cages with the material used by these animals to build their nest (paper clippings, handkerchiefs) and to provide them with empty bottles, plastic tubes or boxes used by the animals to play with.

- The rooms in which the cages are stored should maintain stable conditions with respect to temperature, humidity, ventilation and lighting, appropriate for a particular animal species (see Table 1). Low humidity (below 45%) maintained for a longer period of time is responsible for the occurrence of local narrowing of the tail in rats called ringtail. The cages placed in higher rows should be covered to protect the animals from the excessive exposure to light as it may cause retinal changes in albino animals. Rats are sensitive to respiratory tract infections, therefore, ventilation should be under control so as to avoid draught and increased ammonia levels. Too high temperature (exceeding 28°C) together with increased humidity (over 70%) may be responsible for miscarriages in pregnant guinea-pigs and infertility in rabbits.

- Mice and rats are sensitive to ultrasounds emitted e.g. by a computer and the presence of such equipment in animal quarters may cause hearing defects and low fertility in laboratory animals. It is believed that a silent music playing in animal quarters muffles noise and reduces stress.

- The chow should be balanced and standardised as far as possible (in order to avoid differences in the content of nutrients). The consistency of food ought to be appropriate for each animal species (too hard chow which can not be eaten by mice, may be given to rats). It is recommended to use pre-processed granulated pellets. After being weaned, young animals should receive soft chow (e.g. flaked oats for young mice) in view of their incomplete dentition. The chow to be sterilised before use should contain more nutritional components as some of them will be eliminated by high temperature. The deficit of nutrients in the diet and lack of movement may lead to obesity, particularly in rats. Unlike other rodents, guinea-pigs do not synthesise vitamin C, which should be supplied in this case together with chow, as vitamin C deficiency is responsible for hair loss, the damage of joints and gingivorrhoea. The rooms where chow is stored should be protected against the invasion of parasites and infection-spreading insects such as flies or cockroaches.

- The animals should have constant access to water which had been purified with filters, ster-

<table>
<thead>
<tr>
<th>Environmental requirements</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamster</th>
<th>Guinea-pig</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>20-24</td>
<td>20-24</td>
<td>20-24</td>
<td>20-24</td>
<td>15-21</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>50-60</td>
<td>60</td>
<td>50-60</td>
<td>50</td>
<td>50-60</td>
</tr>
<tr>
<td>Ventilation (no. of air exchanges/h)</td>
<td>15</td>
<td>10/15</td>
<td>10-15</td>
<td>10-15</td>
<td>5-15</td>
</tr>
<tr>
<td>Light/dark (h)</td>
<td>14 / 10</td>
<td>12-14 / 12-10</td>
<td>12-14 / 12-10</td>
<td>14 / 10</td>
<td>12 / 12</td>
</tr>
</tbody>
</table>
ilised or additionally chlorinated or acidified in order to eliminate biological pollution (hydrochloric acid may be used to obtain pH = 2.0–3.5 and the same pH will be obtained with 12–20 ppm chlorine). Water containers ought to be replaced twice a week. Automatic watering devices should be fixed outside the cage so as to avoid flooding the cage accidentally with water and the death of animals [1,4,5,6].

PRINCIPLES OF PROPER MANAGEMENT OF LABORATORY ANIMALS

The staff responsible for everyday care of laboratory animals should be qualified, experienced and friendly towards the animals. The personnel should watch the animals for any changes in their behaviour and correct environmental conditions, if necessary. The animals should be treated gently so as to minimise the stress connected with the presence of people. The animals ought to be grasped skillfully, without gloves whenever possible so that they have an opportunity to get used to the smell of their caretakers.

- Mice are grasped with a hand or handled with tweezers by the tail making sure that the animals do not hang in the air without support with forefeet for too long. Keeping a mouse hanging in the air for a few seconds is unacceptable.

- Rats are handled by their tails, just like mice or they are grasped with a hand in the middle of their trunk. Rats bred in artificial conditions are usually mild and therefore, laboratory staff in fact need not protect their hands with gloves while grasping these animals.

