Animal Care Best Practices for Regulatory Testing

Denise Fillman-Holliday and Margaret S. Landi

Abstract

Best practices result from a partnership between law, science, and the people working with the animals on regulated studies. In an ideal setting, people working with animals observe and study animal behavior as influenced by different housing and handling paradigms. These observations are published to create a body of science, and laws are promulgated based on the science. The ideal world does not exist, but there are certain components of best practices common to all species. These components include study design, housing, social contact, diet/feed, enrichment devices, and human interaction. This paper outlines how the forces of law, science, and people work to create best practices for species in regulated studies, specifically mice, rats, rabbits, dogs, and nonhuman primates.

Key Words: animal care; enrichment; husbandry; regulated studies; regulatory; socialization

Introduction

"Practice is the best of all instructors."
(Publilius Syrus, approximately 300 BC)

The old axiom historically attributed to an ancient Roman is still true. Best practices evolve; they cannot by definition be stagnant. They instruct us, but we create them by observation and assimilation.

The three fundamental forces for best practices are the policies of a particular country, the science known about a specific practice, and the people involved with the animals. The laws, rules, or regulations that govern the use of animals vary between countries. Consider the housing of dogs as one example. In the United Kingdom, it is common practice, indeed the law, for dogs to be housed in kennels or pens. Housing dogs in cages is permitted within the United States as long as minimum floor space requirements are met.

The second force is science. The study of animal behaviors within laboratory settings help us understand the needs of the animals in our care. Ideally, regulations should be promulgated based on scientific evidence. Currently, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes is developing recommendations on basic laboratory animal housing and welfare, based on the science of animal husbandry and behavior. However, science may not always be the genesis of regulations. For example, the provision to provide "exercise for dogs" in the 1985 amendments of the Animal Welfare Act was written with little scientific evidence to justify the requirement. There was little proof then, as there is now, that providing a "minimum amount of exercise" would be beneficial for dogs. What has been shown is the importance of contact with humans, not necessarily a specific need for exercise.

We believe the most important force that puts the word "best" in best practices is the third element—people. People who are caring, observing, and recording the resultant behavior of the animal when a practice is in place or modified are crucial.

In light of the forces of law, science, and people, we review in this presentation the best practices for species often used in regulated studies. Due to our area of experience, we address studies required through regulatory bodies based primarily in North America, to some degree in Europe, but not necessarily in Asia.

Within the force of law, policy, or regulation, the International Conference on Harmonization (ICH[1]) and the Organisation for Economic Co-operation and Development (OECD[1]) have played significant roles. Both of these bodies are described elsewhere in this issue of *ILAR Journal*. Neither the ICH nor the OECD has in their mission any statement on "animal welfare"; however, the benefits to animal use on regulated studies have been great. The ICH and OECD conventions have had a positive effect on reducing the number of animals required for a study or in giving guidelines to define endpoints better. For example, the new ICH guidelines have reduced the number of animals required in segment II reproductive toxicology studies from 25 rodents per dosage group to 20 rodents per dosage group (ICH 1993). The OECD's recent publication, "Guidance

Denise Fillman-Holliday, D.V.M., Dipl. ABT, is Manager of the Veterinary Medicine East Campus & Diagnostic Laboratory; and Margaret S. Landi, V.M.D., Dipl. ACLAM, is Vice President in the Department of Laboratory Animal Science, GlaxoSmithKline Pharmaceuticals, King of Prussia, Pennsylvania.

[1]Abbreviations used in this presentation: ICH, International Conference on Harmonization; OECD, Organisation for Economic Co-operation and Development.

Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation" (OECD 2000), is another example of the effect these bodies have on best practices for animals used in regulated studies. The reader is directed to the web site of each organization (ICH: <http://www.mcclurenet.com/>; OECD: <http://www.oecd.org/ehs>) or to other presentations in this issue for relevant information.

General Best Practice Guidelines

In this paper, we review best practices for the primary species used in toxicology studies, although they are limited to those that have been observed and documented. Many more exist in the different institutions performing safety evaluation studies. For each of the species presented, the following topics will be discussed: study design, housing, social contact, diet/feed, enrichment devices, and human interaction.

Study Design

The first step in providing best practices is the study design. The group size and number of groups should be the minimum number considered acceptable for the particular test. Although the absolute number of animals to test is often not mandated by a regulatory agency, norms have been established that are based on the experience of the regulated investigator with the regulatory body. When it is determined that statistical analyses will be used to facilitate data interpretation, it is important to determine that the number (n) is appropriate by performing a statistical power analysis. Although it is important to try to reduce the numbers of animals used on a particular study, it is as important not to use too few animals as it is to use too many. The combination of the regulation, the regulated party, and the science of the study must always be considered and challenged.

There are many ways to alter the study design to improve animal care practices on a study or even reduce animal numbers further. For reproductive toxicology studies that require dosage administration to pregnant rodents or rabbits during the period of organogenesis (segment II studies), it is now possible to obtain pregnant females, thereby reducing the necessity of maintaining male breeder animals. This practice reduces the number of animals on site, and it allows the vendor to use the stud males for multiple breedings.

Methodology to be used for blood sampling should be considered when looking for "best practice." As technology improves, allowing for smaller and smaller blood samples, the need for controversial techniques such as retro-orbital bleeding in rodents is reduced or eliminated. The method of retro-orbital bleeding has been questioned by some investigators as possibly causing pain and distress to the animals. It is also considered to be aesthetically displeasing by most individuals performing the technique. One study conducted in rats concluded that retro-orbital bleeding in combination with anesthesia caused more distress than tail vein bleeding with anesthesia and saphenous vein bleeding without anesthesia (van Herck et al. 2000).

