

Laboratory Animal Science Issues in the Design and Conduct of Studies with Endocrine-active Compounds

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Abstract

The use of rodent models for research and testing on endocrine-active compounds necessitates an awareness of a number of laboratory animal science issues to standardize bioassay methods and facilitate reproducibility of results between laboratories. These issues are not unique to endocrine research but are particularly important in this field due to the complexities and interdependencies of the endocrine system, coupled with the inherently sensitive and variable nature of physiological endpoints. Standardization of animal models and the control of animal environments depend on the establishment of strong scientific partnerships between research investigators and laboratory animal scientists. Laboratory animal care and use programs are becoming increasingly complex and are constantly changing, fueled in part by technological advances, changes in regulations concerning animal care and use, and economic pressures. Since the early 1980s, many institutions have moved to centralization of animal facility operations concomitant with numerous changes in housing systems, barrier concepts, equipment, and engineering controls of the macro- and microenvironment. These and other changes can have an impact on animals and the conduct of endocrine experiments. Despite the potential impact of animal care and use procedures on research endpoints, many investigators are surprisingly naive to the animal facility conditions that can affect *in vivo* studies. Several key animal care and use issues that are important to consider in endocrine experiments with rodent models are described.

Key Words: animal science; endocrine-active compound; environment; experimental design; rodent

Introduction

Studies of endocrine active compounds (EACs¹) in rodent models have been fraught with numerous inter-laboratory inconsistencies and lack of reproducibility (Ashby 2001; Ashby and Elliot 1997). These findings are

not unexpected considering the relative infancy of the endocrine disruptor field and challenges surrounding the standardization of rodent assays with EACs (Ashby 2003). In addition to the complexities of the endocrine system, there is not yet consensus on the experimental endpoints suitable for assessing whether exposure to EACs has the potential to produce adverse effects. Unlike many other areas of toxicology, health effect studies with EACs often evaluate endpoints that consist of subtle changes rather than overt indices of toxic change. These subtle reproductive, endocrine, behavioral, and developmental changes can have intrinsic variability within individuals and populations (Elswick et al. 2000). Toxicology endpoints in rodent studies are increasingly more complex and more sensitive. This trend is best exemplified by the increasingly widespread use of genetically engineered rodents coupled with high-throughput biology approaches incorporating ever more powerful “omic” technologies (Misra and Duncan 2002).

Advances in molecular biology are being coupled with increasing sophistication in animal monitoring systems such as telemetric assessment of physiological parameters (Kramer et al. 2001), automated dosing and sampling techniques (Xie et al. 2003), and noninvasive imaging methods (Balaban and Hampshire 2001). These technologies make rodent molecular medicine a reality and contribute to an improved understanding of endocrine-related pathophysiology. Despite great technological advances, it is important to recognize that experimental studies with rodents, especially those that involve endocrine endpoints, have many potential methodological pitfalls that can entrap investigators who do not have a thorough understanding of laboratory animal science issues. Minimizing biological variation in rodent endocrine studies necessitates an understanding of the interplay between the animal test system, the experimental objectives and methods, and the animal facility environment. For example, sources of animal stress attributable to the animal facility environment that are capable of confounding study results are often not apparent to investigators (Jain and Baldwin 2003).

Various factors in animal experiments can have an impact on data and account for variability that must be controlled (Table 1). These factors should be considered during the planning and conduct of a research or testing study with EACs. This article highlights selected laboratory science issues that have the potential to influence experimental outcomes in rodent studies with EACs. Several of the more important facets of laboratory animal science are discussed elsewhere in this issue (e.g., genetics issues, reviewed in

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¹Abbreviations used in this article: BPA, bisphenol-A; EAC, endocrine-active compound; IVC, individually ventilated microisolator caging.