- Guinea-pigs should be lifted with a quick, decisive grasp using one hand to clasp them by their chest and back. Guinea-pigs, like rats, are rather mild and do not pose problems during the procedures but they are characterised by considerable motor hyperexcitability; pregnant guinea-pigs should be handled with care.

- Rabbits ought to be grasped with both hands. One hand holds rabbit ears together with the skin on their back, while the other hand grasps the skin fold in the posterior part of the back. In this position, rabbits may be placed in wicker or metal baskets used for internal transport. In case the rabbits must be held in the arms, one hand holds rabbit ears and the skin of the back and the other is slipped under its abdomen between hind legs, so that its rump rests on the forearm of the carrier. The rabbit should never be grasped by its skin, by its skin or by its hind legs only. In such cases, the animal tries to defend itself, moving vigorously its hind legs which often leads to the damage of the spine [3]. This is also dangerous for the personnel as the animal may hurt or scratch its offender.

PROTECTING ANIMALS AGAINST INFECTIONS AND THE SPREADING OF DISEASES

One of the most important responsibilities of the laboratory personnel is to protect the animals against the diseases which may lead to great losses caused by:

- death of animals;
- reduced fertility;
- delayed growth;
- abnormal reaction of animals used for the experiments.

There may be many coexistent factors responsible for the development of a given disease. A good example is proper bacterial flora which may contribute to the occurrence of pathological symptoms in unfavourable conditions (cold, too many animals in one cage, inappropriate nutrition). These pathogenic micro-organisms include but are not limited to Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris. Thus, the development of diseases may be prevented by appropriate diet, care, hygiene with respect to the equipment and animal quarters, i.e. their periodic cleaning and sterilisation. It is also important to ensure an appropriate micro-climate for each animal species (temperature, humidity, ventilation).

Laboratory animals should also be protected against the exposure to pathogenic organisms which may penetrate to animal headquarters from outside. To achieve this, the contact with persons which may have been involved in taking care of other animals should be reduced to minimum. The animal house may become infected through dirty hands, footwear and clothing of the staff, therefore, the personnel ought to be advised to change the clothing and footwear before entering the animal house. The staff should remember to wash their hands frequently and wear protective gloves. It is necessary to monitor closely health condition of laboratory animals, to isolate the infected animals as soon as possible and to disinfect thoroughly the cages and the equipment used for infected animals. Ill animals should remain under the care of a
It is also necessary to quarantine new animals, which should be looked after by special personnel, regular cleaning and sterilisation of cages and the whole equipment as well as maintaining the animal house clean (in the case of varnished or tiled walls, they should be cleaned at least once every three months and the same refers to windows). Each room should be supplied with a separate set of instruments and these ought to be marked so as to avoid using them with other animals. Husbandry animal facilities and the rooms in which experimental animals are housed should be located in two different buildings at a certain distance from each other. If that is not possible, these two should be isolated and the contact between the personnel working in each part of the building should be limited [3].

**MICROBIOLOGICAL MONITORING OF INFECTIONS IN LABORATORY ANIMALS**

Most of infections in laboratory animals are asymptomatic, but these latent diseases may be dangerous because firstly, they lead to changes in animal immune system; secondly, they may be followed by the occurrence of clinical symptoms as a result of stress, the deterioration of environmental conditions or additional infections and this in turn may be responsible for the distortion of experiment results and/or the death of animals. Therefore, it is recommended to monitor health condition of animals including healthy ones at regular time intervals, usually every 3 months. It should include bacteriological, virusologic and parasitologic examinations [6–9].

Table 2 is an overview of an extensive monitoring programme with a range of micro-organisms examined.

When pathogenic micro-organisms are detected, infected animals should be isolated and the staff ought to undertake appropriate action in order to eliminate the infection from the animals (treatment, eliminating infected individuals). One of the method includes isolating the litter obtained after caesarean section from the infected mother and taking them to healthy female individuals serving as wet-nurses. Infected mothers or male individuals are excluded from further husbandry. Infections with Mycoplasma are particularly difficult to eradicate as they are manifested as late as in 7th—8th month after birth, and Mycoplasma crosses the placental barrier. In this case, the foetuses born after caesarean section are kept in isolation for one year. Afterwards, the Mycoplasma-free individuals are fit for husbandry or reproduction.