In nonterminal large animal studies, it is important to establish whether the control animals and animals that received test compound can be used on subsequent studies. Under most circumstances, any animal that was dosed only with vehicle is considered acceptable for use on a future regulated study. It is often possible to assign animals that were dosed with test compound to a nonregulated study after an acceptable washout period.

Housing

Careful selection of the type of housing used for animals on regulated studies has an enormous impact on the animal's well-being during the study. The animal's immediate environment must be clean, dry, of an appropriate temperature and humidity for the species, and large enough to allow for normal postural movements and species-specific behavior. The material for the primary enclosure is important because the ease of cleaning and comfort of the animal must be considered. Finally, the primary enclosure must be safe for both the animals and the animal handlers.

Social Contact

Social contact of some form is very important for the well-being of all species of animals typically used in regulated studies. The different types of social contact include auditory, olfactory, visual, tactile, pair housing, and group housing. The research community and regulatory agencies are becoming increasingly aware of the enormous benefit to animal welfare when certain species are paired or group housed while on regulated studies (USDA 1999). However, there are many well-founded arguments for maintaining test animals in single caging units. Social housing does not allow for accurate reporting of individual feed consumption data or cage pan observations, and dominant individuals may actually affect body weight changes in other individuals. Fighting, particularly in socially housed nonhuman primates, is an important concern. Stable social units of nonhuman primates must be established before study initiation, which makes randomization to study groups problematic.

Diet/Feed

It is critical to ensure nutritional consistency for all of the animals used in a study and to be able to guarantee that consistency. However, the types of feed offered and the way in which the feed is offered can be modified to provide additional enrichment to many laboratory animal species. Typically animals on toxicology studies are fed certified diets that are commercially available and guarantee both that minimum levels of specific nutritional components have been met and that the feed does not exceed certain maximum limits for specified contaminants (e.g., pesticide residues, fungi, and processing by-products).

As enrichment, some laboratory species are frequently offered treats in addition to the certified diet. The treat should make up only a small portion of the total diet, and it should be nutritious as well as accepted by the majority of the animals on study. The animals should be acclimated to the treat before initiation of the study so that the treat is familiar to them. If animals are offered treats on regulated studies, the treat must be offered consistently to all animals on the study at the same time points.

Enrichment Devices

There is a multitude of enrichment devices commercially available for all laboratory species, and many can be homemade. Each device should be carefully considered for whether it is safe, easy to clean, and preferred by most of the animals on a study. The device should be made of a material that is not easily ingested; or, if it is ingested, certificates of analyses must be provided along with the study data to ensure that the device will have no effect on the outcome of the study. Any device used should facilitate in-life observations. For rodents, new enrichment tubes are available that are translucent and constructed of polycarbonate. The rat tubes are tinted amber, and mouse tubes are tinted red. These colors will make the tubes dark, at least in the eyes of the rats or mice staying in them, but they still allow observation of the animals by the care staff. Polycarbonate mouse huts are expected to be commercially available soon. The provision of a tube, hut, or box in a rodent's environment allows the animal to control light levels, withdraw from frightening stimuli, and use the structure for climbing (Baumans 1997; Manser et al. 1998).

Many laboratory animal species, especially dogs and nonhuman primates, lose interest and stop using a particular device after exposure to it for some time (authors' experiences). It is best to have an array of several different enrichment devices available that can be alternated through the animal's environment to prevent boredom. It is also advisable to provide animals on study with prior exposure to an enrichment device. This technique should minimize the variable of a novel device within the animal's environment.

Human Interaction

The value of human interaction is variable, depending primarily on the species of animal. Most laboratory species benefit from controlled, positive interactions with humans such as handling and acclimatization (Baumans 1997; Van de Weerd and Baumans 1995). The benefits are most easily seen in species such as rats, rabbits, dogs, and nonhuman primates; the animals are easier to handle and are less stressed by the interaction. When human interaction is used intentionally to enrich the lives of laboratory animals on regulated studies, it is important to acclimate the animals to the type of interaction well before the initiation of the study.

Species-specific Best Practices

The species described in this section are those often used in toxicology research. The specific species include mice, rats, rabbits, dogs, and nonhuman primates.

Mice

In all studies, the study design should allow for the use of the least number of animals that will provide sound data. There have been many forces for reduction in the number of mice on regulated studies. The paramount reason has been an overall improvement of the health of laboratory rodents, specifically elimination or reduction in confounding pathological lesions due to rodent bacteria and viruses. Carcinogenicity studies often require large numbers of mice due to the prevalence of mammary tumors caused by mouse mammary tumor virus. Elimination of this virus, along with Sendai virus and other complicating diseases, has reduced the number of animals used per study (Pakes et al. 1984).

The optimal type of housing for mice involved in regulated studies has been an area of debate for many years. Traditionally, mice in toxicology studies in the United States have been housed in wire bottom cages. In some areas of toxicology testing, there are sound justifications for continuing the use of wire bottom cages. One example is when checking for the presence of copulatory plugs in reproductive toxicology studies. Another example is with compounds that have active metabolites; animals that are coprophagic may increase their exposure to the metabolite. However, the use of solid bottom caging, with bedding and additional enrichment tools, is becoming the norm in other countries (Purchase et al. 1998). In fact, the Council of Europe recommends providing rodents with solid bottom cages with bedding unless there are strong experimental or veterinary reasons for not doing so (Council of Europe 2001). The *Guide for the Care and Use of Laboratory Animals* also recommends the use of solid bottom caging with bedding for rodents (NRC 1996). If given a choice, rodents prefer solid bottom caging to wire bottom caging (Blom et al. 1996). It is important for each institution's animal care and use program to consider all options and formulate their own policies.

