

The International Symposium on Regulatory Testing and Animal Welfare: Recommendations on Best Scientific Practices for Safety Evaluation Using Nonrodent Species

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Introduction

Toxicologists involved in regulatory testing are responsible for collecting data on the toxicity of substances, for determining how these substances produce their effects, and for making reasonable predictions of their hazard and impact on humans, animals, and their environment. The main tasks of safety evaluation in nonrodent species include evaluation of the spectrum of toxicity, prediction of adverse effects, and evaluation of safe exposure levels. This Breakout Group addressed current best practices and future possibilities for safety evaluation using nonrodent species, in terms of reducing, refining, and replacing nonrodent species within the regulatory testing framework for safety evaluation (Russell and Burch 1959). Internationally, there is a spectrum of approaches currently in place, some of which have more of an impact on nonrodents than others.

Current Considerations

Participants in the group (listed at the end of this report) had previously reviewed a number of key background references (also listed at the end of the report), which had been selected by the group's leaders before the meeting. Nonrodent species were considered to include the beagle dog, rhesus monkey, cynomolgus monkey, marmoset monkey, and miniature swine. Participants were asked to consider the questions listed below as part of their general discussion:

1. What are current experimental practices involving safety evaluation with nonrodent species?
2. What criteria are used in dose selection for single and repeat dose exposure?
 - a. What information should be obtained from each dose group and how many dose groups are needed?

- b. Are surrogate markers used as endpoints and what is their level of acceptability by regulatory agencies?
 - c. What dose-escalation strategies are used in pilot studies: How can studies be designed to provide for accumulation of toxic effects with repeat dosing or accommodation to effect with repeat dosing?
3. How can endpoints be selected that meet the 3Rs and provide characterization of potential toxicity?
 - a. What scientific justification is needed to establish an endpoint involving pain/distress: Does the animal care committee (ACC¹) provide a cost-benefit evaluation before approval?
 - b. What endpoints are necessary before human exposure?
 - c. Are surrogate endpoints accepted by regulatory agencies?
4. What criteria are used in animal model selection for toxicology studies in nonrodent species? Provide:
 - a. Scientific justification/mechanistic interpretation;
 - b. Historical database;
 - c. Regulatory acceptance.
5. How can animal use be minimized in toxicology studies involving nonrodent species? Provide
 - a. Number of animals per sex per group;
 - b. Size of control and treatment groups;
 - c. Reuse of animals: number of times and criteria to discontinue.
6. Consider international consistency among agencies involved with welfare of animals used in regulatory testing in the categorization of pain and distress.

Future Improvements

1. How can the use of nonrodent species be minimized?
 - a. Could the use of a control group be avoided?
 - b. Can regulatory testing be conducted in only one sex?

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¹Abbreviations used in this presentation: ACC, animal care committee; MTD, maximum tolerated dose; NOEL, no observed effect level; OECD, Organisation for Economic Co-operation and Development; SOP, standard operating procedure.

2. To what extent are housing refinements compatible with safety evaluation?
3. What criteria should be used/developed for endpoints and anticipated euthanasia?

Report on Group Discussion: Current Best Practices

For the large majority of substances, nonclinical safety studies are generally successful. The use of nonrodent species largely contributed to this achievement by supporting the selection of appropriate doses and safe substances (Gerbracht and Spielmann 1998; Morgan et al. 1994; Parkinson and Grasso 1993). The group discussed current experimental practices, including restraint, dose administration (volume and route), and blood collection.

Training of animals and acclimation to procedures comprise standard practice and are critical to the success of the study. Acclimation to restraint and other experimental procedures is commonly achieved by training dogs in a breeding colony. It is in the interests of good science, as well as animal welfare, that stress be kept to a minimum.

Dose administration is a broad topic: Many substances, vehicles, or adjuvants may be administered by a variety of routes for a variety of purposes. The route of administration is usually dictated by the anticipated action of the substance or by protocols that are generally accepted for toxicity testing. For dose volume and route of administration, institutions generally develop standard operating procedures (SOPs¹) based on the current literature, and justification is required for exceeding these limits. Furthermore, acceptance by regulatory agencies should be considered when establishing SOPs for these practices.

Blood collection is commonly performed in regulatory toxicology studies to assess exposure to the test compound or for evaluation of hematological and clinical chemistry parameters. Guidelines for volume to be collected are published in literature (Diehl et al. 2001; Morton et al. 2000). For large volume removal, fluid replacement is not standard and presents many clinical and scientific issues. Despite guidelines, it is often difficult to monitor actual blood volume collected from animals versus the amount approved for collection by the ACC as detailed in the protocol. Additionally, there are no published guidelines on the frequency or maximum number of times venipuncture should be conducted, or on the criteria for use of alternative collection methods (e.g., catheter placement).

