

# Future Improvements and Implementation of Animal Care Practices Within the Animal Testing Regulatory Environment

*Pierre Guittin and Thierry Decelle*

## Abstract

Animal welfare is an increasingly important concern when considering biomedical experimentation. Many of the emerging regulations and guidelines specifically address animal welfare in laboratory animal care and use. The current revision of the appendix of the European Convention, ETS123 (Council of Europe), updates and improves on the current animal care standardization in Europe. New guidelines from the Organisation for Economic Co-operation and Development and the European Federation of Pharmaceutical Industries Association focus specifically on safety testing. These guidelines will affect the way toxicity studies are conducted and therefore the global drug development process. With the 3Rs principles taken into account, consideration regarding animal welfare will demand changes in animal care practices in regulatory safety testing. The most significant future improvements in animal care and use practices are likely to be environmental enrichment, management of animal pain and distress, and improved application of the humane endpoints. Our challenge is to implement respective guidelines based on scientific data and animal welfare, through a complex interplay of regulatory objective and public opinion. The current goal is to work toward solutions that continue to provide relevant animal models for risk assessment in drug development and that are science based. In this way, future improvements in animal care and use practices can be founded on facts, scientific results, and analysis. Some of these improvements become common practice in some countries. International harmonization can facilitate the development and practical application of “best scientific practices” by the consensus development process that harmonization requires. Since the implementation of good laboratory practices (GLP) standards in safety testing, these new regulations and recommendations represent a new way forward for animal safety studies.

**Key Words:** animal care; harmonization; housing; husbandry; practices; safety testing; welfare

## Introduction

Animal welfare is an increasingly important concern when considering biomedical experimentation. The future within the regulatory environment is moving toward further implementation of the principles of the 3Rs as defined by Russell and Burch (1959). In this discussion, we review trends in the improvement of the animal care practices and use of animals in regulatory toxicity tests.

Among the many opportunities and challenges facing those of us engaged in the use of research animals, some of the most acute and persistent ones target toxicology testing in the pharmaceutical, agricultural, and chemical industries. For animal care practices within the regulatory testing environment, our challenge is to implement practices that make sense and are science based. Based on this approach, global animal welfare harmonization could significantly improve animal care practices.

Toxicology and regulatory data can be influenced by animal care practices. For example, many of our institutions are being challenged to enrich the animals’ environment. In this context, our goal should be to evaluate whether the results of a study in an enriched environment versus a non-enriched one make a significant difference in absorption, distribution, metabolism, or the toxicity profile of a compound. More and more studies are prospectively looking at questions like these; however, unfortunately, the legislative process and public perception sometimes proceed before the data are available. If regulations or guidelines are put in place before a scientific basis is established, scientists will likely be required to justify to an animal review committee, based on a negative impact on the objectives of the study, why a guideline cannot be applied.

## Guidelines and Regulatory Testing

International harmonization, especially in the United States, Europe, and Japan, is heavily influenced by the presence of transnational regulatory harmonization processes. Several institutions (e.g., the European Federation of Pharmaceutical Industries Association [EFPIA]<sup>1</sup>, the Organisation for Economic Co-operation and Development [OECD]<sup>1</sup>, the

Pierre Guittin, D.V.M., Ph.D., D.A.B.T., is Research Advisor of the Toxicology Laboratory in the Department of Drug Safety Evaluation; and Thierry Decelle, D.V.M., is Section Manager in the Laboratory of Animal Science & Welfare, Aventis Pharma, Vitry, France.

<sup>1</sup>Abbreviations used in this presentation: EFPIA, European Federation of Pharmaceutical Industries Association; OECD, Organisation for Economic Co-operation and Development.

Association for the Assessment and Accreditation of Laboratory Animal Care International, and the Federation of European Laboratory Animal Science Association) have prepared or are preparing new recommendations.

In December 2000, the OECD published the *Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation*, in which an approach to the use of clinical endpoints to replace endpoints that would unnecessarily involve, death, pain, or distress is suggested. This guidance publication is an example of an animal care and use initiative meant to harmonize preclinical safety testing protocols that emerged from the OECD. Then in January 2001, the EFPIA and the European Center for the Validation of Alternative Methods jointly published another guideline titled *A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes of Administration*, which proposes limits for dosing and sampling (Diehl et al. 2001).

Dominant among harmonization efforts in animal care and use is the revision of the Appendix of the Council of Europe's Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). The revision to Appendix A, which is the substance of the species-specific requirements of European animal welfare law, is aimed at updating and improving the current standards on caging, housing, and enrichment. These mandatory changes in animal care practice will undoubtedly influence the conduct of toxicology studies in the future.