Laboratory animals may be dangerous for people, spreading various zoonoses such as tularemia, salmonellosis, pseudotuberculosis, leptospirosis, Acetilobacillus moniliformis (rabite fever), Hantaan and Sendai virus infections, mycotic infections (Trichosporum and Microsporum) or tapeworm Hymenolepis nana. These zoonoses may be avoided in people providing the health condition of animals is closely monitored which allows for an early detection of infectious micro-organisms [1,6].

On the other hand, the staff looking after laboratory animals should undergo appropriate tests to

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**Table 2.** Microbiological monitoring of health condition of mice and rats with the range of investigated micro-organisms [6,7,8,9].

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minute virus of mice (MVM)</td>
<td>Rat coronavirus (RCV)</td>
</tr>
<tr>
<td>Reovirus type 3</td>
<td>Reovirus type 3 (Reo 3)</td>
</tr>
<tr>
<td>Lactic dehydrogenase (LDV)</td>
<td>Hantaan virus</td>
</tr>
<tr>
<td>virus</td>
<td>Kilham rat virus</td>
</tr>
<tr>
<td>Entomelina virus</td>
<td>Sendai virus</td>
</tr>
<tr>
<td>Hantaan virus</td>
<td>Theliers murine encephalomyelitis virus (TMEV)</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis virus (LCM)</td>
<td>Pneumonia virus of mice (PVM)</td>
</tr>
<tr>
<td>Theliers murine encephalomyelitis virus (TMEV)</td>
<td></td>
</tr>
<tr>
<td>Pneumonia virus of mice (PVM)</td>
<td></td>
</tr>
<tr>
<td>Mouse hepatitis virus (MHV)</td>
<td></td>
</tr>
</tbody>
</table>

**Bacteria and mycoplasma**

- Bordetella bronchiseptica
- Citrobacter freundii (only in mice)
- Corynebacterium kutscheri
- Escherichia coli
- Klebsiella pneumoniae
- Mycoplasma spp.
- Pasteurella spp.
- Proteus spp.
- Salmonellae
- Staphylococcus aureus
- Streptobacillus moniliformis
- Yersinia pseudotuberculosis

**Parasites**

- Endoparasites:
  - Protozoons:
    - Eimeria spp., Entamoeba muris, Giardia spp., Hexamita muris
    - Helminthes:
    - Tapeworms, Nematodes
    - Ectoparasites:
  - Arthropods: (lice, fleas, house-bugs, mites)
exclude the carrier state of certain micro-organisms (e.g. Salmonella) as they may be passed on the animals [3,6].

**STRESS**

Stress in laboratory animals may be the result of numerous factors, but it is always an undesirable reaction, leading to changes in the organism related to the ejection of catecholamines and corticosteroids from adrenal cortex. These changes cause the disturbances in homeostasis (i.e. a physiological balance in the organism), they have undesirable effect on mental and physical health of the animals and they may also distort the results of the experiments.

Long-term stress may lead to the development of the following diseases:

1) disturbances in immune function of the body;
2) arteriosclerosis associated with the activation of sympathetic system (increased concentration of catecholamines) and a resultant myocardial ischaemia;
3) myocardial necrosis and fibrosis;
4) renal dysfunction (in mice it is usually related to the retention of urine when older individuals are around – this leads to nephritis);
5) amyloidosis in mice – amyloid deposits in internal organs (heart, kidneys, liver, spleen, bowels); this disease is usually observed among male individuals kept together in one cage;
6) gastric ulcerations – frequent in rats;
7) adrenomegaly;
8) weight loss;
9) changes in retina.

The factors responsible for stress reaction in laboratory animals may include:

- keeping animals in non-physiological environment for a given species;
- poor zoo-hygienic conditions in animal headquarters;
- improper animal care and handling;
- procedures performed on animals
- transport;
- inappropriate combination of animals in groups.

