CHAPTER

14

Opportunities for Implementing the 3 Rs in Drug Development and Safety Assessment Studies Using Nonhuman Primates

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OUTLINE

INTRODUCTION

The "3 Rs" Principles

The concepts of replacement, reduction, and refinement as guiding principles for humane science were first elaborated by Russell and Burch [1]:

- Replacement of animals with nonanimal methods
- Reduction of the number of animals used to the minimum necessary to obtain information of a given amount and precision
- Refinement of scientific procedures and husbandry to minimize pain and distress and improve animal welfare.

The principles of the "3 Rs" have since become accepted internationally and adopted in legislation on the protection and use of laboratory animals. They are increasingly viewed as an integral part of mainstream scientific practice, and there is recognition that application of the 3 Rs during the design and conduct of scientific research studies can benefit not only animal welfare but also the quality of the science and business efficiency. This has led to the 3 Rs also being included in international legislation on the development of pharmaceuticals for human use.

Effective implementation of the 3 Rs requires a team approach, with information sharing, buy-in from senior management, and collaboration within and between organizations. It is worth mentioning, however, that the 3 Rs is not a competitive part of drug development. Hence, more than 30 international pharmaceutical companies, contract research organizations, and regulators have collaborated with the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) to identify opportunities to reduce and refine the use of nonhuman primates. Most of this work has been done outside the demands of the regulatory framework and has led to, for example, opportunities to reduce by up to 64% the number of nonhuman primates used per monoclonal antibody in development.

To fully apply the 3 Rs and maximize the benefits there is a need to ensure that as new knowledge, technologies, and approaches emerge there is timely assessment and evolution of scientific procedures and husbandry, research strategies, and study designs to meet best practices. Ideally, this would be a continuous process. The NC3Rs website (www.nc3rs.org.uk) is one source of contemporary information and advice.

The Current Global Legislative Environment

The use and care of nonhuman primates in research and testing is regulated by national and international laws and governmental regulations and policies on the use of animals for scientific purposes [2]. These are often supplemented by nongovernmental systems of oversight, such as voluntary participation by institutions in accreditation programs (e.g., the Association for Assessment and Accreditation of Laboratory Animal Care International), company global animal welfare policies, and the peer-review processes and grant terms and conditions of research funding bodies (e.g., the National Institutes of Health, European Commission, Wellcome Trust). Together, these oversight mechanisms help to provide the structures to support application of the 3 Rs to nonhuman primate use. Key elements common to many laws on the protection of animals used in science include a requirement for scientific and ethical evaluation of research projects before their authorization, appropriate veterinary care, trained and competent staff, and implementation of measures to minimize pain and distress, such as anesthesia, analgesia, and humane end points (Table 14.1).

The use of nonhuman primates in regulated scientific procedures raises ethical issues [3] and is of great concern to the public [4]. Accordingly, some laws place restrictions on nonhuman primate use, and most contain special provisions aimed at improving the health and psychological well-being of these animals, such as minimum space allowances and requirements for social housing and environmental enrichment (Table 14.2). It is worthwhile to note that while adherence to the prescriptive engineering standards recommended or mandated by law is a starting point for good animal welfare, often much more can be done to refine housing and care to deliver truly high welfare standards. Another important issue is the international supply of nonhuman primates for research, which raises logistical and animal welfare concerns [5,6]. For practical advice on refining supply and transport, see Jennings and Prescott [7] and Swallow et al. [8].

STUDY DESIGN CONSIDERATIONS TO MINIMIZE NONHUMAN PRIMATE USE

Nonclinical safety testing of new chemical and biological entities is a mandatory regulatory requirement before initiating clinical trials in humans. Nonhuman primates are increasingly being used in nonclinical safety testing of pharmaceuticals, which is in large part due to the significant increase in the number of biologics that are in development across the global pharmaceutical industry [9]. While there is a comprehensive set of International Committee of Harmonisation (ICH), US Food and Drug Administration, and Committee for Medicinal Products for Human Use guidelines that lay out the framework, end points, relative timing, and requirements for nonclinical safety testing, none of these guidelines makes

TABLE 14.1 General Legal Provisions Supporting the 3 Rs (Not Intended to be Exhaustive)

Provision	Europe	USA	China	Singapore	Thailand	India
Use of alternatives	E1	U1, U2	C1	S1, S2	T1	I1
Minimization of animal numbers	E1	U1, U2	C1	S1, S2	T1	I1
Minimization of pain, suffering, and distress	E1	U1, U2, U3	C1	S1, S2	T1	I1
Use of humane end points	E1	U2	C1	S1, S2	T1	I1
Use of anesthesia	E1	U2, U3	C1	S1, S2	T1	I1
Appropriate veterinary care	E1	U2	C1	S1, S2	T1	I1
Trained and competent staff	E1	U1, U2, U3, U4	C1	S1, S2	T1	I1
Ethical review committee or animal care and use committee, with advice on the 3 Rs	E1	U2	To be established at national and provincial levels	S1, S2	T1	I1
Project evaluation before authorization, including compliance with the 3 Rs	E1	U2, U3, U6	To be established	S1, S2	T1	I1
Retrospective review	E1	–	–	–	–	–
Harm-benefit assessment as part of the project evaluation	E1	U2	IACUC to review justification for and objectives of animal use	S1	T1	I1
National committee, with advice on the 3 Rs	E1	–	CALAS Laboratory Animal Welfare and Ethics Committee	National Advisory Committee for Laboratory Animal Research	The National Research Council of Thailand	Committee for the Purpose of the Control and Supervision of Experiments on Animals
Minimum enclosure sizes/space allocations (i.e., national animal welfare standard)	E1, E2, E3	U2, U3	C2	S2	T1: The type of animal cage depends upon the standard required for the species, weight, and number of animals in the cage	I1