The decision to house mice in a regulated environment singly or in a group should take into account the type of study to be conducted, the type of data to be collected, and, of particular importance, the strain of mouse to be used. The males of many strains of mice that are commonly used on toxicology studies will fight each other once they reach puberty. Fighting also causes some degree of stress, with concurrent corticosteroid elevations (Haseman et al. 1994). Even changes such as low stocking rate per cage and the use of enrichment devices will not prevent the dominant male from injuring or killing subordinate males. Frequently, subordinate males are smaller and do not thrive because dominant males limit their access to the feed. Even female mice of many strains will fight once they reach sexual maturity. Cage side observations become more difficult with group housed mice. If test compound-related mortality occurs within a group of mice, the carcass may be cannibalized. Some studies have revealed that there are sound scientific reasons to avoid group housing mice on longer-term studies. Group-housed male mice were found to have a lower T-cell proliferative response than singly housed males (Grewal et al. 1997). Group-housed B6C3F1 mice tend to have a higher incidence of dermal/subcutaneous tumors and lower survivability than individually housed mice (Haseman et al. 1994). However, this same study demonstrated an increase in liver tumor incidence in the individually housed mice, perhaps related to increased body weights that typically occur compared with group-housed individuals.

Mice are often fed ad libitum on toxicology studies. Generally, the feed consists of a pelleted ration with a controlled and consistent nutritional content. Treats or nutritional supplements have not been commonly offered to mice on regulated studies. Some devices that are offered to mice are used extensively for the purpose of gnawing (authors' experiences). Several of these devices are now available with nutritional analysis information, and some are "certified." Some studies suggest that diet optimization may improve survivability in mice and reduce the incidence of certain spontaneous neoplasms (Haseman et al. 1998; Turturro et al. 1996). Diet optimization is defined as providing adequate calories and nutrition to maintain a normal body weight. The National Toxicology Program has carried out studies that have shown that the incidence in liver tumors in mice is higher in mice with increased body weights (Haseman et al. 1998).

Many commercially available enrichment devices for mice exist. Most of these devices are inexpensive and simplistic, and they allow the mouse to carry out species-specific behavior such as burrowing. Devices that allow the creation of a burrow or house are favored by most strains of mice. Many institutions use plastic bottles, tubes, or plastic balls cut in half with a hole for a doorway to provide a place to hide (Figure 1). Nesting material is also used by almost all strains of mice. The mice spend time shredding the nesting material and arranging their nest. Several commercially available forms of nesting material are certified, which is important because a certain amount of material may be ingested by the mouse. Other devices such as running wheels can provide enrichment for mice without compromising the science (Figure 2).

Human interaction appears to be of little or no value for enriching the lives of mice. Regular handling of mice does seem to make them more docile, but mice do not appear to respond to the interaction in any other positive manner. On studies where mice are handled on a regular basis, it is important to minimize stress by acclimating the animals to the handling before initiation of the study.

Figure 1 Plastic water bottles are frequently used to provide a shelter for rodents on regulated studies.

Rats

As stated above with mice, the study design should allow for the least number of animals that would provide sound data. In addition, like mice, the number of rats needed on studies has been decreasing due to improved health. Complication of studies due to sialodacryoadenitis virus, *Mycoplasma pulmonis,* and other rodent diseases has drastically decreased over the past 20 yr (Pakes et al. 1984).

There is more information published about the effect of housing of rats in solid bottom cages than there is for mice.

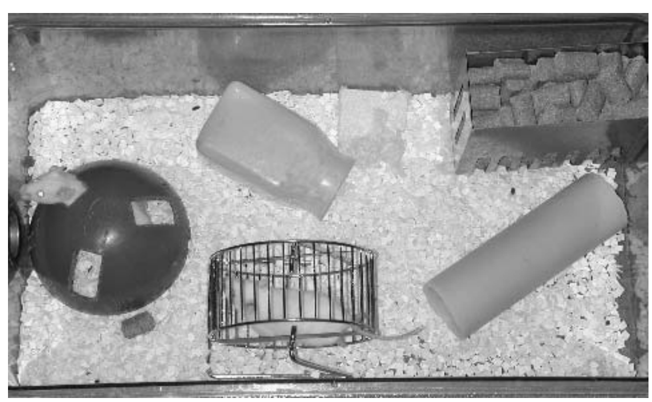

Figure 2 Several different enrichment devices are available for use in mouse cages.