Judicious selection of dosages at the beginning of a study governs the outcome of the study, the quality of the data generated, and, ultimately, the value of the study for safety evaluation. Pain and distress may be minimized by conducting pilot studies using dose escalation. The initial dose should be based on estimated therapeutic dose or rat acute toxicity data. A control group can be excluded if historical data exist, although a control group is needed if a new vehicle is involved. The control group can be very

important for assessment of toxicity inasmuch as it provides information on environmental or other factors that may affect studies. Generally, three dose groups (no observed effect level [NOEL¹], mid-dose, and maximum tolerated dose [MTD¹]) are evaluated. There was some debate by the group about which criteria should be used to establish an MTD.

Pharmacological properties of the compound should be considered in establishing a surrogate marker. Surrogate markers may include clinical pathology, endocrine or immune parameters, or QT interval (i.e., time required for complete electrical excitation of the heart and recovery of the ventricles) prolongation. However, professional judgment is needed because strict guidelines cannot be applied. Toxicokinetic data (i.e., saturation) may also be used in evaluating surrogate markers.

The decision process for the euthanasia of an animal should be described in an SOP approved by the local ACC. Communication between the study director and the veterinarian is critical. Ideally, scientific and humane endpoints should be the same. The group discussed the case of maximum tolerated dose in anticancer studies in particular.

The consensus of the group was that the number of animals used for toxicology studies should be science driven. On average, three nonrodents are used for 1- and 3-mo studies, and four nonrodents are used for each 6-, 9-, or 12- mo study. On average, two nonrodents are used for recovery arms. The reuse of nonrodent species is a common practice, particularly for pharmacokinetic studies and in studies in which telemetry is used. Early pilot non-good laboratory practice studies for dose selection purposes may also involve the reuse of animals. However, it may not be appropriate in all instances to reuse animals based on the level of invasiveness of the procedure, the degree of pain and distress, or the potential for underlying toxicity from a previous compound.

When testing is performed on a novel compound or an innovative family class, or when a rare effect is expected, minimization of animal numbers per study group could be risky or counterproductive. Repetition of studies may result in the use of more animals. The working group suggested several approaches to developing future guidelines for reducing the number of nonrodents used without compromising the scientific integrity of studies (see recommendations below).

To investigate a possible gender effect, regulatory testing should be performed on both genders. The working group agreed that one gender could be used for investigative and exploratory studies (e.g., mechanistic studies). Such an approach may also be possible in pilot studies when the absence of a gender effect had already been confirmed. In the European Union, phase I clinical trials in male human subjects can be supported by studies in one gender only.

In safety and toxicity studies, the use of a control group is justified based on scientific and/or regulatory need. Current guidelines recommend the use of control groups in regulatory studies. In most toxicity studies, control groups

are necessary to evaluate compound and noncompound related effects. Individual animal variation among nonrodent animals remains an important variable. The control group is critical when evaluating subtle clinical effects or histological lesions because such findings can be hidden by spontaneous anomalies and individual animal variability. In most toxicity studies, a control group is required. However, in some cases, a control group may not be required (e.g. in pilot, exploratory, or dose-finding studies, or when there are adequate in-house historical data of the vehicle used in the formulation). Consideration should be given to factors such as the impact of environmental conditions and the value of a control group on study results. Use of *ex vivo* organ studies could reduce the total number of studies.

The group discussed issues relating to the compatibility of group housing with toxicity study evaluation. In particular, they discussed the suitability of solid-bottom caging for toxicity studies and the impact that environmental enrichment may have on toxicity studies.

The consensus of the group was that consistency among international agencies monitoring animal welfare is needed and that studies on nonrodent species are important and essential for safety assessment. Careful attention to experimental procedures used, dose selection criteria and justification of endpoints, or, when appropriate, the use of surrogate markers can improve the welfare of nonrodent species used in regulatory testing.