Provision of environmental enrichment	E1, E2, E3	U2, U3	C1: The special husbandry demand of animals in certain pathogenic and physiological conditions, including welfare requirements, shall be satisfied and be subject to IACUC review and where possible, appropriate items or devices shall be provided to laboratory animals based on their species and intended uses	S2	T1	-

E1: European Community (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September on the Protection of Animals Used for Scientific Purposes, OJ L276/33. Brussels: European Commission. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

E2: Council of Europe (2005). European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986 - Text amended according to the provisions of the Protocol (ETS No. 170) as of its entry into force on 2 December 2005. Strasbourg: Council of Europe. http://conventions.coe.int/Treaty/en/Treaties/Html/123.htm#ANX.

E3: Council of Europe (2006). Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). Guidelines for Accommodation and Care of Animals (Article 5 of the Convention). Approved by the Multilateral Consultation. Cons 123 (2006) 3. Strasbourg: Council of Europe. http://conventions.coe.int/Treaty/EN/Treaties/PDF/123-Arev.pdf.

U1: Interagency Research Advisory Committee (1985). U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples.

U2: National Research Council (2011). Guide for the Care and Use of Laboratory Animals, Eighth Edition. Washington, D.C.: National Academies of Science Press. http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf.

U3: US Animal Welfare Act—7 US Code 2131-2157 (Agriculture) 9 CFR Parts 1, 2, and 3. http://www.gpo.gov/fdsys/pkg/CFR-2013-title9-vol1/xml/CFR-2013-title9-vol1-chapI-subchapA.xml.

U4: National Institutes of Health (1985). Health Research Extension Act of 1985, Public Law 99-158, November 20, 1985, Animals in Research. Bethesda, MD: National Institutes of Health. http://history.nih.gov/research/downloads/PL99-158.pdf.

U6: National Institutes of Health (2002). Public Health Service Policy on Humane Care and Use of Laboratory Animals. Bethesda, MD: National Institutes of Health. http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf.

C1: National Standard of the People's Republic of China, Laboratory Animal Facilities—General Requirements for Quality and Competence, Draft Standard for Approval, 12th April, 2013, Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China, 2013.

C2: National Technical Committee on Laboratory Animal of Standardization Administration of China (2010). National Standard of the People's Republic of China, Laboratory animal---Requirements of Environmental and Housing Facilities.

S1: Ministry for National Development (2004). Animals and Birds Act (Chapter 7) Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/12801/Attach24_legislation_AB_CareandUse_rules.pdf.

S2: National Advisory Committee for Laboratory Animal Research (2004). Guidelines on the Care and Use of Animals for Scientific Purposes. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/13557/Attach3_AnimalsforScientificPurposes.PDF.

T1: National Research Council of Thailand (2006). Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes. https://www.aaalac.org/resources/Ethical_Principles_and_Guidelines_for_the_Use_of_Animals_for_Scientific_purposes.pdf.

I1: Ministry of Environment and Forestry, India (2010). Standard Operation Procedure (SOP) for Institutional Animal Ethics Committee (IAEC), Guidelines on the Regulation of Scientific Experiments on Animals, Guidelines for Laboratory Animal Facility and Breeding of and Experiments on Animals (Control and Supervision) Rules, Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). https://www.aaalac.org/resources/SOP_CPCSEA.pdf.

TABLE 14.2 Legal Provisions on the Use of Nonhuman Primates[a]

Provision	Europe	USA	China	Singapore	Thailand	India
Requires justification of nonhuman primates over other vertebrate species	E1	U2: Guide requires justification of any species proposed for use	C1: Animals higher in the phylogenetic scale shall be replaced with lower ones C3: Nonhuman primates can only be used for experiments where they are indispensable	–	–	I1
Restrictions on the purpose of use	E1: Limited to basic research, preservation of the nonhuman primate species, or translational/applied research for the avoidance, prevention, diagnosis or treatment of disease, ill health, or other abnormality of their effects in human beings	–	–	–	–	–
Ban on the use of great apes	E1: Effective ban on great ape use, save exceptional circumstances approved by the European Commission (safeguard clause)	National Institutions of Health has ceased funding most types of studies. The CHIMP Act provides funds a sanctuary system for retired research chimpanzees	–	–	–	–
Species/ genera specific provisions	E1	U2, U3	C3: Playground and access to it should be established for breeding stock and enrichment items appropriate for the species should be placed in the playground	S2	–	I1: Nonhuman primates should have a run for free ranging activities