As with mice, the use of solid bottom caging is the standard in Europe. In the United States, the trend is to move away from the use of wire bottom cages as the norm for primary housing. Over the last few years, many pharmaceutical companies and contract research organizations in the United States have moved to the use of solid bottom caging for rodents on toxicology studies (Smiler et al. 2001). Toxicologists, regulatory bodies, and accrediting institutions (AAALAC 2000) in the United States are becoming more accepting of this housing, and the most recent edition of the *Guide for the Care and Use of Laboratory Animals* recommends solid-bottom caging (NRC 1996). Rats housed long term in wire bottom cages and fed ad libitum often develop painful foot lesions after they reach a certain weight (Peace et al. 2001). If rats are provided with a solid substrate within a wire bottom cage, they will tend to rest on the solid area and defecate and urinate over the wire area (authors' experience). In one study, it was noted that rats housed in solid bottom cages were less active than rats housed in wire bottom cages (Rock et al. 1997). This same study demonstrated that rats housed in wire bottom cages ate significantly more feed than those housed in solid bottom cages. As with mice, there are some sound scientific justifications for housing rats on wire bottom cages. For example, it is difficult to observe copulatory plugs when rats are housed in solid bottom caging, and it is possible that the rat will be exposed to higher levels of the test compound through contact with the urine and/or the feces.

Rats appear to benefit more from group housing than mice. Male rats do not tend to fight as readily as mice, particularly if they are provided a cage that is large enough for the subordinate male to retreat. Rats appear to prefer each other's company and will interact as they explore their environment. As with group-housed mice, cage side observations are more difficult than with individually housed animals, and cannibalization may occur if there is an unexpected death.

Within the past decade, a decrease in longevity in certain strains and stocks of rats has been documented. One factor in this decrease has been ad libitum feeding in rats (Goodrick et al. 1983), not generally practiced with other species such as dogs and monkeys. Diet optimization for rats on long-term studies is becoming increasingly accepted in the industry. There have been many studies that have proved survivability is increased and the incidence of specific neoplasms are lower when rats are not allowed to become overweight (Christian et al. 1998; Goodrick et al. 1983; Haseman et al. 1998; Klurfeld et al. 1991). Feeding treats to rats on regulated studies is not routine; however, rats are often provided devices for gnawing, and they may ingest a small amount of the device material. Many of these devices are commercially available, along with nutritional analysis and certification.

Rats accept and use a number of enrichment devices. As with mice, nesting material and a shelter or place to hide (e.g., plastic or stainless steel tubes) may provide an enrichment benefit (Baumans 1997; Manser et al. 1998) (Figure 3). Vertical cage space can be used advantageously if climbing ropes or mesh are provided. Rats also spend time rolling large marbles or stainless steel balls, especially if they are housed in wire bottom cages, and they use hanging toys. As with mice, it is important to consider the substrate of the enrichment device and whether it could be ingested. Obviously, the device should not be used if ingestion of the device could be harmful or jeopardize the integrity of the data.

Human interaction is most likely beneficial to rats that are acclimated to it. Most laboratory rats do seem to respond in a positive manner to gentle handling. It is important to acclimate rats to human interaction before initiation of a study. It is interesting to note that rats housed in an extensively enriched environment tend to be more aggressive to humans (A. Haynes, GlaxoSmithKline, personal communication, 2001) and are reported to be more highly reactive to physical restraint (Spencer et al. 1993) than rats housed in less enriched environments (Figure 4).

Rabbits

Rabbits are generally the nonrodent species of choice for reproductive toxicology studies and other regulated studies such as eye irritation studies. The group size and number of groups required are often mandated, and recent ICH and OECD changes have reduced the numbers required in a specific study design (ICH 1993, 1996).

Generally rabbits are housed in plastic or stainless steel cages with grids or slats for floors on reproductive toxicology studies. The floor slats should be wide enough for the majority of the foot to be supported by the slat; otherwise, foot lesions may develop as the rabbits gain weight. The slats should also be close enough together that the foot does not slip down between the slats, allowing for the possibility of leg fractures. Rabbits housed in wire bottom cages have

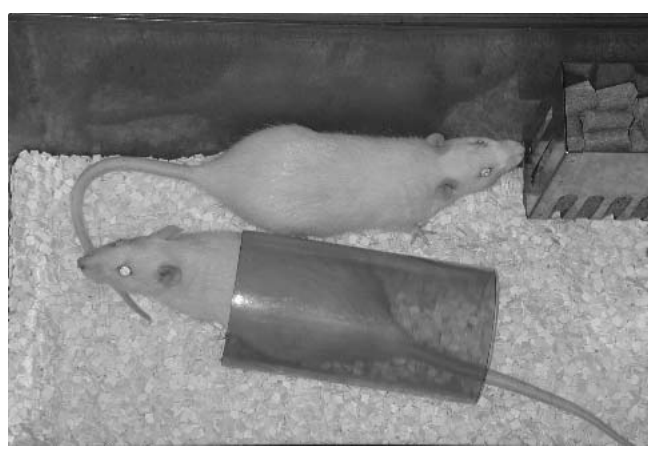

Figure 3 Amber-tinted translucent plastic tubes provide a darkened shelter for rats while still allowing observation by staff.

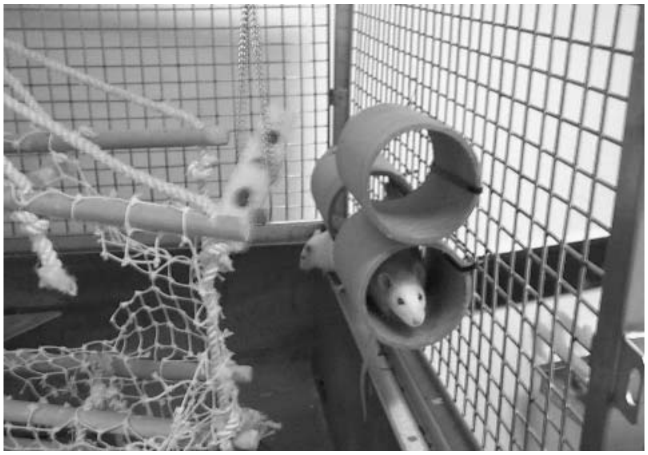

Figure 4 Rats housed in an extensively enriched environment are noted to be more aggressive to humans.

a high incidence of ulcerative pododermatitis (Love 1994); this type of housing should be avoided. Gridded plastic flooring seems to be associated with less foot lesions and leg fractures while still allowing for a clean, dry environment. Rabbits have also been successfully housed in large pens (Love 1994), although this housing is not standard on reproductive toxicology studies.