One key change proposed in ETS 123 is related to caging and social housing. Another major item will be the emphasis on environmental enrichment. Future changes may affect the management of pain and distress.

## Caging

The Council of Europe's strong recommendation regarding the use of solid-bottom caging for rodents is emerging as an important concept. Solid-bottom bedded caging is recommended because foot lesions can develop when rats are housed on wire for long periods of time and because bedding, as material for foraging and nesting, is a source of enrichment. In addition, rodents are less active when housed in bedded cages, suggesting a more relaxed emotional state (Dean 1999). Nevertheless, wire caging can be used for rats in study designs of short duration without adversely affecting health and humane care. It is argued, however, that the use of solid-bottom caging in toxicology studies presents a risk of additional exposure to the compound due to consumption of bedding soiled by urine or feces. Observation of stools, a very useful clinical sign, is also made more difficult when bedding is present.

There are other concerns related to the Council of Europe proposals for safety and toxicology studies. The requirement of larger cages could increase occupational risk

for technicians when they handle animals. Provision of larger cages allows animals to avoid being captured rapidly and effectively, which increases the risk of trauma and stress. A larger surface area for dogs will produce a decreased number of animals per room, significantly reducing the capacity of existing facilities. At this time, there is a lack of clear scientific data that support any current chosen cage or enclosure size. Provisions of nesting for rodents and platforms for rabbits make daily observations without handling more difficult. Increased floor area for dogs may make urine collection more difficult. Exercise areas and larger cages for monkeys will also be a concern when frequent capture or handling is required. Addressing these concerns has been proven to be possible without greater problems.

The immediate consequence of such changes would be a complete replacement of all racks and cages in the coming years. The cost of caging conversion would be significant. Renovation and expansion of animal facilities may be needed to accommodate new caging and address increased space requirements. Furthermore, the improvements in animal care and welfare resulting from these proposed changes would require more resources because daily observation and animal handling would be done quite differently.

## Social Housing

Future guidelines will encourage social housing. For example, the draft of the Council of Europe revision recommends that dogs not be housed singly for more than 4 hr. This approach attempts to encourage group housing while at the same time allowing for the need to monitor food intake and postdosing observations. A limit of 4 hr for single housing of dogs is not always compatible with toxicology evaluations, either for the evaluation of clinical signs or for the recording of food intake. In such ways, social housing of large animals may have an impact on study designs and data collection. Harmonious group-housed animals are often less stressed, and stress effects, both beneficial and deleterious, are magnified.

Nonhuman primates exhibit a high level of complex social interaction, which is in large part dependent on the distribution of animals (Novak and Suomi 1989). Social needs of nonhuman primates are a component of psychological well-being (Menck 1998). Nevertheless, human and animal interactions may be more useful for animal welfare than the housing system.

Social housing of incompatible animals may result in injury and affect the conduct of toxicology studies (i.e., there is a potential of incompatibility injury). If one rodent dies and is cannibalized, the toxicology study is adversely affected. Social housing may influence access to food and increase stress. There is also risk involved in splitting an established group in accordance with study design (e.g., sequential termination of the study) or when major drug effects have an impact on group hierarchy. Methods to provide social housing should be reconsidered when unbal-

anced group sizes exist. In rabbits and mice, only females and castrated males can be group housed.

Social housing may improve the welfare of animals, but the evaluation of clinical observations is more difficult and there is a risk of lost data. Adapting to new social housing guidelines would require significant changes in toxicology study design. This adaptation and improvement are ongoing and are already possible without modifying the regulatory studies. Determining which components of particular housing designs are most important to the different species of nonrodents is not intuitively obvious and will require careful study before science-based recommendations can be made.

## Examples of Other Environmental Enrichment

Effective enrichment often requires a detailed analysis of patterns of behavior and consequences on the goal of the study. One example of environmental enrichment is food enrichment, such as treats for monkeys or hay for rabbits. The Council of Europe revision will also encourage the use of nests and cage boxes or tubes for rodents.

Animals may be hidden by the nest or the box, requiring handling of the animals for clinical sign observations. Changes in animal observations and daily handling will need to be implemented. Continuous refinement of toxicity studies will require the introduction of competence, commitment, and collaboration of all toxicology and animal care staff categories. An increased number of person-hours to perform animal husbandry is a possible consequence of future improvements in environmental enrichment. An overall increase of the workload in the animal facilities is expected. A higher degree of education and training will be necessary for improvement in animal care practices.

The enrichment of the animals' environment will not likely affect the interpretation of the results, particularly if adequate controls are used. Appropriate enrichment methods can be chosen to be compatible with the aims of the study.