Transport is one of the most important stressors. Even a very short-distance journey may cause an alarm reaction and changes in the immune system of an animal. It has not been established how much time is necessary for all the components of immune system to re-assume their normal values. It is believed, however, that stabilisation will be re-achieved in at least 24–48 hours. After transport, an optimum quarantine period before starting experiments is 1–3 weeks.

The animals should be transported in well-ventilated cages, making escape impossible. It ought to be remembered that cages piled one on top of the other do not ensure sufficient access to oxygen. SPF cages (Specified Pathogen Free) should be equipped in filters. The animals of various species and genders must not be mixed. Journey duration should be reduced to minimum. The cages with animals should be loaded last and unloaded first immediately after reaching the destination. Animals have acute sense of smell and hearing, therefore, it is recommended not to expose them to excessive noise or unfamiliar smells during the journey.

When the journey duration exceeds 12 hours, the animals must be given water (in juicy fruit or vegetables), and when the journey lasts more than 24 hours, the animals should be fed with the chow they are used to.

The animals that are exported or imported from a different country should have health certificates with the specification of the diseases which were investigated and excluded in them. During the quarantine period and after it is completed, the animals undergo microbiological monitoring. In case of any doubts concerning the health condition of the animals, the place of their origin should be verified [4,6].

Another important stressor in addition to transport are the procedures performed on laboratory animals as a part of their treatment or experiment. It is impossible to eliminate the stress accompanying them entirely, but one should always remember that:

- experiments must be planned properly in order to avoid the procedures that would eventually turn unnecessary or useless;
- animals must be treated properly;
- suffering should be maximally reduced or eliminated during and after the investigation;
- unsuccessful experiment ought to be analysed carefully so as to avoid failure in the future.

Reactions to pain or stress may vary greatly (depending on the species, type of stimulus, possi-
bility to escape or hide, earlier experiences, etc.). The knowledge of physiological patterns of animal behaviour and the types of reactions to pain or stress helps to prevent the suffering. The discomfort of laboratory animals may be manifested in [1,10]:

- changes in motor activity (creeping, hobbling);
- hiding in a safe refuge;
- decreased mobility, apathy, depression;
- vocal reactions;
- anorexia.

Apart from behavioural changes, physiological parameters may also serve as an alarming signal. These parameters include [10]:

- mydriasis;
- increased pulse rate and respiratory rate;
- hypersalivation;
- perspiration;
- urinating and defaecation.

Avoiding or reducing the stress and suffering of laboratory animals is not only a moral obligation of their caretakers but it is also practically significant as the discomfort of animals may distort experiment results.

**BLOOD SAMPLING**

Experiments conducted on laboratory animals often require blood sampling. Up to 10% blood volume may be collected from a healthy animal without any negative effect on its condition. A single removal of 30–40% blood will lead to a hypovolaemic shock (with 50% mortality). The symptoms of hypovolaemic shock include:

- filiform pulse;
- pale, dry mucous membranes;
- cold skin and extremities;
- anxiety;
- hyperventilation;
- subnormal temperature.

When these symptoms occur, the animal should receive intravenous or intraperitoneal infusion of physiologic saline of 30–35°C of the same volume as the volume of removed blood.

If less blood is sampled in too short time intervals, anaemia may develop. This is manifested in:

- pale mucous membranes;
- fatigability;
- increased respiration rate.

Below is the list of approximate blood volume for selected species of laboratory animals [11]:

- mouse – 70–80 ml / kg body mass
- rat – 50–70 ml / kg body mass
- hamster – 78–80 ml / kg body mass
- guinea-pig – 67–92 ml / kg body mass
- rabbit – 44–70 ml / kg body mass

**ANAESTHESIA**

As already mentioned, experiments conducted on animals may cause pain which ought to be eliminated or reduced to minimum for ethical and scientific reasons. The suffering associated with surgical procedures may be prevented with injection or inhalation anaesthetics. Most of them have an effect on physiological functions of animal body which may distort the results of an experiment, but this is also true for the pain which triggers stress reaction.