Rabbits are generally individually housed during reproductive toxicology studies. If male rabbits are kept as sires, they must be physically separated from other males due to fighting (Huls et al. 1991; Whary et al. 1993). Pregnant females must be kept separate because they will be stressed by the immediate presence of a conspecific and will also fight. Young rabbits may be group housed after weaning, but it is important to keep them in large pens where subordinate animals can escape from the dominant ones. One study compared nonpregnant females in individual housing versus a small group setting and concluded that there were no significant differences in physiological and immunological parameters, but group housing appeared to be a successful method of enrichment for females (Whary et al. 1993). Another study found that 46% of females that were group housed showed signs of aggressive attack by the age of 63 days (Bell and Bray 1984). If rabbits are housed individually, it is beneficial to allow them to have visual, auditory, olfactory, and even tactile association with conspecifics (Council of Europe 1997).

The typical diet fed to rabbits on reproductive toxicology studies consists of a pelleted rabbit chow that is fairly high in fiber (generally 15-20%) to help prevent the formation of trichobezoars. The diet is usually limited to prevent overeating and associated disorders such as obesity, bloat, and hemorrhagic enteritis. Ad libitum feeding in pregnant females is associated with decreased numbers of embryo implants, decreased numbers of live born and decreased fetal body weight (Clark et al. 1991). Some institutions routinely use various supplements to help prevent trichobezoars. "Certified" alfalfa cubes and chew sticks, loose irradiated hay, kale, pineapple, and other fruits and vegetables are examples of treats that can be given in moderation.

Many different enrichment devices are suitable for rabbits (Huls et al. 1991). Rabbits spend time ringing bells that are attached to the sides of their cages and will kick around and toss small dumbbell-shaped dog toys (Figure 5). Balls, keys, hanging toys, and other devices have also been provided to enrich their environments. It is imperative that appropriately sized structures such as tubes are present to provide refuge for the subordinate animal housed in a pen with other rabbits. Low-volume music is often provided to rabbits for some period of time during the day. Rabbits that are acclimated to music or white noise are observed to react less violently to noise stimuli than rabbits kept in a quiet environment (N. Lefebvre, Covance Research Products, personal communication, 2001).

Human interaction appears to be an important aspect of enriching the lives of rabbits on toxicology studies (Anderson et al. 1972; Podberscek et al. 1991). When appropriately handled, most rabbits become more tractable as soon as they have become accustomed to the handling. If males are kept as sires, they require periodic grooming as they age, and they usually look forward to their grooming sessions. Typically on reproductive toxicology studies, does are not allowed to deliver their litter. If a study requires that the doe deliver and raise the litter, then only familiar human interaction should be allowed to continue close to the time of parturition, and all other interaction should be kept to a minimum.

Dogs

The typical species of choice for nonrodent toxicology studies is the dog. The study design should minimize the number of dogs used on study; typically, three dogs per dosage group per sex are used for a 1-mo toxicity study. Due to the

Figure 5 Rabbits utilize enrichment devices such as bells and dog toys.

size of the dog and its relatively tractable nature, many methods have been developed to facilitate and reduce the invasiveness of dosing and data collection procedures. For intravenous access of relatively short duration, indwelling intravenous catheters can be placed and maintained in superficial veins for administration of test compounds or for blood collection. Before the initiation of a study, dogs can be surgically implanted with vascular access ports and telemetry devices that will facilitate test compound administration, blood collection, and collection of data such as blood pressure measurements, heart rate, specific vessel and cavity pressures, body temperature, and other measurements without hampering the dog's activity or causing it stress or pain.

Dogs on toxicology studies can be cage or kennel housed. Kennel and pen housing for dogs is considered to be the same within the context of this paper. Kennel housing offers many advantages, especially the dog's ability to exercise at will (Hughes et al. 1989). The floors of kennels are usually supplied with sawdust or other suitable bedding material. Kennels will often have raised platforms installed to allow the dog more vertical space (Figure 6).

Socialization with conspecifics should be considered of paramount importance for dog studies (Beerda et al. 1997). Dogs that are pair or group housed generally exhibit less stereotypic behaviors than individually housed dogs. Dogs of both sexes will play together and rely less on other forms of enrichment when they are group housed. It is relatively easy to establish appropriate pairs or groups of dogs, especially if they are young. Fighting occurs occasionally in older males and is usually prompted by disagreements over food or a toy. Dogs should be able to be pair or group housed while on toxicology studies; however, special attention must be paid to feed consumption. If dogs cannot be paired while on study, they should be allowed to have visual, auditory, and olfactory socialization with conspecifics (Wells and Hepper 1998).

Meal feeding with an optimum amount of feed, rather than ad libitum feeding, should be the norm for all dogs. Overeating in dogs can lead to obesity and other problems. Frequently, the volume of feed offered must be adjusted for individual dogs within a group closely matched in age and weight to maintain optimal body scores. Typically pair- or group-housed dogs must be separated at the time of feeding due to individual differences in feed consumption rate and the possibility for fighting. Because most dogs will readily accept treats in lieu of their balanced diet, treats are often not used for dogs in regulated studies.