TABLE 14.2 Legal Provisions on the Use of Nonhuman Primates[a]—Cont'd

Provision	Europe	USA	China	Singapore	Thailand	India
Social housing	E1	U2, U3	C1: In general, where possible, social husbandry of animals shall be provided	S2	–	I1
Environmental enrichment	E1	U2, U3	C3: Playground and access to it should be established for breeding stock, and enrichment items appropriate for the species should be placed in the playground	–	–	I1: Provision should be made for animals with specialized locomotor patterns, especially when held for long periods, e.g., artificial trees, ropes, bars and perches for nonhuman primates
Inspection by competent authorities	E1: At least once per year for breeders, suppliers and users of nonhuman primates Drug agencies for Good Laboratory Practices	USDA (annual) AAALAC (triennial) FDA (for GLP studies) OLAW IACUC (local oversight)	Semiannual by the IACUC	S1, S2 (all species/facilities): Annually by IACUC and as required by AVA	–	–
Retrospective assessment	E1: All projects involving nonhuman primates must undergo a retrospective assessment of the outcomes, harms, and implementation of the 3 Rs	–	–	S2: Retrospective reporting of all adverse events for all species	–	–
Individual marking and history file	E1: Individual marking and history file, including social information	USDA U2	C1: Methods used shall be subject to IACUC review	S1, S2	–	I1: Required for all species

(*Continued*)

TABLE 14.2 Legal Provisions on the Use of Nonhuman Primates[a]—Cont'd

Provision	Europe	USA	China	Singapore	Thailand	India
Breeding strategy / supply	E1: Requirement to move to use of nonhuman primates of F2 or greater generation or else sourced from self-sustaining colonies; subject to feasibility study	National Institutes of Health	In general experimental animals shall be procured from qualified producers and have known sources and acceptable quality	–	–	–
Thematic reviews	E1: Thematic reviews by the European Commission of the 3 Rs and nonhuman primate use especially	–	–	–	–	–

E1: European Community (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September on the Protection of Animals Used for Scientific Purposes, OJ L276/33. Brussels: European Commission. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

U2: National Research Council (2011). Guide for the Care and Use of Laboratory Animals, Eighth Edition. Washington, D.C.: National Academies of Science Press. http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf.

U3: US Animal Welfare Act—7 US Code 2131-2157 (Agriculture) 9 CFR Parts 1, 2, and 3. http://www.gpo.gov/fdsys/pkg/CFR-2013-title9-vol1/xml/CFR-2013-title9-vol1-chapI-subchapA.xml.

C1: National Standard of the People's Republic of China, Laboratory Animal Facilities—General Requirements for Quality and Competence, Draft Standard for Approval, 12th April, 2013, Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China, 2013.

C3: Ministry of Science and Technology (2006). Instructive Guideline on Humane Care of Laboratory Animals.

S1: Ministry for National Development (2004). Animals and Birds Act (Chapter 7) Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/12801/Attach24_legislation_AB_CareandUse_rules.pdf.

S2: National Advisory Committee for Laboratory Animal Research (2004). Guidelines on the Care and Use of Animals for Scientific Purposes. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/13557/Attach3_AnimalsforScientificPurposes.PDF.

I1: Ministry of Environment and Forestry, India (2010). Standard Operation Procedure (SOP) for Institutional Animal Ethics Committee (IAEC), Guidelines on the Regulation of Scientific Experiments on Animals, Guidelines for Laboratory Animal Facility and Breeding of and Experiments on Animals (Control and Supervision) Rules, Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). https://www.aalac.org/resources/SOP_CPCSEA.pdf.

[a]This list is not intended to be exhaustive.

clear recommendations on study design, in particular in relation to the number of animals to be used. This flexible approach to study design within the guidelines allows for scientific and evidence-based decisions depending on the specific drug candidate in development. Table 14.3 shows how and where the regulatory guidelines refer to numbers of animals in study design.

The ICH guidelines describe a stepwise process for evaluating both animal and human efficacy and safety information as a drug progresses through development. With respect to nonclinical safety study objectives, it is stated that these studies should characterize toxic effects, identify target organs, and assess the dose-toxicity relationship. Furthermore, when appropriate, the potential reversibility of adverse effects should be demonstrated.

TABLE 14.3 Regulatory Guidelines and Group Sizes for Repeat Dose Regulatory Toxicology Studies

Relevant guideline	Typical group size for nonrodent studies	Notes
ICH M3 (R2)	Main study: 3 males+3 females Recovery: 2 males+2 females	See footnote to Table 3 within the ICH M3 (R2) guideline relating to extended single dose studies
ICH S6 (R1)	No numbers given	Indicates that small group sizes may in part be mitigated by increasing the frequency and duration of monitoring of individual animals
CHMP (previously CPMP) note for guidance on repeated-dose toxicity	No numbers given	Size of treatment groups should be sufficient to allow for meaningful scientific justification of the data generated
OECD 409	At least four per sex per group	Studies of 90 days' duration for chemicals

Drug-specific and scientifically driven study design is critical to ensure that all human dose and safety predictions are accurate. There are risks associated with routinely following regulatory guidelines without a tailored case-by-case study design because specific questions related to the individual drug may not be answered and safety concerns may be missed. This is particularly important when considering the nonhuman primate as a nonclinical species; although they may be perceived as the gold standard because of their closer phylogenetic relationship to humans, this may not always be a correct assumption. Thus appropriate scientific and ethical justification is needed for the choice of species as well as the number of animals used to generate safety data, and opportunities to minimize the use of animals without affecting study outcome should be explored [10–12].