Table 1 Factors that influence data in animal studies

• Microbial status	• Diseases and lesions
• Genetic manipulation	• Background genotype
• Environment	• Study methods
• Animal care and use program	• Personnel conducting study

Stokes 2004; diet composition and feeding, Thigpen 2004) and are not reviewed in depth in this paper.

Choice of the Rodent Model

Choice of stock or strain is a critical determinant of outcome and must be considered carefully in the design of endocrine disruptor and other types of toxicology experiments (Kacew and Festing 1996; Long et al. 2000; Spearow et al. 1999). In addition to scientific factors that dictate choice of stock or strain, the availability of the animals to other investigators and laboratories should also be considered. Genetic drift and colony maintenance methods can lead to changes over time in small breeding colonies and have been postulated to account for important differences in findings between laboratories (Ashby 2001). In other words, substrains that have limited availability should not be utilized, and, where possible, rodents of identical genotype should be available to multiple laboratories so that results can be compared and confirmed. Such practices will help avoid controversies inherent in past studies of EACs (Ashby 2001) and facilitate reproducibility between laboratories.

Investigators should consider the husbandry and environment of the source colony of the selected rodent model. Laboratory animal facility managers and veterinarians generally perform quality control checks on genotype, microbial integrity, and health status of source colonies; but less attention is paid to the husbandry and environmental details within vendor facilities. Numerous aspects of environment can affect rodent studies (Table 2) (reviewed in Clough 1982), and endocrine researchers should familiarize them-

Table 2 Animal facility environmental influences that affect data

• Housing methods	• Light
• Bedding	• Noise
• Diet	• Transportation
• Temperature	• Chemicals in feed and bedding
• Humidity	• Air quality
• Ventilation	• Water treatment
• Animal handling	• Vibration
• Urinary chemosignals	• Caging and accessories

selves with the husbandry and environment of animals that are purchased and then taken to novel environments and used relatively soon after arrival.

An understanding of the production environment of rodent vendors is warranted because many colony management practices can affect the final product. Potential factors that should be considered include genetic management, microbial and pathogen status, breeding schemes, housing and husbandry methods, diet, and physical environment. Consideration should be given to how animals are selected for filling orders and how they are transported to research facilities. Upon the arrival of the animals at research facilities, consideration must be given to methods of acclimation to rooms and equipment. Ideally, methods for minimizing bias in the assignment of animals to study groups should be used. One such method is to randomize (Martin et al. 1986), using both age and weight considerations with rigorous restraints on acceptance criteria.

Investigators who have difficulty obtaining sufficient numbers of sex-, age-, and weight-matched animals have a tendency to randomize “outliers” across study groups, rather than conduct studies with stringently specified parameters. The advent of expensive genetically engineered rodent models that are difficult to breed has exacerbated this practice because the production and availability of many genetically modified rodent models are often limited. Investigators should understand production limitations within vendor and in-house source colonies. Ideally, one would want to obtain animal orders so that littermates are not ultimately in the same experimental group. To avoid this result, the production colony must be of sufficient size and the investigator must adhere to appropriate selection practices. Many toxicologists and pathologists have been perplexed by the finding of multiple, supposedly “rare” findings that occur in a single experimental group within a given study. However, such occurrences could be due to a genetic event involving a single litter that was unknowingly distributed into the same treatment group (Hard et al. 1994).

Laboratory Animal Facility Environment

The effects of the physical environment on laboratory animals are well described in the biomedical literature and have been the subject of extensive review (e.g., Clough 1982). Modern animal care and use facilities have numerous engineering features to control and document environmental conditions. There is increasing awareness of the effects of physical environment that is increasing as study endpoints become more sensitive and thus more likely to be influenced by environmental perturbations. This is especially true for studies of EACs in rodent models, in which the intrinsic variability of many key parameters is assessed (Ashby 2002). Various environmental factors have the potential to influence *in vivo* data (Table 2), and many of the individual factors have complex interrelationships. Unfortunately, researchers are periodically reminded of the im-

portance of environmental control in endocrine studies when animal facilities are subjected to renovations and construction projects during which noise and vibration can provide significant environmental variables. In addition to altering sensitive endpoints such as stress hormone levels, unanticipated effects such as disruption of energy balance have been noted (Dallman et al. 1999).