Numerous commercial dog toys are available and many are suitable for use in a laboratory setting. The toy should be constructed of a material that is not easily ingested, and if it is ingested, it should be innocuous. Dogs enjoy chewing and will ingest small amounts of most of the toys that are available. Analysis and certification are available on some of the dog toys specifically marketed for the research laboratory.

Human interaction is extremely important for dogs, and it is a form of enrichment that is relatively easily attained (Hubrecht 1995; Loveridge 1998). Such interaction is also mutually beneficial for dogs and their care givers. Dogs can be easily acclimated to specific research-related needs such as staying in a sling for blood sampling or lying on their side for electrocardiograms. Dogs also enjoy being groomed. Care givers enjoy interacting with dogs and a regular schedule for interactions such as training, acclimatization, and use of special devices should be encouraged and developed (Figure 7).

Nonhuman Primates

In regulated studies, nonhuman primates are the alternative nonrodent species of choice when a dog is not acceptable.

Figure 6 Kennels with raised platforms allow dogs to utilize more of the available vertical space.

Figure 7 Regular exercise programs and interaction with care givers are important forms of enrichment for dogs.

The most commonly used genus of nonhuman primates in regulated studies is *Macaca.*

Dogs may not be acceptable for a number of reasons including the following:

- The potential drug to be studied is active in vivo on receptors or enzymes that occur only in primates (both human and nonhuman);
- The potential drug would be destroyed by the immune system of other species but would be biologically active in both human and nonhuman primates;
- The anatomy and/or physiology of a disease syndrome (e.g., osteoporosis) is similar in humans and nonhuman primates but different in other species;
- The drug or vehicle used to administer the drug is tolerated only in humans and nonhuman primates and not in other animal species (e.g., dopamine agonists);
- The assessment for a disease process can be studied only in primates (e.g., the assessment of certain cognitive abilities and behaviors that are only exhibited in nonhuman primates and humans); and
- A disease such as polio, which occurs only in primates.

Transgenic rodents, specifically mice, have replaced the use of nonhuman primates in some studies (Herzyk et al. 2001), but there are still many studies in which nonhuman primates are needed.

When it is necessary to use nonhuman primates, it is essential to consider and address all aspects of enrichment to attempt to enhance their lives while serving on a study. As with dogs, there are several surgical models available to facilitate and improve the quality of data collected and minimize study-induced stress, such as telemetry systems. Consistent collection of higher quality data may in turn allow for a smaller group size.

Nonhuman primates assigned to toxicology studies have been traditionally housed in stainless steel cages. However, there is a trend toward housing in pens that are larger and allow for greater movement, exercise and preferences for perching and resting. The use of pens works best with smaller species that can be safely hand or net captured. The use of pens may prove to be impractical and unsafe for humans and nonhuman primates when the study requires that larger species are handled frequently. The cage or pen must be constructed so that the animals cannot escape or become trapped or entangled, and all surfaces must be free of sharp edges. Some cages are constructed with panels that can be removed to open up two to four consecutive cages to a compatible group of nonhuman primates. All enclosures for nonhuman primates must allow the animal to stand to its full height and move freely about the enclosure. Perches must also be provided.

Conspecific socialization is extremely important for all nonhuman primates, and there are numerous studies that reveal the benefits to be gained by providing adequate socialization (Novak and Suomi 1991; Reinhardt 1991, 1994; Woolverton et al. 1989). However, if social groups change frequently, aggression and injuries also increase (Woolverton et al. 1989). Some institutions routinely pair or group house their nonhuman primates on toxicology studies. It is essential that paired or grouped nonhuman primates are observed very closely to ensure that the animals are compatible. This observation is particularly important with some of the larger, more aggressive species such as macaques. It is also important to provide a large enough enclosure that a nonhuman primate can escape from an aggressor if necessary. If it is not possible to pair or group house macaques, it is important to provide them with other forms of contact such as visual, auditory and olfactory socialization. The cage should also provide a visual barrier to allow subordinate individuals to retreat behind an area. Some cages are constructed with panels that allow nonhuman primates to touch fingertips without allowing fingers to be damaged.

There are many opportunities for enriching the lives of nonhuman primates by providing a variety of feed treats or supplements. Most nonhuman primates are fed a commercially available chow as the major component of the diet. Certified diets are available for toxicology study use. The main diet should be supplemented with fresh fruits and vegetables and other treat items in small quantities. When selecting the types of produce to feed to nonhuman primates, it is important to make choices based on how well the item is favored, and whether the item could have any effect on the data, such as changing clinical chemistry parameters. All produce must be thoroughly washed or peeled in order to reduce pesticide residue ingestion. Other treats such as raisins, grapes and cereals may be offered in small quantities and can be used for hand feeding purposes or as rewards. Also, ice cubes, grapes frozen in ice cubes and frozen cubes of fruit juice provide enjoyment for many nonhuman primates. It is important to acclimate the animals to the type of produce or treat before initiation of the study so that unexpected occurrences can be avoided. It is also advisable to generate a specific list of appropriate and acceptable items to feed and then to rotate through the list so that individuals do not become bored with the selection.