A typical design, including the number of animals per group and the number of dose groups, of a nonhuman primate toxicology study is shown in Table 14.4. Individual companies may implement alternatives to this template design during the course of drug development, all of which are acceptable within the current regulatory guidance. Some of this variation is related to unique aspects of the individual drug target, whereas some is due to company practices. Where the variation in study design is associated with company practice rather than a case-by-case approach, there are opportunities to minimize the number of nonhuman primates in the nonclinical program, which yield scientific, business, and ethical benefits. There is substantial evidence in the literature to suggest that in many cases the design of nonhuman primate nonclinical safety studies is driven largely by company practice and internal standard (or default) study designs, rather than an assessment of the scientific requirements for each molecule [13, 15–16]. For example, according to an industry-wide survey on the design of chronic toxicity studies of 54 monoclonal antibodies using nonhuman primates, many instances of variation in the numbers of animals used despite similar study objectives were reported [13]. Furthermore, there was evidence to suggest that findings from short-term toxicity studies were not leveraged to inform the design of subsequent chronic long-term studies in terms of the number of dose groups needed, the number of animals

TABLE 14.4 Alternative Study Designs for Repeat Dose Regulatory Toxicology Studies

Dose group	Low	Medium	High	Control
FREQUENTLY USED STUDY DESIGN				
Animals in main study group (n)	3M+3F	3M+3F	3M+3F	3M+3F
Recovery animals (n)	2M+2F	2M+2F	2M+2F	2M+2F
			Total per study	40
ALTERNATIVE STUDY DESIGN 1				
Animals in main study group (n)	3M+3F		3M+3F	3M+3F
Recovery animals (n)			2M+2F	2M+2F
			Total per study	26
ALTERNATIVE STUDY DESIGN 2				
Animals in main study group (n)			5M+5F	3M+3F
Recovery animals (n)			2M+2F	2M+2F
			Total per study	24

Chapman et al. [13] and Baldrick [14].
M, male; F, female.

per group, or the inclusion of recovery animals. This is perhaps related in part to the limited available (regulatory) guidance on study design. However, general risk averseness within the pharmaceutical industry is also likely to play an important role.

Opportunities to Reduce Nonhuman Primate Use in Individual Studies

A number of nonclinical safety studies are required over the course of drug development. For each of these studies, a range of scientific questions and end points, as well as a priori knowledge about the compound/class, influences the numbers of animals used. For example, is the drug a novel entity or are there similar molecules in the class? What toxicity profile might be expected based on findings in previous studies (e.g., non-Good Laboratory Practice studies or those determining dose ranges)? Was the high dose used in short-term (subchronic) toxicology studies considered the no observed adverse effect level?

There are many opportunities to reduce the number of animals in a typical study design by considering both an individual study and how an individual study integrates into the whole drug development program. When initially focusing on the design of an individual study, the key factors that should be considered are the number of animals in a group, the number of dose groups, and the number of recovery animals. The group size for nonhuman primate studies generally varies from 6 (3 males plus 3 females) to 10 (5 males plus 5 females). The goal of general toxicity studies is hazard identification, and studies are not routinely

designed to be statistically powered. One opportunity to minimize the number of nonhuman primates is to use a group size of six unless there is specific scientific justification to increase the number. On some occasions, group size for chronic studies tends to be larger than that in short-term studies [13]. However, this may be because of a default decision based on standard company practice rather than case-specific scientific rationale. Individual group size should also be balanced with consideration of the number of dose groups. For example, if only the control and one dose group is used, increasing the number of animals in the one dose group to 10 (5 males and 5 females) may be necessary; however, in such a study the total number of animals would be fewer. There are some situations where statistical power may be required, for example, in reproductive toxicology studies. Calculations to determine group sizes that yield group sizes of six to eight infants are available in Chapter 9 and have been provided by Jarvis et al. [17] and Cappon et al. [18].

Typically, a control and three dose levels are included in a general toxicology study to determine a dose response. In some instances there may be opportunities to reduce this to one or two dose levels, for example, if the drug is a monoclonal antibody and pharmacokinetic/pharmacodynamic information is available to optimize dose selection. From a scientific perspective, if 100% saturation of the receptor has been demonstrated at the lowest dose, including an additional two doses above this may be unnecessary. In reproductive toxicology studies, the opportunity to use fewer dose groups is high because there is already information about dose response as well as the toxic effects of the drug candidate from prior nonclinical and clinical studies.