Recommendations: Current Best Practices

Experimental Practices: Dose Administration, Restraint, Acclimation, and Blood Collection

- The route of administration that causes the least discomfort and least anticipated pain response should be used, without compromising the scientific goals of the study. Appropriate route and volume (e.g., maximum acceptable volume) and physiochemical properties (e.g., formulation, solubility, concentration, viscosity, biocompatibility) should be considered.
- SOPs should guide decisions, and justification should be provided for dose administration procedures outside the SOPs. The scientific literature should be used to establish guidance (e.g., acceptable volumes) (Diehl et al. 2001). Acceptability of the procedure and dose levels tested by regulatory agencies assessing safety must be considered in developing SOPs.

Dose Selection

- Pilot dose escalation studies should be used to provide data to help minimize pain and distress in subsequent studies. The starting dose should be based on the estimated therapeutic dose or rat acute toxicity data.
- Control groups can be excluded if historical data are

available but should be included if a new vehicle is used. Typically, three dose groups (NOEL, mid-dose, and MTD) should be evaluated. Toxicokinetics and pharmacodynamic endpoints should be used in dose selection strategies to improve the evaluation of toxicity with repeat dosing.

Surrogate Markers

- Surrogate markers should meet certain criteria, including occurrence before signs of toxicity and a well-established relation between the surrogate marker and signs of toxicity. In the case of therapeutics, surrogate markers should provide an assessment of clinical benefit.
- In selecting surrogate markers, the pharmacological properties of the compound must be considered. Strict guidelines cannot be applied inasmuch as professional judgment is necessary.

Justification of Endpoints

- With advances in knowledge and technology, investigators in animal research will be able to identify more specific, earlier humane endpoints and reduce pain and distress. Ideally, this advance would permit international harmonization of endpoints.
- Accurate and detailed observations of the animals during studies should be encouraged to improve the scientific quality of studies, allow earlier detection of toxicities, and improve animal welfare.
- Each institution should delineate key processes for a euthanasia decision. Institutions should clearly define the euthanasia decision process in an SOP based on the country's regulations.
- Each institution should define potential endpoints for the euthanasia of nonrodents in an SOP using references such as the Organisation for Economic Co-operation and Development (OECD¹) document (OECD 2000) on endpoints and/or any local guidance documents. The SOP should provide flexibility, and the final decision should be made by the study director and veterinarian in close cooperation with the animal care committee. Protocol specific guidance may be necessary (Stokes 2000; Toth 2000).
- Regulatory agencies and the OECD should state clearly that the criteria for endpoints and the ultimate decision for the euthanasia of an animal rest with the institutional veterinarian or, in the case of a conflict, with the ACC.

Minimization of Animal Use

- Selection of the numbers of animals, as well as the reduction of nonrodent use, should be based on scien-

tific analysis. Knowledge of the drug substance is key to establishing the numbers of animals used.

- Studies should be designed to maximize the data assessed per study to reduce the number of studies.
- The collection, dissemination, and review of data (e.g., vehicle control) would avoid unnecessary duplication of studies.
- Using methods such as statistical analysis of in-house data could optimize animal numbers.
- In preliminary studies, the use of a control group may not be required. For example, in pilot, exploratory, and/or dose-finding studies, the objective of the studies is to assess dose level or acute toxicity. In addition, when adequate in-house historical data of the vehicle used in the formulation are known, it may be possible to minimize the control group in preliminary studies.
- Pooling of data from both sexes could increase the power of the analysis and thereby help reduce the number of animals. Such an approach is possible when the absence of gender effect has been confirmed. Additionally, the use of one sex to support initial clinical trials could be considered for harmonization.
- Consideration should be given to the elimination of some repeat dose toxicity studies. Accelerated development plans may, in some situations, reduce the number of studies. Thus, the number of nonrodents used should be evaluated by compound rather than by studies.
- In some cases, clinical trials may be supported by using data obtained from noninvasive or nondestructive techniques such as biomarkers, surrogate markers, or imaging techniques. Such investigative techniques would help in supporting clinical trials in combination with in vivo studies.
- Mechanistic investigations involving the use of ex vivo organ studies could reduce the total number of studies.
- Animals used in telemetry studies may be used for multiple studies.
- It may not be appropriate in all instances to reuse animals based on the level of invasiveness of the procedure, the degree of pain and distress, or the potential for underlying toxicity from a previous compound. The reuse of nonrodent animals should be carefully managed. We recommended a washout period of 3 to 4 wk between studies and a maximum of three uses.

Housing Refinements Compatible with Safety Evaluation

- Further work is needed to evaluate and develop group housing systems with regulatory testing (Reinhardt and Reinhardt 2000). Other forms of enrichment should be developed for incorporation into safety studies with the goal of improving the quality of the data and animal welfare (Diehl et al. 2001).
- A thorough knowledge of the biology and behavior of the animal is necessary for making pertinent observa-

tions. Appropriate education and training of animal care personnel are prerequisites for improving animal care practices. The working group supports these refinements within the constraints of the study requirements.

