Requirements of Biomedical Research in Terms of Housing and Husbandry for Non-Human Primates: Pharmacology & Toxicology

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ABSTRACT

The objective of toxicology and pharmacology studies is to detect change or variation from normal and to interpret the significance of such change, with the intention of assessing risk to man. With non-human primates (NHPs) detection of change related to the experimental procedure in use is made more difficult by confounding factors, such as excessive stress, abnormal behaviour, injury, pre-existing disease or parasitism or pathological abnormality arising from these factors. In order to meet the objectives of toxicology or pharmacology studies these factors need to be eliminated or minimised as far as possible. Many of these factors may be attributable to inappropriate techniques of housing or husbandry. With improving techniques of housing and husbandry there have been clear subjective improvements in the quality of data, allowing more secure interpretation of the data.

The purpose of this short paper is to review the requirements for the successful conduct of pharmacology and toxicology studies in non-human primates (NHPs) and to examine how housing and husbandry systems have affected this, and how they can be modified to produce better data in the future. Studies in pharmacology and toxicology are generally conducted to aid decisions relating to risk to man resulting from exposure to the tested material or stimulus. These studies are crucial in providing data to enable the assessment of risk in administering new drugs to human volunteers in early clinical studies.

The basic objective of such studies is the generation of high quality, well controlled data to enable the detection of change or variation from normal and the interpretation of the significance of any change. It is crucial, at the outset, to have a satisfactory definition of normality In a laboratory context normality is defined by prior experience of the experimental animal in terms of data such as behaviour, growth rates and historical control ranges for clinical pathology values. The greater the variability and extremes in these data the more useless they become as benchmarks for detecting change and the more they confound accuracy in interpretation; therefore increased normality is equivalent to reduced variability in the control range data. This is an important point in the conduct of studies in NHPs, because the numbers of animals in an experimental group is kept to an absolute minimum.

In pharmacology studies it is normal to investigate effects of a chemical on a specific endpoint or organ system, for example the cardiovascular or nervous systems. Usually a single dose is given. There may be many measurements of a single parameter or a set of related parameters. In contrast, toxicology is a complex screening process using repeated doses in studies, which may be up to 52 weeks in length. Because a toxicology study is a screen with no pre-determined or accurate endpoint, many parameters are examined including Baudot and food consumption, ophthalmology, clinical signs, clinical pathology (determined from blood and urine samples) and toxicokinetics; finally, a necropsy is performed and

up to 50 diverse tissues are examined microscopically. Approximately 10,000 numeric data points may result from a simple 6 month study In addition, due to their importance in assessing the safety of new drugs, these studies are subject to Good Laboratory Practices, a stringent set of regulations governing their conduct and reporting.

The assessment of change in NHPs in such studies may be made more difficult or impossible by confounding factors such as abnormal behaviour, pre-existing injury or disease and parasites. The potential presence of significant zoonoses such as hepatitis, might be an important consideration in some species. In laboratory bred animals there may be conditions which mask any effects of treatment, for instance marmoset wasting syndrome which was due to poor husbandry techniques. Stress is also a factor, the effects of which are very difficult to assess. Although some stress is normal (in social interactions for instance) and may be necessary for a "normal" animal, clearly there can be situations where there is excessive or abnormal stress. Manser has reviewed the effects of stress in laboratory animals (MANSER, 1992) and it is clear that excessive stress can have wide ranging effects in NHPs.

The overall effect of these factors is to increase variability amongst individual NHPs and to make the data more difficult to interpret. It should be born in mind that an abnormal animal may give an abnormal response, which may mask a significant effect of treatment and lead to a distorted interpretation of the data. Good housing and husbandry reduce the influence of these factors and make the interpretation of studies more secure.

The basic requirements for housing and husbandry of NHPs may be summarised as follows:

Housing systems for NHPs should:	**Husbandry should provide:**
• Allow easy capture of animals	• A suitable diet
• Facilitate records of measured data	• Environmental enrichment
• Allow clear observation and recording	• Regular handling of animals
• Be easy to maintain	• Isolation of sick animals
• Be safe for the animals	• Avoidance of overstocking
• Recognise the special needs of NHPs	• Thorough staff training

In housing systems the process of removing the animals from the cages should be easy as possible so as to minimise the stress of handling and to reduce the likelihood of minor injury to fingers or limbs. It is important that the animals can be observed clearly without being removed from the cages and that identification of individual animals in the cage is possible. The design of the whole facility and of the systems in use within it should facilitate the recording of measured data, such as food consumption or body weight. Housing should be easy to clean and maintain and above all be safe for the animals; in these respects choice of materials and design are significant. For example, toxic paints, plastic coatings and the presence of loose hanging play chains should be avoided. Above all housing should recognise the special needs of primates in terms of social interactions with and avoidance of cage and avoidance of cage mates. For marmosets, large cages for family groups are essential for animals prior to experimental use; existing cages can be modified with tubes etc. to give more space. The natural grouping of marmosets as families has contributed to the removal of marmoset wasting syndrome experienced by some users of this species. Following on from earlier group housing studies with wild caught baboons (Papio spp.), we have successfully conducted toxicity studies at Ciba, Wilmslow using group housed cynomolgus monkeys and have explored group housing of marmosets in similar general toxicology studies (unpublished data).