The inclusion of recovery animals can also be adapted in study design; for instance, there are opportunities to include only assessment of recovery in the control and one dose (low or high, depending on the drug candidate). Table 14.4 show examples of minimized study designs by using fewer animals in a group, fewer doses, and fewer recovery animals. These study designs are not always appropriate, but they illustrate the flexibility and incentive to carefully consider the study designs used for individual drugs on a case-by-case basis. For assessment of reproductive toxicity in nonhuman primates, an enhanced pre- and postnatal development study design can be used in place of traditional independent segment II and segment III study designs to minimize animal use while still capturing critical measurements and end points [19]. There are potential opportunities to further reduce the number of nonhuman primates in reproductive toxicology studies by reusing maternal animals treated with vehicle control and by reviewing the timing of the studies so that they are carried out later in development (phase III) on fewer compounds overall. In addition, on occasion there is the potential to reuse nonhuman primates, particularly for small molecules, if no terminal end points are required and no adverse toxicology is observed.

Inclusion of multiple end points and combining studies presents a topical and interesting approach to minimizing animal use, particularly nonhuman primates. This has been successfully implemented in the now widely used enhanced pre- and postnatal development study design but can also be expanded to include safety pharmacology or fertility end points and/or robust pharmacokinetic/pharmacodynamic measurements in general toxicology studies [20,21]. One example of this is the use of telemetry devices in short-term toxicity studies to evaluate cardiovascular end points, particularly for biologics. In addition, sexually mature nonhuman primates may be included in chronic toxicology studies for evaluation of reproductive toxicity and fertility-related end points to minimize the need for stand-alone studies.

Opportunities to Reduce Nonhuman Primate Use Across the Whole Drug Development Program

To optimize both the 3 Rs and overall resources during the drug development process, considering how the overall toxicology program can be streamlined—from initial studies supporting first-in-human clinical trials through those required for marketing authorization—is important. Individual toxicity studies should be designed to enable the specific needs of the development program at any given stage and should take advantage of any prior information available on the compound or drug class, rather than using a generic or "default" approach.

Certain oncology indications have different regulatory requirements for safety characterization than, for example, a drug for rheumatoid arthritis. This is defined within ICH S9, which provides opportunities to reduce animal use by limiting the scope of nonclinical safety studies required to support safety in specific patient populations based on risk/benefit assessment. Using a single 12-week study in nonhuman primates to support the entire clinical development program for certain oncology indications may be possible. Similarly, whether a reproductive toxicology study is necessary is also highly dependent on the indication of the drug candidate. The considerations for waiving a reproductive toxicology study or using a species other than the nonhuman primate include if the primary drug indication is for a geriatric population or a life-threatening disease and/or if historical information about the target already exists [22,23].

Additional important considerations to streamline drug development include maximizing the use of information available from studies that have been carried out earlier in development and continued review of whether assessment of certain end points is necessary and when assessment should be carried out. Much information can be gathered from the first pivotal short-term toxicity study to support first-in-human clinical trials. In addition to the importance of these studies in informing early clinical trials, they have critical importance in informing subsequent nonclinical chronic toxicology studies. The findings gathered from short-term (subchronic) studies may provide further opportunities to reduce the number of doses in the chronic toxicology study, such as in circumstances where no toxicity has been observed and/or the no observed adverse effect level is the highest dose tested in the short-term study. In addition, in the case of biologics that are potent in multiple species, ICH S6 indicates that if the toxicological findings in short-term studies of rodents and nonrodents are similar or have been characterized based on the mechanism of action, then longer-term general toxicity studies in one species are considered sufficient—this presents a clear opportunity for reducing the use of nonhuman primates.

ICH M3 indicates that evaluation of recovery from toxicity is necessary in at least one study during the development program. However, it is not necessary to assess recovery in all studies. The study in which recovery animals are included can vary, and this can impact animal use. If recovery has been evaluated in the short-term toxicity study, scientific justification should be provided for inclusion of recovery on the long-term study. One example of this may be if more detail on the nature of recovery from an observed toxic effect is needed. In this case, however, limiting assessment of recovery to only the highest dose and the control in the long-term study may be appropriate. There are different approaches to the inclusion of recovery animals in a development program to reduce animal use. For instance, assessment of recovery during the initial short-term study may enable determination of whether a

candidate drug should be continued/discontinued in development, reducing further animal use for a compound that will be dropped later in development. However, this may lead to the inclusion of recovery animals in more studies overall, as more short-term studies of a greater number of compounds are carried out. Alternatively, recovery groups may be excluded from the short-term study in favor of inclusion on the longer-term study once specific drug-related toxicities have been defined and a need for recovery assessment has been identified.

The number of studies carried out over the course of drug development may vary from one (e.g., 12 weeks for an oncology indication for a biological) to three (e.g., 4 weeks, 12 weeks, 6/9 months). However, for biologics (not for small molecules) repeat-dose toxicity can be evaluated with one short-term (2 weeks' to 2 months' duration) and one chronic study (6 months) to support market authorization [24]. Although additional repeat-dose toxicity studies may be required for small molecules (e.g., 1, 3, and 9 months), the entire development program should be considered at each stage to maximize information obtained from individual studies and minimize the total number of studies needed.