Many commercially available toys and enrichment devices are commonly used with nonhuman primates on toxicology studies (Figure 8). These items seem to entertain and occupy many nonhuman primates, particularly if they are individually housed. Stainless steel mirrors attached to the outside of the cage by a chain are among the devices used the most consistently by cynomolgus macaques at the authors' facility. Macaques have been observed to use the mirrors to look at themselves, and also to angle the mirror to be able to look down the line of cages to see activities at the other end of the room. With hanging devices, it is important to limit the length of the chain or rope in order to avoid accidental hangings. Many dog toys can be given to nonhuman primates to provide something to chew on or toss around the cage. Puzzle toys which challenge the nonhuman primate to work for a small treat are used by many cynomolgus macaques (authors' experiences). Rope or chain swings provide additional enrichment to nonhuman pri-

Figure 8 Different forms of enrichment devices for nonhuman primates include manipulative devices, gnawing objects, and audiovisual media.

mates. However, these devices may interfere with the cage's squeeze back. The use of hammocks has been evaluated at the authors' facility, and they are used by many individuals for resting and swinging (Figure 9). The hammocks do not interfere with the squeeze plate, as it is attached to and pulled up along with the squeeze plate. It is important to alternate the types of enrichment devices periodically so that the animals do not become bored. The periodic use of music and videos is used routinely as enrichment for nonhuman primates on toxicology studies. Radio music was shown to reduce the heart rate of baboons in one study, although blood pressure and behavior did not vary (Weaver 1996). Videos must be selected carefully to ensure that nothing contained in them is frightening or evokes an aggressive behavior in the viewers. Images of snakes even in cartoon form have caused increased incidences of stereotypic behavior and self-biting in groups of macaques in the authors' facility.

Figure 9 Hammocks are used by macaques for resting and swinging in the authors' facility.

Positive human interaction is critical for nonhuman primates involved on studies where handling is routine. Most nonhuman primates will respond favorably to kind human interaction once they have become accustomed to it. Handling must be performed routinely before study initiation in order to acclimate the nonhuman primates to the handlers and procedures. With kind and consistent handling, many macaques learn to cooperate with handlers, which ultimately leads to less stress in the animal and better data overall.

Conclusion

We believe that best practice can be defined as a partnership between law, science and people. It is important to remember that the best practices in place now, are not necessarily the best practices of tomorrow. Only by observation of animals in our care can they instruct. By observation, investigation and publication of findings, best practices will continue to evolve for both the benefit of the animals, and the people who work with them.

References

AAALAC International [Association for Assessment and Accreditation for Laboratory Animal Care International]. 2000. Connection Newsletter, Summer.

Anderson CO, Denednberg VH, Zarrow MX. 1972. Effects of handling and social isolation upon the rabbit's behaviour. Behaviour 43:165-175.

Baumans V. 1997. Environmental enrichment: Practical applications. In: Van Zutphen LFM, Balls M, eds. Animal Alternatives, Welfare and Ethics. Elsevier. p 187-197.

Bell DJ, Bray GC. 1984. Effects of single and mixed sex caging on post weaning development in the rabbit. Lab Anim 18:267-270.

Beerda B, Schilder MBH, Bernadina W, VanHooff J, De Vries HW, Mol JA. 1997. Chronic stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological responses. Physiol Behav 66: 243-254.

Blom HJM, van Tintelen G, Van Vorstenbosch CJAHV, Baumans VJ, Beynen AC. 1996. Preferences of mice and rats for types of bedding material. Lab Anim 30:234-244.

Christian MS, Hoberman AM, Johnson MD, Brown WR, Bucci TJ. 1998. Effect of dietary optimization on growth, survival, tumor incidences and clinical pathology parameters in CD Sprague Dawley and Fischer-344 rats: A 104-week study. Drug Chem Toxicol 21:97-117.

Clark RL, Antonello, JM, Wenger JD, Deyerle-Brooks K, Duchai DM. 1991. Selection of food allotment for New Zealand white rabbits in developmental toxicity studies. Fund Appl Toxicol 17:584-592.

Council of Europe. 1997. Resolution on the Accommodation and Care of Laboratory Animals, adopted by the multilateral consultation on 30 May 1997. Strasbourg: Council of Europe.

Council of Europe. 2001. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123). Future principles for housing and care of laboratory rodents and rabbits. Strasbourg: Council of Europe.

Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider NL. 1983. Differential effects of intermittent feeding and voluntary exercise on body weight and lifespan in adult rats. J Gerontol 38:36-45.

Grewal IS, Heilig M, Miller A, Sercarz EE. 1997. Environmental regulation of T-cell function in mice: Group housing of males affects accessory cell function. Immunology 90:165-168.

Haseman JK, Bourbina J, Eustis SL. 1994. Effect of individual housing and other experimental design factors on tumor incidence in B6C3F1 mice. Fund Appl Toxicol 23:44-52.

Haseman JK, Hailey JR, Morris RW. 1998. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update. Toxicol Pathol 26:428-441.

Herzyk DJ, Gore ER, Polsky R, Nadwodny KL, Maier CC, Liu S. Hart TK, Harmsen AG, Bugelski PJ. 2001. Immunomodulatory effects of anti-CD4 antibody in host resistance against infections and tumors in human CD4 transgenic mice. Infect Immun 69:1032-1043.

Hubrecht RC. 1995. Enrichment in puppyhood and its effects on later behavior in dogs. Lab Anim Sci 45:70-75.

Hughes HC, Campbell S, Kenney C. 1989. The effects of cage size and pair housing on exercise of beagle dogs. Lab Anim Sci 39:302-305.