Lighting is an important environmental parameter that can influence endocrine study endpoints. Aspects of lighting that should be considered include wavelength, intensity, photoperiodicity, and duration of exposure (Bellhorn 1980). In addition, relationships between lighting and temperature affect the occurrence of phototoxic retinopathy in rodents and demonstrate the importance of the interplay of environmental factors. Lighting not only affects the visual system but may also have profound systemic effects, as exemplified by the influence of photoperiod on atherogenesis in hamsters (Smith et al. 2001). While lighting affects endocrine endpoints, the endocrine system itself can affect light-induced photoreceptor damage, thus emphasizing the complexities inherent in controlling biological variables (Rudeen and O'Steen 1979).

Although investigators and personnel are aware of the need to control the macroenvironment of the animal room, it is also important to control the microenvironment in animal cages. The concept of macro- and microenvironment is interrelated because room conditions in part govern the conditions within certain kinds of caging systems (Corning and Lipman 1992). Toxicologists have long been aware of the experimental differences between rodents maintained in suspended stainless steel wire cages and those housed on direct contact bedding within shoebox-style cage environments. In addition to potential issues of contact with excreted metabolites and chemicals, the metabolic and thermoregulatory responses of rodents maintained on either metal or plastic caging materials have important implications for pharmacological studies (Gordon and Fogelson 1994).

The microenvironment within the cage may influence the biological response of the animal test system, and several important trends in laboratory animal science are resulting in profound changes in rodent caging. One major trend is to enlarge cages and add objects and complexity for the purpose of environmental enrichment to address animal welfare concerns (Olsson and Dahlborn 2002). This trend has arisen with changes in the regulatory and animal welfare environment and the push to develop a greater understanding of the natural needs of laboratory rodents. Changes in animal husbandry for purposes of animal welfare considerations, such as the use of social housing, have not been rapidly embraced within the regulatory toxicology community, but these changes are now slowly occurring along with validation of methods (Turner et al. 2003).

Another important trend in laboratory animal science is the development of new and technologically advanced filter-capped isolator cages, which serve the simultaneous purpose of excluding pathogens while containing chemical

substances, animal allergens, and exogenous administered compounds within the cage (Lipman 1999). Although it remains difficult to assess scientifically the environmental comfort of rodents and their physiological well-being, there have been many attempts to improve husbandry and caging within the past decade. Much more scientific data are needed for laboratory animal scientists to understand which environmental factors are most important.

Effects of Housing Systems on Research Data with Rodent Models

Housing may have an important environmental influence on rodent research results. Whether animals are individually or group housed can have significant research implications. The literature is replete with examples of hormonal influences and altered endocrine-related parameters affected by housing arrangement (e.g., Nyska et al. 2002). Depending on the endpoint and variable being examined, the literature has conflicting data concerning so-called stress-like responses in group-housed or individually housed rodents (Sharp et al. 2003). There is a trend toward group or social housing in mice and rats for animal welfare reasons, but there is little consensus concerning factors that constitute optimal housing. Optimal rodent housing appears to depend on age, sex, and strain, as well as the experimental objective and situation. In some experimental situations, group housing of rodents has led to the predisposition to stress and endocrine-related pathology (Weinreich et al. 1996).

Another important variable in housing is the size of the cage. Recent studies in rats suggest that interaction between group-housed rats appears to be more important to stress reduction than providing increased floor space per animal (Sharp et al. 2003).