REFINEMENT OF HUSBANDRY AND PROCEDURES TO IMPROVE ANIMAL WELFARE AND QUALITY OF SCIENCE

Key aspects of the refinement of nonhuman primate use include spacious and complex accommodation, effective environmental enrichment, housing in stable social groups, well-trained staff, careful study design, use of best humane technical practices, and training the animals with positive reinforcement techniques. All of these important elements are linked and ultimately help to support good animal welfare and high-quality science (e.g., see the three-pillar housing concept presented by Müller [25]). For more extensive reviews of opportunities to refine nonhuman primate use and care see Refs. [2,7,26–29].

Housing Design and Environmental Enrichment

Accommodation for nonhuman primates in the laboratory should be designed to meet the biological and behavioral needs of the species and give them a degree of choice and control over their environment [30]; it has a primary impact on the welfare of the research animals [31]. General requirements are specified in Appendix A of the European Convention ETS 123 [4] and the ILAR Guide for the Care and Use of Laboratory Animals [5]. There should be sufficient space in which to provide a complex and enriched environment, so that the animals can exhibit a wide range of natural, species-specific behaviors (e.g., resting, running, climbing, leaping, foraging, grooming and other social interactions). Full use should be made of the enclosure volume (i.e., the three-dimensional space) by incorporating fixed and moveable substrates (e.g., shelves, ladders, hammocks, swings, ropes). Enclosure height is important; nonhuman primates flee upward when alarmed and prefer to rest on elevated perches; ideally enclosures should be floor-to-ceiling high, allowing the animals to perch above human eye level. Elevated verandas/balconies on the front of the enclosure are particularly attractive resting places and improve the animals' visibility of the holding room and other pens, which can help habituate them to human activity. To meet these requirements many research organizations house

their nonhuman primates in customized rooms rather than standard, freestanding metal cages, which has resulted in less aggressive, more cooperative animals [6]. Even free-standing conventional caging can be converted into linked gang-housing by removing the partitions between adjacent and tiered units [32]. Figure 14.1 shows a flexible approach with pens for three to five macaques each and the flexibility to combine them to accommodate larger groups.

A good amount and complexity of space is also required to enable social housing of nonhuman primates, which is crucial for their psychological well-being [33]. Adequate perching at different heights, visual barriers, and multiple escape routes help hierarchical species such as macaques to manage their social interactions; subordinate animals can more easily modify their behavior to avoid conflict with dominant animals. Solid floors are preferable to grid floors because they allow foraging for fine food items scattered in suitable litter such as wood shavings; this occupies the animals for extended periods and helps promote calm behavior. Enrichment items, such as toys that the animals can manipulate, mirrors, foraging devices (e.g., puzzle feeders), and swimming pools should be provided and rotated to maintain novelty as part of a documented environmental enrichment program. No evidence of an effect of enrichment on within- or between-experiment variation has been documented thus far [34]. Therefore these elements of good nonhuman primate housing and care in the laboratory do not conflict with scientific aims and are commonly provided in regulatory toxicology and other studies by contract research organizations in Europe and increasingly elsewhere [7,35–37].

Housing design also needs to consider the operational needs of the laboratory facility and research projects. These include the ease of capturing the animals for dosing and sampling, ease of visual inspection for monitoring clinical signs and welfare, and safeguarding the health and safety of the staff. Flexible enclosure designs with moveable panels allow the pen

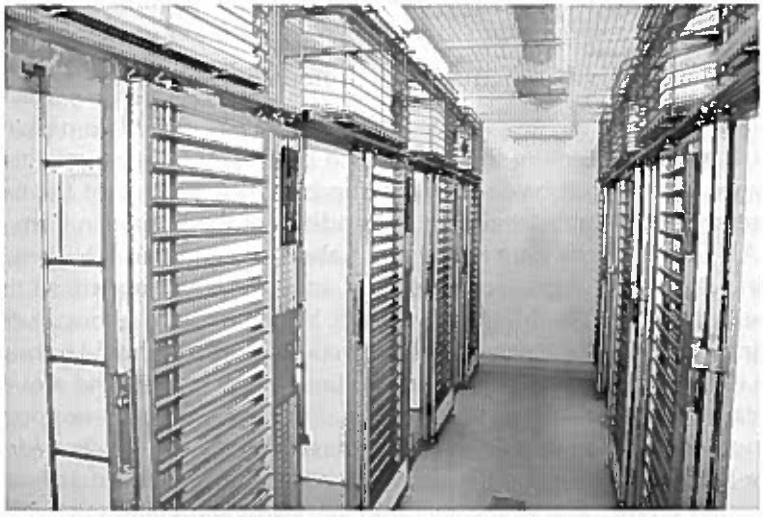

FIGURE 14.1 Gang housing system for macaques, showing pop holes to connect adjacent enclosures, elevated verandas, and widely-spaced horizontal bars on the enclosure fronts—the latter give better visibility down the room than do vertical bars, facilitate human-animal interaction and can be used for climbing.

space to be divided to temporarily separate individuals or groups of animals (e.g., for capture or cleaning) or to combine adjacent enclosures to allow additional space or to house larger groups. Innovative enclosure design can help to facilitate training of the animals and to refine procedures; for example, areas within the enclosure that allow temporary individual separation and collection of urine or feces avoid the need for extra metabolism cages and hence reduce the stress induced by cage change and removal from social companions (Figures 14.2 and 14.3).