Huls WL, Brooks DL, Bean-Knudsen D. 1991. Response of adult New Zealand White rabbits to enrichment objects and paired housing. Lab Anim Sci 41:609-612.

ICH [International Conference on Harmonization]. 1993. ICH Guidelines: Detection of Toxicity to Reproduction for Medicinal Products. Jun. Geneva: ICH Secretariat.

ICH [International Conference on Harmonization]. 1996. ICH Guidelines: Single Dose Acute Toxicity Testing for Pharmaceuticals. Aug. Geneva: ICH Secretariat.

Klurfeld DM, Lloyd LM, Welch CB, Davis MJ, Tulp OL Kritchevsky D. 1991. Reduction of enhanced mammary carcinogenesis in LA/N-cp (corpulent) rats by energy restriction. Proc Soc Exp Biol Med 196:381-384.

Love JA. 1994. Group housing: Meeting the physical and social needs of the laboratory rabbit. Lab Anim Sci 445-11.

Loveridge GG. 1998. Environmentally enriched dog housing. Appl Anim Behav Sci 59:101-113.

Manser CE, Broom DM, Overend P, Morris TM. 1998. Investigations into the preference of laboratory rats for nestboxes and nesting materials. Lab Anim 32:23-35.

NRC [National Research Council]. 1996. Guide for the Care and Use of Laboratory Animals. Washington DC: National Academy Press. 7th ed. p 23-24.

Novak MA, Suomi SJ. 1991. Social interaction in nonhuman primates: An underlying theme for primate research. Lab Anim Sci 41:308-314.

OECD [Organisation for Economic Co-operation and Development]. 2000. Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Paris: OECD.

Pakes SP, Lu YS, Meunier PC. 1984. Factors that complicate animal research. In: Fox JG, Cohen BJ, Loew FM, eds. Laboratory Animal Medicine. New York: Academic Press. p 649-665.

Peace TA, Singer AW, Niemuth NA, Shaw ME. 2001. Retrospective analysis of foot lesion development in rats housed on wire-bottom versus solid-bottom caging, and between rats from different Sprague Dawley suppliers. Contemp Top Lab Anim Sci 40(5):17-21.

Podberscek AL, Blackshaw JK, Beattie AW. 1991. The effects of repeated handling by familiar and unfamiliar people on rabbits in individual cages and group pens. Appl Anim Behav Sci 28:365-374.

Purchase IF, Botham PA, Bruner LH, Flint OP, Frazier JM, Stokes WS. 1998. Scientific and regulatory challenges for the reduction, refinement, and replacement of animals in toxicity testing. Toxicol Sci 43: 86-101.

Reinhardt V. 1991. Group formation of previously single-caged adult rhesus macaques for the purpose of environmental enrichment. J Exp Anim Sci 34:110-115.

Reinhardt V. 1994. Pair-housing rather than single-housing for laboratory rhesus macaques. J Med Primatol 23:426-431.

Rock FM, Landi MS, Hughes HC, Gagnon RC. 1997. Effects of caging type and group size on selected physiologic variable in rats. Contemp Top Lab Anim Sci 36:69-71.

Smiler K, Schroder M, Goulet S, Turner PV, Koch MA, Mack PA, Hargaden M. 2001. Animals in safety and toxicology studies: Validation of new procedures in your GLP facility. Presented at the national meeting of the American Association for Laboratory Animal Science, Baltimore, MD, October 23, 2001.

Spencer PJ, Mattsson JL, Johnson KA, Albee RR. 1993. Neurotoxicity screening methods are sensitive to experimental history. Int J Psychophys 14:5-19.

Turturro A, Duffy P, Hart R, Allaben WT. 1996. Rationale for the use of dietary control in toxicity studies—B6C3F1 mouse. Toxicol Path 24: 769-775.

USDA, APHIS [United States Department of Agriculture, Animal and Plant Health Inspection Service]. 1999. Final Report on Environmental Enhancement to Promote the Psychological Well-Being of Nonhuman Primates. Riverdale MD: USDA.

Van de Weerd HA, Baumans V. 1995. Environmental enrichment in rodents. Environmental Enrichment Information Resources for Laboratory Animals 1965-1995 AWIC 1965-1995: Birds, Cats, Dogs, Farm Animals, Ferrets, Rabbits, and Rodents. AWIC [Animal Welfare Information Center] Resource series no. 2. US Department of AWIC/ UFAW. Agriculture, Beltsville, MD, and Universities Federation for Animal Welfare, Potters Bar, Herts, UK.

van Herck H, Baumans V, Brandt CJWM, Boere HAG, Hesp APM, van Lith HA, Schurink M, Beynen AC. 2000. Blood sampling from the retro-orbital plexus, the saphenous vein and the tail vein in rats: Comparative effects on selected behavioural and blood variables. Lab Anim 35:131-139.

Weaver BL. 1996. The physiological and behavioral affects of radio music on singly housed baboons. J Med Primatol 25:370-374.

Wells DL, Hepper PG. 1998. A note on the influence of visual conspecific contact on the behaviour of sheltered dogs. Appl Anim Behav Sci 60:83-88.

Whary M, Peper R, Borkowski G, Lawrence W, Ferguson F. 1993. The effects of group housing on the research use of the laboratory rabbit. Lab Anim 27:330-341.

Woolverton WL, Ator NA, Beardsley PM, Carroll ME. 1989. Effects of environmental conditions on the psychological well-being of primates: A review of the literature. Life Sci 44:907-917.