Perhaps the greatest technological change that has affected laboratory animal science has been the widespread generation and use of genetically engineered rodent models. Use of these invaluable models has changed many of the traditional rodent housing practices and concepts and led to many advances and changes in caging systems. Laboratory animal care facilities now struggle to house rodents from multiple sources concurrent with an increasing daily census. The procurement of pathogen-free rodents from a few well-screened commercial vendors and the maintenance of barrier conditions at the facility and room level, a practice utilized in most toxicology testing facilities, is no longer a viable management paradigm for many facilities. Modern toxicology facilities often must now obtain animal models from a wide variety of sources of varying microbial and genetic background, as has been the situation in large academic animal facilities for many years. Genetically engineered rodents are often procured from numerous small colony sources and subsequently housed together in close proximity in the same facilities. This situation has contributed to substantial changes in rodent housing with a movement toward barrier systems at the cage level.

Many genetically engineered rodents have undergone manipulations that can alter immune parameters and predispose the animals to microbial infections that would otherwise be innocuous. The need for flexibility in the animal facility to maintain rodents of multiple sources and differing microbial status led to the concept of “microisolation” caging (Lipman 1999). This system consists of filter-capped shoebox cages that serve as the “barrier.” Cages are handled aseptically as units, in biological safety cabinets, and they effectively prevent the spread of pathogens. They also can serve as containment tools for any exogenous compounds that are administered. The first generation of this caging consisted of “static” filter-capped cages without delivered air. It is now recognized that ventilation is poor in these cages (Keller et al. 1989), and that volatile contaminants can build up due to microbial degradation of waste products, which affects rodent metabolism and the nasal epithelium (Bolon et al. 1991). Because rats and mice are obligate nose breathers whose nasal epithelium has a high level of metabolic activity, this “poorly ventilated” environment can result in potentially significant systemic metabolic effects. A variety of implications can result from the use of these caging systems for toxicology and pharmacology experiments.

In an effort to provide the benefits of microbial protection with excellent air quality, many rodent facilities now use individually ventilated microisolator caging (IVCs¹) (Lipman 1999). Many investigators have begun to examine and define the microenvironmental parameters within these cages (Hoglund and Renstrom 2001; Reeb et al. 1998). Others have examined the physiological parameters of rodents housed in these systems (Chaguri et al. 2001; Tsai et al. 2003). It is important to note that cage environment within the various types of IVC systems can vary substantially with respect to location of air supply in the cage, ventilation rates, cage size, and how objects are placed in the cage. These differences can affect behavior and other parameters (Baumans et al. 2002). It is now more necessary than ever for animal care and use operations to develop in-house databases and an understanding of facility-specific responses.

Olfactory Cues

Olfactory cues are extremely important environmental influences in the animal facility, and numerous chemosignals can affect endocrine experiments. How rodents perceive chemosignals depends in part on the caging and ventilation systems within the facility. Cyclical variation in hormone levels in groups of rodents should be considered in the experimental design of certain endocrine studies, based on knowledge that urinary chemosignals have potentially major effects on cyclicity. One well-known example of this influence is the Whitten Effect in mice, whereby exposure to male urine can induce estrus in female mice. The phenomenon has been used to standardize estrus in research protocols (Dalal et al. 2001). Similarly, urinary chemosignals from female mice can lead to pregnancy termination

and female-biased litters in mice (Drickamer 1999). Both ventilation and caging equipment are important means by which to control the effects from chemosignaling and olfactory cues. Problems are increasingly common in rodent facilities that breed large quantities of genetically engineered mouse models in the same facilities where experiments are conducted. Therefore, research investigators must use caution when co-housing experimental animals with breeding animals.

Urinary Chemosignaling

The effects of urinary chemosignaling must also be considered in certain housing and experimental situations, particularly with group housed rodents. One common experimental procedure in the toxicology laboratory, where airborne chemosignals and olfaction effects may not be fully appreciated, is during the conduct of whole-body inhalation exposure to potential EACs. These studies often mix sexes and species within single inhalation exposure chambers. The same chambers often serve as domiciliary housing during the course of the experiments, and include open hanging stainless steel wire cages. Significant physiological differences may result in animals maintained in this type of housing compared with those animals exposed to compounds within IVCs, where there is no mixing of air between cages. The responses of rodents housed in inhalation exposure chambers may differ substantially from those animals housed in solid bottom shoebox caging. For example, the control incidence rates of lesions, particularly tumors in F344 rats in National Toxicology Program studies, differ substantially in these two types of housing situations (Hase-man et al. 2003).