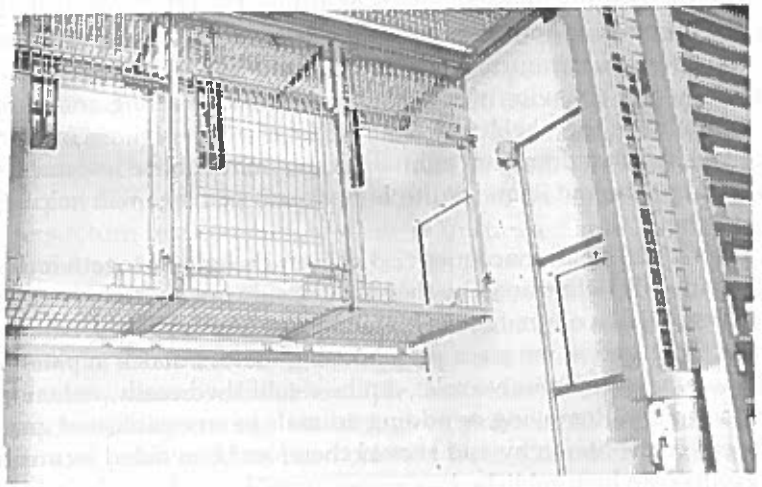

FIGURE 14.2 Multifunctional box at the rear of the enclosure which provides: (a) a high resting place; and (b) a means to temporarily separate individuals for short periods for procedures such as urine collection whilst allowing interaction with the social group.

FIGURE 14.3 Macaque temporarily housed in the multifunctional box for urine collection.

Managing Social Groups

Attending to the social environment of captive primates is fundamental to their welfare; it helps to decrease the incidence of behavioral abnormalities, expands the behavioral repertoire, provides stimulation, and reduces the impact of stress [38,39]. Allowing cage mates to continuously and fully interact with each other is more beneficial than providing temporary or partial social contact [40]. In the wild cynomolgus and rhesus macaques live in multimale, multifemale groups with strong hierarchical social structures. While it is rarely possible to replicate these same structures in a laboratory environment, macaques used in pharmaceutical studies can be successfully housed in pairs or groups [37,41]. Careful selection of companions and ongoing monitoring and management is necessary to ensure stable and harmonious pairings/groupings and to minimize any aggression [42–44]. Staff should have a basic knowledge of the ecology and behavior of the relevant species. Housing and enrichment should be designed to minimize the likelihood and impact of aggressive encounters; for example, care should be taken so that dominant animals cannot monopolize resources such as feeders, water spouts, and enrichment items; multiple resources per cage will help limit competition and potential conflict.

Stock groups of 10-20 small macaques (2-3 kg) can be housed together. The size of study animal groups is usually determined by the study design but should generally be as large as the design permits so that a normal social hierarchy can be established. Although a pair is not a natural social configuration for macaques, housing mature males in pairs may be preferable so that the dominant/submissive roles can be established easily, reducing the likelihood of aggression and injury. Removing or adding animals to an established group often results in reestablishment of the hierarchy and should therefore be avoided as much as possible. If a new social group is to be established or a new animal is to be included in an established group, moving all of the animals into a novel enclosure simultaneously may be preferable so that the newcomer is less disadvantaged and can more easily find their own position within the social structure.

Successful establishment and maintenance of a group with a stable hierarchy is greatly facilitated by using knowledge from the breeder/supplier about the social history, behavior, and health of individual animals, previous and current caging, as well as socialization methods. Breeding centers generally hold young adults in nonfamilial, same-sex groups. Consideration should be given to selecting experimental groups from within the same holding cage so that the animals are familiar with each other before reaching the laboratory environment. If the information available from the breeder does not provide sufficient information to assign cage mates, the likely compatibility of prospective social partners can be assessed by first observing their behavior in a large group and recording incidents of affiliative and hierarchical behaviors between the animals (snapshot ethograms) [25]. These data can then be used as the basis for assigning cage mates for smaller enclosures. Comingling in the enclosures should occur under the direct observation of caretakers. The animals should be allowed adequate time to get used to the enclosure and their cage mates before the start of a study. The duration of this acclimatization period may depend on the study type; changes in caging or cage mates can influence reproductive parameters such as menstrual cyclicity for several months [45].

Some laboratories house males and females (particularly sexually mature animals) in separate rooms to limit visual, auditory, and olfactory contact between the sexes and reduce

competition within same-sex groups. Another factor to consider in the establishment of stable hierarchies is position within the room, with particular regard to foreknowledge of impending procedures or interruption by staff. Provision of balconies, "windows," or glass doors to enable animals within all cages to have good visibility of the room and corridor and an opportunity to vocalize to inform the group of human activity may facilitate equality between the cages.