General anaesthesia results in loss of consciousness, while local anaesthetics insensibilise only parts of the body (frequently used anaesthetics include procaine and lidocaine). General anaesthesia may be achieved with a single anaesthetic (e.g. inhalation halothane or injection pentobarbital) or a combination of these (e.g. ketamine/xylazine).

A laboratory animal is frequently premedicated before receiving the anaesthetic proper in order to reduce side effects of anaesthesia and ensure a mild regain of consciousness. Anticholinergic drugs (e.g. atropine) decrease salivation and the secretion of bronchial mucus and reduce other undesirable effects related to the stimulation of autonomic nervous system. Tranquillisers and sedatives also used for premedication lower stress and help the animals to regain consciousness mildly.

After the procedure, the animals need peace and warmth (25-35°C for adult animals, 35-37°C for new-borns – possibly in the incubator) and the alleviation of postoperative pain. Analgesia may be achieved with opiates (e.g. morphine), non-steroid anti-inflammatory drugs (e.g. aspirin) and local anaesthetics [1,6].

The examples of the dosage of drugs used in premedication, anaesthesia and analgesia of laboratory animals are demonstrated in tables 3, 4 and 5.
EUTHANASIA

Most experiments end up in the death of a laboratory animal or euthanasia which relieves suffering. Euthanasia is defined as an act of killing an animal, preceded by its loss of consciousness. There are several methods of euthanasia [6]:

- overdose of injection or inhalation anaesthetic;
- dislocation of cervical vertebra;
- a blow in the back of the head;
- exposure to CO₂ (carbon dioxide);
- decapitation.

The choice of these methods is determined by the following factors [6]:

- death should be painless;
- the animal should lose consciousness as soon as possible;
- the method ought be unfailling and irreversible;
- the method should be associated with as little stress as possible;
- euthanasia must be safe for the person who is doing it;
- the method ought to be simple;
- necessary devices should be easily available.

MICROBIOLOGICAL CLASSIFICATION OF LABORATORY ANIMALS

Laboratory animals may be classified into the following groups with respect to their microbiological status [6]:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamster</th>
<th>Guinea-pig</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine (anticholinergic)</td>
<td>0.05 mg/kg SC</td>
<td>0.05 mg/kg SC</td>
<td>0.05 mg/kg SC</td>
<td>0.05 mg/kg SC</td>
<td>0.05 mg/kg SC</td>
</tr>
<tr>
<td>Diazepam (sedative)</td>
<td>5.0 mg/kg IP</td>
<td>2.5 mg/kg IP</td>
<td>5.0 mg/kg IP</td>
<td>5.0 mg/kg IP</td>
<td>2.0 mg/kg IV</td>
</tr>
<tr>
<td>Hypnorm (fentanyl fluanisone)</td>
<td>0.3 ml/kg IP/SC</td>
<td>0.4 ml/kg IP</td>
<td>0.5 ml/kg IP</td>
<td>1.0 ml/kg IP</td>
<td>0.5 ml/kg IM</td>
</tr>
</tbody>
</table>

SC - subcutaneous injection; IM - intramuscular injection; IP - intraperitoneal injection; IV - intravenous injection

Table 3. Examples of premedication drug doses [1].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamster</th>
<th>Guinea-pig</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol</td>
<td>26 mg/kg IV</td>
<td>10 mg/kg IV</td>
<td></td>
<td>10 mg/kg IV</td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>100 mg/kg IP</td>
<td>90 mg/kg IP</td>
<td>200 mg/kg IP</td>
<td>40 mg/kg IP</td>
<td>35 mg/kg IM</td>
</tr>
<tr>
<td>Xylazine</td>
<td>40 mg/kg IP</td>
<td>40-55 mg/kg IP</td>
<td>50 mg/kg IP</td>
<td>37 mg/kg IP</td>
<td>45 mg/kg IV</td>
</tr>
<tr>
<td>Pentobarbitalone</td>
<td>26 mg/kg IV</td>
<td>100 mg/kg IP</td>
<td>100 mg/kg IP</td>
<td>90 mg/kg IP</td>
<td>5 mg/kg IM</td>
</tr>
</tbody>
</table>