Long-term Rodent Experiments

For many years, scientists have noted that animal facility procedures should include careful consideration of cage allocation designs for long-term rodent experiments (Herzberg and Lagakos 1991; Young 1987, 1989). Accordingly, investigators should carefully consider the experimental objectives and potential effects of the following: how animals will be allocated to cages and cages to racks, how cage racks are distributed within individual rooms, and how rooms are situated within the animal care facility. Many animal care facilities develop standard operating procedures that govern how cages and racks are rotated on study to minimize the bias of differing environmental conditions such as light and ventilation levels. Consideration of these issues can be complex. For example, with dosed-feed studies, it is necessary to consider the appropriateness of housing control animals in the same room with treated animals to avoid the airborne spread of one or more compounds on particulate matter.

If it is necessary to house controls in the same room, special containment procedures such as microisolation caging should be used. Likewise, although statisticians might

suggest complete randomization of cages on racks in a room, the reality is that on long-term dosed-feed studies, there would be great possibility of a feeding error if animal cages were distributed in that fashion. Those factors must be considered and procedures instituted where necessary to minimize bias and variation. The experimental needs, facility design, equipment, and personnel factors all weigh into the decision making process. The research investigator should discuss these issues with laboratory animal science professionals during protocol design and review.

Potential for Recent Changes in Rodent Housing to Affect Experiments with EACs

The *Guide for the Care and Use of Laboratory Animals* (NRC 1996), an important reference used by the Association for Assessment and Accreditation of Laboratory Animal Care, International, emphasizes a preference to provide direct contact bedding in rodent cages. This emphasis has raised issues for toxicology studies. Many facilities that housed experimental rodents on suspended wire-bottom caging changed to solid-bottom cages, thus changing in-house databases and facility experience. This change in rodent housing has been substantial because more than 80% of rodents housed in toxicology facilities in a 1999 survey were housed on wire-bottom caging (Stark 2001). Investigative staff must be aware of the types of direct contact bedding used in studies of EACs, and must ensure that the bedding avoids potential contaminants such as mycotoxins and other chemicals that can affect these experiments.

Recently, in efforts to provide environmental enrichment, animal care and use programs have embraced policies to make cage environments more complex with the addition of "play objects," nesting boxes and materials, and other types of accessories (Turner et al. 2003). Additional data are needed to determine fully whether these enrichment schemes contribute to rodent well-being. It is important to recognize that these changes in environment have the potential to affect experiments significantly. One example is the effect of rodent environment on the development of synaptic connections in the brain (Benefiel and Greenough 1998). In some instances, polyvinylchloride objects have been used as enrichment devices, although this material contains phthalates, which are known to be endocrine-active materials (Mylchreest et al. 1999).

The rearing of rodents in more complex cage environments can contribute to changes in behavior and the developing brain (Benefiel and Greenough 1998; Wurbel 2001). In addition, enrichment of cage environment is known to have effects on stress physiology, which is important to investigators working with endocrine-related endpoints (Haemisch and Gartner 1997). As one of many examples, enriched housing environment was found to exacerbate amyloid precursor protein in a transgenic mouse model of Alzheimer's disease, thereby influencing the progression of

the pathology (Jankowsky et al. 2003). It should be noted that the effects of different enrichment designs on physiology and behavior in rodents may be species and strain dependent (Tsai et al. 2003).