Study Design and Scientific Procedures

Refining the study design and scientific procedures is a process based on continuous reassessment of routine practice. The ultimate goal is an experiment in which the animal feels, does, and expects exactly what would occur without the experimental intervention having been imposed. Throughout the monkey's life and experience, the highest standards of handling, procedural techniques, and empathy must be used. With such a culture of care, all experimental interventions will become more tolerable to the animals. Furthermore, physiological biomarkers return to a common baseline in unstressed animals, making experiments more sensitive and reproducible [46].

Single housing of nonhuman primates can have a major negative effect on their welfare and should be avoided whenever possible. Separation not only causes stress for the affected animal but for the cage mates as well. Therefore, the need for any data requiring single housing should be critically reassessed. For example, individual food consumption data are not routinely needed in toxicity testing; for biologics (monoclonal antibodies), urinalysis is a largely irrelevant end point and has been omitted from a number of studies without comment from European regulators. When total collection of urine from individual animals is required and the normal housing does not permit this, metabolism cages may be required. The duration of time the animals spend in the metabolism cages should be the minimum possible, and the cages should be designed to minimize negative impacts on animal welfare [7]. Whenever there is a strong scientific need to separate animals from their cage mates, they should be given visual and auditory access to them so they can continue to interact socially; there should never be a need for social isolation. The positioning of freestanding cages must be mindful of dominance hierarchies in the home colony.

Instrumentation (pumps for port catheter systems, telemetry devices, etc.) are often carried in jackets. The use of such devices is often associated with single housing because of concerns about cage mates interfering with the instrumentation, causing loss of data, repeat surgery, and so on. Tailoring each monkey's jacket to its own body shape greatly improves comfort and reduces interference from cage mates, enabling significant durations of social housing. The Joint Working Group on Refinement has published guidance on the refinement of telemetry procedures [47] and husbandry [48].

Social housing enhances the accurate assessment of individual well-being because socially housed animals show behaviors that animals housed singly do not. Any deterioration in well-being shows a marked contrast against both the behaviors of cage mates and the previous behaviors of the affected animals. Furthermore, the possibility of detecting changes in locomotion, balance, coordination, and peer interaction mean that socially housed animals allow a range of end points that are not detectable in depressed individuals housed singly.

The duration and intensity of procedures, such as wearing jackets and chair/tube restraint, should be built up stepwise over time as part of a documented training program. Most important, the procedures should be conducted in a calm, empathetic way with gentle handling. Animals showing appropriate, desired behavior should be rewarded with kind words and food treats.

Staff-Animal Interactions

The quality of interaction between staff and the nonhuman primates they work with can have a major impact on both animal welfare and science. All staff interacting with the nonhuman primates should be trained to ensure that they can carry out their work competently, safely, and with minimal stress to the animals. Competence and opportunities for continuing professional development should be assessed regularly. An important part of this is training to understand and interpret the monkeys' gestures, facial expressions, and vocalizations and to respond appropriately when performing routine husbandry and procedures; in the case of common marmosets, see www.marmosetcare.com. Even simple modifications to behavior can benefit welfare, such as knocking on the door or ringing a bell before entering a room to avoid startling the animals and increase the predictability (signaling) of potentially adverse events, allowing the animals to relax at other times [49,50].

Using positive reinforcement techniques to desensitize and habituate animals to handling and procedures, and to gain their voluntary cooperation, is a major refinement goal [51]. Such techniques help to reduce stress and give the animals greater control over their lives, which is beneficial for welfare. For example, a study comparing trained versus untrained animals resulted in a statistically significant reduction in cortisol, lower heart rates, and normal stress leukograms for trained subjects [52]. It is important to be clear about the behavior modification technique being employed:

- Desensitization: Systematically pairing positive rewards directly with an uncomfortable or aversive experience or stimulus to reduce any associated fear or anxiety response.
- Habituation: The waning of a response as a result of repeated stimulation, but not fatigue. This kind of learning is of importance in familiarizing an animal with aspects of the environment to which it is not expected to react. Ignoring nonthreatening stimuli (e.g., the sound of clippers, wearing jackets, restraint in a chair) is of value in the training of nonhuman primates.
- Training: Shaping the behavior of an animal so that it actively responds in a way that is desired by the trainer (e.g., offers a limb for injection, stands on a weighing scale, waits and allows subordinate individuals to feed uninterrupted).

While developing equal animal training skills in all animal care personnel may not be feasible, all should have a basic working knowledge of the techniques involved to ensure consistency across staff and facilities. Establishing a formal institutional behavioral management program assists with this, improving the quality of care and outcome of research procedures [53]. The most effective program for nonhuman primates would begin at the facility of origin and continue through all other institutions in which the animals are housed. The desired training goals are more likely to be achieved if these are in line with the behavioral preferences of the monkeys; for instance, areas of the enclosure for capture of the animals should be situated in an elevated position.

Institutions may encounter challenges during the implementation of an effective behavioral management program, such as start-up and maintenance costs, resource implications, achieving buy in, and securing the long-term commitment of key personnel. These can usually be addressed by collecting quantitative data to demonstrate to technical staff, toxicologists, and senior management the effectiveness and benefits of the program for animal welfare and science. A progressive program should be subject to continual review in light of empirical data, experimental design, and the behavior and welfare of the animals.

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