SC - subcutaneous injection; IM - intramuscular injection; IP - intraperitoneal injection; IV - intravenous injection

Table 4. Examples of the dosage of anaesthetics [1].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamster</th>
<th>Guinea-pig</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>120 mg/kg PO every 4 hours</td>
<td>100 mg/kg PO every 4 hours</td>
<td>85 mg/kg PO every 4 hours</td>
<td>100 mg/kg PO every 4 hours</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>300 mg/kg PO every 4 hours</td>
<td>100-300 mg/kg PO every 4 hours</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>2.5 mg/kg SC every 2-4 hours</td>
<td>2.5 mg/kg SC every 2-4 hours</td>
<td>2.5 mg/kg SC or IM every 2-4 hours</td>
<td>2.5 mg/kg SC or IM every 2-4 hours</td>
<td></td>
</tr>
</tbody>
</table>

SC - subcutaneous injection; IM - intramuscular injection; PO - per os

Table 5. Examples of the dosage of analgesics [1].
I. Germ free animals (GF)

Obtained in histerectomy and free of any detectable forms of life. They are extremely sensitive to those infections to which conventional animals are usually resistant. They cannot be bred in ordinary conditions – they require sterile environment in isolators.

II. Gnobiotic animals (GN)

Obtained through histerectomy, too, or in caesarean section; they may also be born to GN animals in isolators. They possess known microflora/fauna (they are microbiologically standardised) or are entirely free of micro-organisms. Among GN animals, mono-, di- and polybiontes are distinguished, depending on the number of micro-organism species found in the animals. GN animals, just like GF ones, must be bred in isolation.

III. SPF animals

These animals are free from specific micro-organisms which are potentially pathogenic for given species (Specified Pathogen Free). Thus, they have unknown microflora but we are sure that some of the known micro-organism species are not found in them. In order to maintain their status, these animals ought to be bred in a closed system. Open husbandry will not eliminate the risk of SPF animals becoming infected with new, unknown microflora.

IV. Conventional animals

They have a number of unknown, harmless species of micro-organisms, therefore, their microbiological status is not determined. These animals do not require protection against micro-organisms, and hence they may be bred in open husbandry.

Microbiological status of laboratory animals is maintained thanks to the use of a number of hygienic barriers and systems protecting the animals against the exposure to microbiological contamination.

An isolator is an absolute barrier used for the husbandry of GN and GF animals. The whole necessary equipment, litter, chow and water undergo sterilisation in an autoclave before being placed in the isolator.

A classic barrier for SPF animals protects them against the penetration of potential pathogenic micro-organisms only. It is possible to disinfect the equipment which cannot be autoclaved. The number of people working behind the barrier should be reduced to minimum (these staff members ought to enter the animal quarters through a special ‘sluice’ room after taking a shower and changing their clothes into sterile protective clothing, putting on gloves, masks and special headgear).

Hyperbaric pressure maintained in SPF animal headquarters ensures that the air flow is directed outward preventing the polluted air from outside.

In reversed classical barrier, hypobaric pressure is maintained in animal headquarters and the personnel take a shower and change clothing after coming from behind the barrier. In this case, the outside environment is protected against harmful micro-organisms found in animals. The air and materials used from behind the barrier are sterilised. If the animals are the carriers of human pathogenic micro-organisms, it is vital to ensure that all these micro-organisms are kept behind the reversed barrier [1,3,6].

Conventional animals do not require the use of the barriers discussed above, but it is important to remember about other requirements such as the following [6]:

1) The building housing animal headquarters must be free from wild rodents and insects;
2) The number of persons entering the animal headquarters should be reduced to minimum and all of them ought to wash their hands, wear protective clothing, gloves and headgear;
3) It is important to keep the cages, equipment, chow, drinking water and litter clean;
4) Laboratory animals should receive special chow and litter.

GENETIC STANDARDISATION

The husbandry of laboratory animals (mainly mice and rats) should be aimed at the greatest genetic standardisation possible as this ensures high repeatability of experiment results.