## Pain and Distress

Another European guideline published in January 2001 generated as a joint effort of EFPIA and the European Centre for the Validation of Alternative Methods (Diehl et al. 2001), proposes limits for dosing and sampling in the species most commonly used in toxicology. The ad hoc Animal Welfare and Research Working Group of EFPIA will support and encourage research among its member companies that leads to information on possible ways to improve the well-being of laboratory animals. In addition, the absence of a negative impact of new guidelines on the quality of the studies may need to be confirmed, especially for safety studies of pharmaceuticals and biologicals.

As a result of these efforts, we are seeing more and more serious, scientific efforts to integrate research and testing requirements with animal welfare concepts so that both endeavors can benefit. Good examples of attempts to integrate science and welfare are the Canadian Council on Animal Care guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing (CCAC 1998), the OECD Humane Endpoints document (OECD 2000), and the dosing/sampling publication from the EFPIA and the European Centre for the Validation of Alternative Methods (Diehl et al. 2001). Although these papers have no regulatory force, they should be seen as tools for improving the conduct of science. For example, the suggested volumes for subcutaneous administration of fluid are descriptions of sound practice, which may be helpful for providing advice and guidance on study design. Providing a recovery period related to the percentage of blood volume removed is critical. Hypotension and hypovolaemia after blood sampling should be monitored because such recovery is necessary for red blood cell regeneration. These examples should be considered for application during protocol review process at the discretion of the institution animal care and use committee, ethical committee, or animal welfare officer.

One goal of future improvements in regulatory testing is to reduce the distress that can be an unavoidable consequence of drug safety testing in animals. The objective of toxicity testing is to characterize the potentially toxic effects of the active substance: first, to protect the consumer; second, to estimate the degree of in-use hazard; and third, to understand the biological mechanism of toxicity. Thus, toxicity is a necessary endpoint of any *in vivo* study that meets these objectives. Pain, especially in rodents, may not always be obvious, and careful observation of changes in appearance and behavior over time will aid in recognizing signs of pain. The clinical management of pain and distress is dependent on the training of animal caretakers, technical staff, and veterinarians, who make the critical observations (Carstens and Moberg 2000) and assessments.

Management of pain and distress must be carefully considered inasmuch as their alleviation may interfere with the evaluation of the maximum tolerated dose (e.g., for the starting dose in clinical trials of the anticancer compound [DeGeorge et al. 1998]). In this case, it may limit the detection both of toxic effects at the highest doses and of side effects, and it may mask any possible drug tolerance. Nevertheless, we should consider that "humane endpoints" will very rarely limit the detection of toxic effects, side effects, and drug tolerance.

## Humane Endpoints

Another animal care and use initiative meant to harmonize preclinical safety testing protocols emerged from the OECD (OECD 2000). This test guidance document published in December 2000 suggests an approach to the use of clinical endpoints to replace endpoints that would unnecessarily in-

volve death, pain, or distress. The purpose of the document is to apply the principles of the 3Rs to the use of animals in regulatory toxicity tests.

It is expected that with increasing knowledge and experience, investigators in animal research will be able to identify more specific and earlier humane endpoints in the form of clinical signs rather than using impending death or severe pain and distress. Clearly defined experimental objectives are essential to the development of both scientific and humane endpoints. The nature, intensity, and duration of the study design determine the potential consequences of the experiment for the animals. The experimental objective will determine the scientific approach, animal model, and group sizes and will define the scientific and humane endpoints (Stokes 2000). This process would permit international harmonization of these humane endpoint policies. Animal care practices could sometimes interfere with the evaluation of toxicology parameters. To improve the evaluation of endpoints, accurate and detailed observations of the animals during studies will be required. As a consequence, more time and effort will be spent on developing clinical landmarks, performing animal observations, and improving clinical examinations.

## International Welfare Harmonization

Lastly, we must consider approaches to improvements in animal welfare that are science based. One possible approach is the use of international harmonization as a tool for the implementation of improved animal care practices. In the harmonization of animal care and use practices, a primary consideration is the reduction or control of variability. We can agree that the reduction of variability in experimentation is a universal scientific tenet. As a consequence, harmonization can contribute to the interpretation of results from animal studies, particularly when one is attempting to reproduce or interpret the results of a study in another laboratory. In-house programs to ensure a high health standard must also be set up and harmonized between sites of a same company.

Consequently, it seems clear that international harmonization can facilitate the development and practical application of “the best scientific practices” by the process of consensus development that harmonization requires. We already see, through examples cited in this discussion, how evolution of the international guidelines are driving and/or magnifying the harmonization of animal care and use.