Potential Effects of Plastic Cages in Experiments with EACs

Recently there has been a great deal of concern in the scientific community concerning low-dose effects of EACs such as bisphenol-A (BPA¹) leaching from plastic products and affecting human health. Although BPA is clearly estrogenic at high dose levels, the data supporting low-dose effects of BPA are still somewhat controversial and were the subject of an Environmental Protection Agency expert panel (Kaiser 2000). The panel concluded that doses of some endocrine disruptors significantly less than those normally found to be safe can cause biological effects in laboratory animals. The panel members cautioned, however, that scientists do not understand the relevance of these subtle developmental changes in animals to human health. Several recent studies indicate that caution is warranted in the use of certain polymer plastic caging and other products in rodent studies of EACs (Howdeshell et al. 2003; Hunt et al. 2003). For example, BPA has been shown to leach from polycarbonate flasks during autoclaving, and the estrogenic activity of the leached material was demonstrated in *in vitro* studies with the MCF-7 cell line (Krishnan et al. 1993).

Two recent studies incriminate polycarbonate caging with endocrine effects. The first study, by Hunt and colleagues, linked a sudden spontaneous increase in meiotic disturbances in oocytes from control mice with accidental exposure of animals to an environmental source of BPA (Hunt et al. 2003). The investigators identified the source of the exposure as polycarbonate caging material that had been damaged by the inadvertent use of a harsh alkaline detergent. They were able to reproduce the meiotic abnormalities by deliberately damaging caging and water bottles with detergent. They further determined that BPA induced meiotic effects, and that these effects were dose dependent and could be induced by environmentally relevant doses of BPA. A subsequent study by Howdeshell and colleagues showed that BPA leaches from polycarbonate and polysulfone cages into standing water with BPA levels highest in old, visibly worn polycarbonate cages (Howdeshell et al. 2003). Based on their findings, these authors stated that laboratory animals maintained in polycarbonate and polysulfone cages may be exposed to BPA via leaching.

At a recent symposium sponsored by the Institute for Laboratory Animal Research, a speaker stated that housing mice in polycarbonate caging could lead to endocrine disruption effects (NRC 2004). This statement created concerns among the laboratory animal specialists in the audience due to the fiscal and operational effects of discarding existing polycarbonate rodent housing systems. It is

Table 3 Laboratory animal science issues to be addressed by the endocrine-active compound research team

Institutional Animal Care and Use Committee (IACUC) Issues

Review each experiment from the standpoint of optimizing animal numbers and ensuring proper statistical design.
Review issues of animal pain and distress and consider methods to minimize these factors.
Review personnel training and training documentation issues.
Define humane endpoints/criteria for euthanasia and the decision tree for determination.

Study Issues

Ensure compliance with applicable regulatory test guidelines.
Review staffing and associated concerns (e.g., training, logistics).
Determine methods of allocation of animals to study without bias (randomization).
Determine animal identification and cage identification methods.
Determine acclimation and quarantine periods (length, location, methods).
Review dosing methods and issues related to test compounds (e.g., dose schedule, volume, vehicle, special monitoring needs).
Establish any chain of custody record and compound archiving requirements in the animal facility.
Review all experimental manipulations of animals, including timing of events.
Establish Standard of Practice for removing animals unscheduled from study and determine methods for handling and disposition of these animals.

Animal Model Selection and Use

Determine species and strain, and review scientific rationale for model selection.
Establish age, gender, weight, physiological status, and genetic needs.
Choose source or supplier.
Review genetic history and health history of source colony.
Establish need for any prestudy health or genetic testing of supplied animals.
Review strain-related causes of morbidity and mortality and institutional experience with chosen animal model.

Animal Environment

Review animal room environmental issues (temperature, humidity, lighting, noise, air flow) on both room and cage levels.
Establish methods and intervals for environmental monitoring.
Review housing issues (group vs. single housing, type of caging, type of bedding, frequency of cage change, room allocation, and methods to prevent bias in housing and handling such as rotation of cage or rack placement).
Review special housing needs (e.g., inhalation or metabolism caging) and methods for acclimation to equipment and special procedures.
Determine whether other animals (e.g., sentinel animals) or studies (e.g., microbial infections in facility) pose danger to study animals and institute protective procedures if warranted.
Determine whether various groups of animals housed in proximity to study will have effects on study subjects (e.g., urinary chemosignal effects).