The most effective method to obtain animals with identical genetic material is based on inbreeding. Thus, it is more likely that an individual will inherit identical alleles after its ancestors, i. e. it will become homozygous. Brothers and sisters are usually mated; after 20 generations having been crossbred according to this system, a population of
homozygotes with identical genotype called inbred tribe is obtained.

Homozygosity is measured with inbred ratio, which indicates the probability for both alleles of each individual to be found in homozygosity.

The husbandry for inbred strain is rather arduous, it takes around five years and is often associated with the loss of many animals due to their homozygosity. Lethal recessive genes found in heterozygous population are masked by dominant alleles which do not occur in homozygotes. A result of this is the manifestation of those lethal genes and the formation of maladjusted or inanimate animals.

The use of genetically homogenous material is only possible with those species for which inbred tribes have been bred. The remaining animals are outbred, but the material is not homogenous. However, in this case too, it is recommended to strive for the highest possible repeatability of experiment results. The animals are bred and mated in closed herds, without the addition of new animals. As long as the herd is big (as it is observed in natural conditions) and the mating is random, the genetic make-up of individual animals is heterogeneous, but the whole herd possesses certain characteristic properties [3].

GENETIC MONITORING

Genetic contamination of inbred strains leads to their heterogeneity. Thanks to genetic monitoring it is possible to maintain good genetic quality of homozygotes. There are numerous methods to control homogeneity and genetic authenticity with the use of DNA, biochemical (e.g. isozymes), morphological, pathophysiological and cytogenetic markers [1].

GLP PROCEDURES

In most countries, the procedures of experiments with laboratory animals should correspond to the requirements of Good Laboratory Practice (GLP). These requirements were presented for the first time in late 1970s by Food and Drug Administration, a federal agency of the American government. They regulate the issues related to experimental methods and procedures.

According to GLP, the experiment itself should be preceded by designing a detailed schedule taking into account all the stages from the beginning to the end of the experiment.

Documentation should also contain the data of an investigator responsible for conducting the experiment as well as observation results. Each action (e.g. examination of animals, experimental techniques) must be documented in detail. GLP also specifies the requirements concerning the location and the equipment of laboratories and animal headquarters [1]. More information on GLP procedures can be found in the publication by Rydzyński and Stetkiewicz [12].

LEGAL REGULATIONS CONCERNING EXPERIMENTAL PROCEDURES WITH ANIMALS

Below is the list of selected legal issues related to the use of laboratory animals regulated by the Animal Protection Act dated 21st August, 1997 (Law Reports no. 111, item 724):

- Experiments and tests on animals may be performed only in appointed scientific institutions and only when they are necessary for scientific research, university education or the protection of the health of people or animals if these objectives cannot be achieved otherwise as no alternative methods exist (art. 28 of the above Act);
- The experiments and tests must be approved of by the Local Ethics Committee supervised by the National Ethics Committee (art. 28);
- The husbandry of laboratory animals must be licensed by the Ministry of Agriculture and Food Economy (art. 29);
- Animal headquarters should ensure the conditions appropriate for given animal species (art. 29);
- Experiments associated with pain ought to be performed in general or local anaesthesia only once on one individual, unless the nature of the experiment requires its repetition on the same animal. The experiments may be performed without anaesthesia only in exceptional cases, when it is necessary from the scientific point of view.

RECAPITULATION

Reliable results of experiments on laboratory animals are largely dependent on the standardisation of the factors affecting physiological reactions of these animals and on the broad idea of the well-being of laboratory animals. The main factors influencing the quality of experiments conducted on laboratory animals include:
• genetic and microbiological quality of the animals;
• their biological status (sex, age, body mass);
• health condition;
• nutrition;
• maintenance conditions (type of cages, litter, number of animals in one cage);
• animal headquarters (ventilation, temperature, humidity, lighting, noise);
• exposure to stressogenic stimuli;
• proper care;
• the choice of appropriate experimental techniques.

Meeting these requirements allows to obtain repeatable, reliable results of experiments and to create proper living conditions.

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