Husbandry systems should provide an appropriate diet - low vitamin E, selenium and protein were considered responsible for marmoset wasting syndrome (WADSWORTH, 1994), although stress was also probably a factor. There should be provision for environmental enrichment, as given by forage food, swinging furniture, perches etc., which reduce boredom and give fitter animals. Before the collection of experimental data the animals should be handled regularly to accustom them to procedures; for instance in recording electrocardiograms from restrained unsedated animals a reduction in heart rate is commonly seen with repeated examinations. Where animals are group housed, it should be possible to isolate sick animals or animals which are too dominant or too submissive as this definitely alleviates an obvious stress. It is important to avoid overstocking, particularly with marmosets; too many animals in one room can aid the spread of disease and increases stress. The training of staff responsible for husbandry is also very important; well trained and confident staff who understand the animals they handle will get better data than those who have not had suitable training or experience.

Over recent years there has been a gradual evolution of NHP housing systems from single to group housing. This has resulted in larger more complex caging, with greater attention to the environment. The old style of cage for a single NHP was usually about a meter cube, with crush backs to assist removal of the animal from the cage. This provided a sterile, boring environment, with no social interaction possible, except with human handlers and was associated with a range of problems, particularly behavioural. Self mutilation through biting and scratching was common and other stereotypic behaviours could be seen. Due to the cage design there could be interruptions to the supply of water or food; automatic water supply to the back of the cage could be interrupted by the animal pulling the cage-back about. Food taken from a cage mounted hopper could fall straight through to the cage tray, where it became inaccessible.

The move towards group housing in larger more complex cages allows greater attention to the environment and provides improved beneficial social interactions, more exercise, and the opportunity to forage for food scattered on bedding. The net result is a fitter animal with better behaviour patterns and less variability due to factors associated with single housing, particularly excessive or undesirable stress. However, such housing is more difficult to design for easy maintenance and capture. In designing facilities for NHPs it is very important to remember that the quality of the ancillary areas is also important and consideration must be given to the use to which they will be put in running the unit. In general, good quality facilities should result in good quality data.

When the general purpose of studies in pharmacology and toxicology is considered, namely the assessment of chemicals for human use, it is vital that data that result from these studies are of the highest possible quality When data are of high quality, interpretation should be more secure and greater confidence may be placed in the conclusions drawn from the studies. In studies with NHPs the number of animals in any experimental group, for ethical reasons, is likely to be small. Additional factors that contribute are the expense of the animals and the space needed for adequate housing and husbandry. Quality data may be achieved by minimising variability between individuals and maintaining a comprehensive data base of control data from previous studies. With reduced variability between animals, the normal ranges in the historical data base become narrower, thus facilitating the detection of variation from normality; in other words with less variability it becomes easier to define normality for the species at the facility Although there are published data bases of controls data for some NHPs (YARBOROUGH et al., 1984; STEWART, J., 1994), caution should be exercised in the use of historical data from other facilities, due to potential variations in the procedures, environment and analytical equipment from one laboratory to another.

It is reasonable to conclude, with the evolution of housing and husbandry techniques and with greater understanding of NHPs as experimental models, that data quality has improved. In particular the change in emphasis from the use of wild caught to the more defined captive bred animals has made a huge contribution. In contrast to captive bred NHPs, wild caught animals were of uncertain age and origin and did not have a known history with regard to parasitic or disease status; they were inherently more variable than the purpose bred animal. For example clinical pathology parameters are potentially affected by injury (increased aspartate amino transferase) by parasitism (increased eosinophil counts) or infections (increased neutrophil and lymphocyte counts). Better management of marmosets has largely eliminated mortality associated with wasting syndrome, which was associated with gastric changes and other confounding pathology In some respects the precision or quality of data has decreased; for instance individual food and water consumption data cannot be obtained for group housed animals and transient clinical signs, such as vomiting or faecal abnormality, cannot always be assigned to an individual unless observed at the time. However, these deficits are not enough to warrant a return to old-style practices of housing and husbandry.

In addition to techniques of housing and husbandry attention must be given to methods used for data collection; the procedures used may themselves produce abnormal data which could obscure changes in a "normal" animal. In particular a recent publication (BERLIN et al., 1996) illustrated the value of new techniques over traditional practices. In a study with a potassium channel opener/vasodilator in cynomolgus monkeys, heart lesions were seen microscopically, although there had been no evidence of electrocardiographic abnormality during the treatment period. It was expected that this class of drug would be associated with increased heart rate and reduced blood pressure which could explain the lesions seen. This hypothesis was confirmed by the use of telemetric implants, which showed that there was a a sustained reduction in blood pressure and a sustained elevation of heart rate, which had been masked by the data collection procedures in the initial study

The housing and husbandry of NHPs are crucial factors in helping to reduce the inherent variability of these animals, thereby enhancing the interpretability of the data produced in toxicology and pharmacology experiments. A normal test system in which the variables are controlled as closely as possible is essential for producing reliable results in biomedical research where the data are used to back up decisions on administration of new chemical entities to humans.

Overall, because the results of toxicology and pharmacology studies with NHPs are used to predict hazard and risk to man from use of new chemicals or drugs, it is sensible to conclude, in order to achieve the objective of the study, that high quality data are essential. Variables should be controlled as much as possible and the use of the best possible housing and husbandry can produce a more "normal", less variable animal. However, it is acknowledged that the improvement in data, that better housing and husbandry give, cannot be easily quantified.

Finally, and most importantly, the issue of animal welfare should not be overlooked. There is no doubt that improved welfare is associated with improved housing and husbandry as discussed above; irrespective of other factors we should ensure that the animals we keep and work with are kept in the best possible conditions and are treated humanely at all times.

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