Diet

Specify type (e.g., open vs. closed formula, natural vs. semipurified ingredients) and form (e.g., pelleted, powder, liquid) of diet.
Review method of feeding (i.e., ad libitum vs. diet restriction).
Review phytoestrogen content of diet.
Determine the need for certified diet or for methods for contaminant analysis.
Review water distribution method (bottle vs. automatic) and treatment method if any (e.g., autoclaved, filtered, acidified, chlorinated).
Determine methods and intervals for monitoring food and water consumption if needed.
In the case of dosing in feed or water, review stability, mixing, analytical issues related to the diet, and palatability.

Conduct of In-life

Establish clinical parameters to be monitored on study as well as schedules and methods of monitoring.
Determine need for monitoring of cycling and other reproductive parameters and methods of monitoring.
Review animal manipulation, sampling, and dosing procedures with respect to circadian rhythm and photoperiodicity issues.
Review chemical safety issues and cage change procedures.

important to recognize the existence of significant knowledge gaps concerning effects of low-dose BPA (Kaiser 2000). Furthermore, there is little compelling evidence that under normal conditions of use, plastic caging leads to endocrine-related effects. The Howdeshell study used a very artificial leaching system. The actual effects on mice in that study (16% increase in uterine weights in prepubertal female mice relative to females housed in polypropylene cages) did not reveal statistically significant differences. Thus, data suggest that endocrine disruption effects may result from low-dose BPA, but additional studies are needed to confirm these results. Furthermore, it is premature to conclude that housing rodents in well-maintained polycarbonate or polysulfone cages leads to detrimental effects. Additional laboratories should corroborate these findings before major changes are warranted in animal facilities. Nonetheless, the findings are provocative and should serve as a warning against improper use of sanitization and autoclave procedures, as well as the development of policies for culling damaged cages from use.

Controlling Variability by Optimizing Husbandry and Experimental Conditions

Animal husbandry procedures such as routine cage changing can affect animal responses and influence endocrine-related endpoints. For example, cage change operations have been associated with transient but significant changes in cardiovascular parameters and behavioral responses (Duke et al. 2001). In certain situations, caging systems such as IVCs can minimize the need for frequent cage changes and thus reduce handling and other stress. Minimizing the effects of human interactions in husbandry and experimental procedures is important for all studies of EACs. Techniques that are non- or minimally invasive and that minimize stress, such as the use of telemetric monitoring and remote automated sampling techniques, are useful to incorporate into study designs (Kramer et al. 2001; Xie et al. 2003). The acclimation of animals to experimental setups and equipment is one of the most important areas in which investigators can reduce in vivo study variables. For example, acclimation to metabolism cages in a rat nephrotoxicity experiment was shown to reduce the LD₅₀ of a nephrotoxicant 60-fold, once animals were acclimated to the cages and learned how to drink to avoid dehydration (Damon et al. 1986).

In rodent experiments with endocrine-related endpoints, investigators should carefully consider and standardize the timing of all handling and manipulative events in husbandry and experimental procedures. Consideration should be given to the timing of events such as light cycles and circadian rhythms. Investigators should review the design of experiments with laboratory animal specialists, and discuss potential confounders and experimental needs in the animal facility during the genesis of experimental protocols (Table 3).

Conclusions

The control of biological variation in rodent endocrine studies necessitates an understanding of the interplay between the animal test system, the experimental objectives and methods, and the animal facility environment. The generation of reproducible data and the control of variables in rodent endocrine experiments depend on a close partnership between the research investigator and the laboratory animal scientist, who should work in concert to optimize the experimental design and conduct of these bioassays.

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