International Consistency Among Regulatory Agencies

- International accumulation of nonproprietary historical data would assist in rationalization of nonrodent species use. The working group strongly encourages the collection, dissemination, and review of data such as vehicle information to avoid unnecessary duplication of studies.
- International consistency is needed among international agencies evaluating safety studies as well as animal welfare regulatory agencies.
- True global harmonization of test requirements is needed for the acceptance of refined endpoints in one country to have an impact on other countries.

Background References

- CCAC. 1998 [Canadian Council on Animal Care]. CCAC guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching and testing. Ottawa: CCAC <<http://www.ccac.ca/english/gdlines/endpts/appopen.htm>>.
- Council of Europe. 1991 (Revision ongoing). European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS123). <<http://conventions.coe.int/treaty/EN/cadreprincipal.htm>>.
- CPMP [Committee for Proprietary Medicinal Products]. 1998. Note for guidance on the pre-clinical evaluation of anticancer medicinal products. CPMP/SWP/997/96. <<http://www.emea.eu.int/index/indexh1.htm>>.
- CPMP [Committee for Proprietary Medicinal Products]. 2000. Note for guidance on repeated dose toxicity. HCH 3BS2a, CPMP/SWP/1042/99. <<http://www.emea.eu.int/index/indexh1.htm>>.
- DeGeorge J, Chan-Ho A, Andrews PA, Brower MD, Giorgio DW, Goheer MA, lee-Hom DY, McGuinn WD, Schmidt W, Sun CJ, Tripathi SC. 1998. Regulatory considerations for preclinical development of anticancer 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.
- Gerbracht U, Spielmann H. 1998. The use of dogs as second species in regulatory testing of pesticides. I. Interspecies comparison. *Arch Toxicol* 72:319-329.
- Hull RM. 1995. Guideline limit volumes for dosing animals in the pre-clinical stage of safety evaluation. *Hum Exp Toxicol* 14:305-307.
- Hull RM. 1995. Guideline limit volumes for dosing animals in the pre-clinical stage of safety evaluation. *Hum Exp Toxicol* 14:305-307.
- Kuijpers MHM, Walvoort HC. 1991. Discomfort and distress in rodents during chronic studies. In: Hendriksen CFM, Koëter HWBM, eds. *Animals in Biomedical Research*. Amsterdam: Elsevier. p 281.
- Morgan DG, Kelvin AS, Kinter LB, Fish CJ, Kerns WD, Rhodes G. 1994. The application of toxicokinetic data to dosage selection in toxicology studies. *Toxicol Pathol* 22:112-123.
- Morton DB, Jennings M, Buckwell A, Ewbank R, Godfrey C, Holgate B, Inglis I, James R, Pasge C, Sharman I, Verschoyle R, Westfall L, Wilson AB. 2000. Refining procedures for the administration of substances. *Lab Anim* 35:1-41.

- 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>>.
- Olson H, Betton G, Stritar J, Robinson D. 1998. The predictivity of the toxicity of pharmaceuticals in humans from animal data—An interim assessment. *Toxicol Lett* 102-103:535-538.
- Parkinson C, Grasso P. 1993. The use of the dog in toxicity tests on pharmaceutical compounds. *Hum Exp Toxicol* 12:99-109.
- Reinhardt V, Reinhardt A. 2000. Social enhancement for adult nonhuman primates in research laboratories: A review. *Lab Anim* 29:34-41. <<http://www.labanimal.com/iacuc/reinhardt0100.htm>>.
- 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/human_exp/het-het-toc.htm>.
- Spielmann H, Gerbracht U. 2000. The use of dogs as second species in regulatory testing of pesticides. II: Subacute, subchronic and chronic studies in the dog. *Arch Toxicol* 75:1-21.
- 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*. Amsterdam: Elsevier. p 897-906.
- Toth A. 2000. Defining the moribund condition as an experimental endpoint for animal research. *ILAR J* 41:72-79. <<http://www.national-academies.org/ilar>>.
- Wallace J. 2000. Humane endpoints and cancer research. *ILAR J* 41:87-93. <<http://www.national-academies.org/ilar>>.
- Zbinden G. 1991. Predictive value of animal studies in toxicology. *Reg Toxicol Pharmacol* 14:167-177.

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