Advances in laboratory animal science are also promoting greater international harmonization. There are now major animal suppliers that can deliver standardized animals to the major research centers around the world. In addition, the animal suppliers and their partners and colleagues in academia are steadily improving animal health, diagnostics, and genetic standardization. We are also beginning to see greater standardization and availability of harmonized diet formulations—another way to reduce experimental variability.

International harmonization does not mean an engineered standard with inflexible rules, but preferably a common approach based on performance, as suggested by the Institute for Laboratory Animal Research (NRC 1996). This performance approach requires professional input and judgment to achieve the best animal care practices. With increasing acceptance of a performance-based approach, global harmonization in animal care and use practices will be enhanced.

## Conclusion

To implement improvements in animal care practice, we need to make every effort to define good animal practices as a guide and not a rule, because we should not impose any change without a strong scientific rationale. We also need to stay focused on the objectives of regulatory safety testing. Thus, for future improvements, we must work together on solutions that make sense and are science based. All changes should be clearly justified and openly debated. Determining which components of particular enrichment are most important to nonrodents is not intuitively obvious and requires further study. The changes should be based on facts, scientific results, and analysis. We must not forget that the determination of toxicity is necessary to minimize human risk. The greatest advantage of using an animal for drug safety testing is that it is an inclusive model of all of the factors involved in human exposure.

In summary, the main areas for future improvements in animal care and use practices are animal environmental enrichment, management of animal pain and distress, and increased use of humane endpoints. Numerous other potential targets of harmonization could have an impact on toxicology testing, including housing (e.g., solid-bottom caging and cage design), group housing, environment enrichment, and pain management.

Harmonization of animal care practices through companies should be beneficial for the implementation of the animal welfare. Decisions to harmonize are not simple “yes” or “no” questions but must be considered in the context of the objectives of the experimentation. Data generated in one country should be acceptable to regulatory agencies in another country, through transnational regulatory harmonization. Harmonization is complex and potentially costly, but it carries real possibilities for added value. Harmonization priorities must be based on realistic assessments of benefits, costs, and implementation practicability. Harmonization and globalization of animal welfare concepts call for an approach to integrate welfare decisions with scientific rationale. Thus, harmonization of animal welfare should be beneficial.

Because animal welfare is a major parameter for the scientific community, it should be afforded further objective and scientific analysis. Since the 1980s, we have witnessed a progressive change from basic animal technology to a combination of laboratory animal science, technology, and

welfare. Future progress will depend on the further development of an international approach to animal welfare harmonization, which it is now possible to validate and implement through international collaborative efforts.

## Acknowledgment

The authors acknowledge John C. Donovan for his fruitful advice and pertinent review.

## References

- Carstens E, Moberg GP. 2000. Recognizing pain and distress in laboratory animals. *ILAR J* 41:62-71.
- CCAC [Canadian Council on Animal Care]. 1998. CCAC guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing. Ottawa: CCAC.
- Dean S. 1999. Environmental enrichment of laboratory animals used in regulatory toxicology studies. *Lab Anim* 33:309-327.
- DeGeorge JJ, Ahn CH, Andrews PA, Brower ME, Giorgio DW, Goheer MA, Lee-Ham DY, McGuinn WD, Schmidt W, Sun CJ, Tripathi SC. 1998. Regulatory considerations for preclinical development of anti-cancer drugs. *Cancer Chemother Pharmacol* 41:173-185.
- Diehl K-H, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal J-M, van de Vorstenbosch C. 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 21:15-23.
- Mench J. 1998. Why it is important to understand animal behavior. *ILAR J* 39:20-26.
- Novak MA, Suomi SJ. 1989. Psychological well-being of primates in captivity. *ILAR J* 31:5-15.
- NRC [National Research Council]. 1996. Guide for the care and use of laboratory animals. 7th ed. Washington DC: National Academy Press.
- 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. (ENV/JM/MONO(2000)7). Paris: OECD. <<http://www.oecd.org/ehs/test/mono19.pdf>>.
- Russell WMS, Burch RL. 1959. The principles of humane experimental technique. London: Methuen & Co. Ltd. [Reissued: 1992, Universities Federation for Animal Welfare, Herts, England.] <[http://altweb.jhsph.edu/publications/humane\\_exp/het-toc.htm](http://altweb.jhsph.edu/publications/humane_exp/het-toc.htm)>.
- Stokes WS. 2000. Humane endpoints for laboratory animals used in toxicity testing. In: Balls M, van Zeller AM, Halder M, eds. Progress in the Reduction, Refinement, and Replacement of Animal Experimentation. New York: Elsevier Science. p 897-906.