

1 Meeting 1.05 Summary

2 2/1/05

3 The meeting consisted of article summaries and discussion.

4 **Bennet, B.T, Brown, M.J., Schofield, J.C (1990). Essentials for Animal Research: A**
5 **Primer for Research Personnel.** The book can be obtained from the Primate Info Net
6 (PIN): <http://pin.primate.wisc.edu/research/welfare/essentia.html>

7 Amy Reviewed Chapter 2: Alternative Methodologies. Excerpts are listed below.

8 **Different meanings of ‘alternatives’ [this helps explain the debate on alternatives]:**

9 It [alternative] is a term that has different meanings to different people and this
10 difference largely depends on which side of the issue one is found. To many biomedical
11 researchers, alternative techniques refer to those which can be used in addition to the
12 more traditional animal models...To the so-called abolitionist who seeks the immediate
13 end to all animal research, teaching and testing, the term alternative refers to those
14 techniques which can entirely replace the use of animals. The dictionary, defines
15 alternative as: "offering or expressing a choice." The dictionary also defines technique as
16 "a method of accomplishing a desired aim." By combining these definitions, the term
17 alternative technique becomes "one which offers a choice in accomplishing a desired
18 aim."...Since a literal definition provides a rather simplistic approach to dealing with our
19 responsibility for reducing the potential pain and suffering of animals that must be used,
20 it is necessary to develop a working definition of the term. In Dr. Rowan's book, Of
21 Mice, Models and Men, he defines the term alternatives to refer **to those techniques or**
22 **methods that replace the use of laboratory animals altogether, reduce the numbers**
23 **of animals required, or refine an existing procedure or technique so as to minimize**
24 **the level of stress endured by the animal.** Since stress can be difficult to describe and
25 quantitate, for the purpose of this manual it will be replaced by the term distress.

26 The working definition of alternative techniques thus evolves to "**those techniques**
27 **which replace the actual use of animals, reduce the numbers used, and/or refine the**
28 **techniques to minimize the potential for the animal to experience pain or distress."**

29 [Comment: Amy thought this was a nice incorporation of the 3Rs into the definition of
30 alternatives, Comments? Email amy@primatesinc.com]

31

32 **The 3Rs:**

33 The 3Rs appeared in a book by Russell and Burch published in 1959 entitled The
34 Principles of Humane Experimental Technique. In the original work, the authors defined
35 the 3 R's as follows: "Replacement means the substitution for conscious living higher
36 animals of insentient material. Reduction means reduction in the numbers of animals
37 used to obtain information of given amount and precision. Refinement means any
38 decrease in the incidence or severity of in-humane procedures applied to those animals
39 which still have to be used."

40 **Definition of Animal**

41 Prior to discussing the replacement of animals with non-animal models, the word animal
42 must be defined. On the surface this appears an easy task. Common sense would tell us
43 that an animal is one of the two major kingdoms of living organisms. The dictionary
44 defines an animal as "any of a kingdom of living beings typically differing from plants in
45 capacity for spontaneous movement and rapid motor response to stimulation."

46 In the Definition of Terms promulgated to implement the amended Animal Welfare Act
47 an animal is defined as: "any live or dead dog, cat, nonhuman primate, guinea pig,
48 hamster, rabbit, or any other warm blooded animal, which is being used or is intended for
49 use for research, testing, experimentation, or exhibition purposes or as a pet. This term
50 excludes: birds, rats of the genus *Rattus* and mice of genus *Mus* bred for use in research,
51 and horses and other farm animals such as but not limited to livestock or poultry used or
52 intended for use as food or fiber, or livestock, or poultry used or intended for use for
53 improving animal nutrition, breeding, management, or production efficiency, or for
54 improving the quality of food and fiber."

55 The PHS Policy defines an animal as "Any live, vertebrate animal used or intended for
56 use in research, research training, experimentation, or biological testing or for related
57 purposes." On the other hand the Guide defines an animal as "any warm blooded
58 vertebrate animal." For the purposes of this manual, and to be consistent with most
59 approaches to discussing alternative techniques, an animal will be any living vertebrate,
60 with the caveat that any model system which moves down the phylogenetic scale from
61 the generally acceptable animal model will be considered an alternative.

62 [this is why at least federally-funded rats, mice and birds are regulated, b/c the PHS
63 definition does not exclude them from the term animal. Comments? Email
64 amy@primatesinc.com]

65
66 **Festing, M.F.W (2004) Refinement and reduction through the control of variation.**
67 **ATLA. 32 (supplement 1), 259-263.**

68 GayeLyn reviewed the article.

69 Summary:

70 The key to doing animal experiments efficiently, while using the minimum number of
71 animals without loss of scientific information, lies in good control of random variation,
72 and recognition and control of “fixed effect” variation, such as the sex or strain of the
73 animals. However, many scientists erroneously assume that the use of outbred,
74 genetically heterogeneous animals is justified, because in some way, they more closely
75 model humans. Unfortunately, all this does is to increase the phenotypic variation, which
76 results in less-powerful experiments. If the aim is to model variation in human responses,
77 this can be done by using a small number of animals from several isogenic strains,
78 without increasing the total number of animals. Reducing inter-individual variation,
79 whether caused by genetic or non-genetic causes, will nearly always result in improved
80 experiments. Fixed-effect variation, such as the sex of the animals, can be taken into
81 account, either by restricting the conclusions to the sex actually used, or by assuming that
82 the other sex would respond in the same way, or by including both sexes in the study, by
83 using a factorial design, without increasing the total number of animals.

84

85 Festing describes how good experimental design can lead to reduction in animal use and
86 describes the types of variation that need to be taken into account: There are two main
87 types of variation that need to be taken into account. First, variation can be caused by so
88 called “fixed effects”, such as the sex, strain and age of the animals, which can be
89 controlled by the scientist at a level thought to be appropriate. Thus, the scientist can
90 choose whether to use males or females or both, and a choice will be made, taking into
91 account the implications of the resulting choice. Second, the variation can be caused by
92 so called “random effects”, such as inter-individual differences and measurement error,

93 which cannot be fixed by the researcher. It is important to understand these two sources
94 of variation and the ways in which experiments can be designed to take account of them.
95 (p 259)

96 Discussion:

97 The group discussed the benefits of variation such as using females and males.
98 First, if a study didn't use both females and males, how would we discover that there
99 were indeed differences between females and males? In addition, variation occurs in the
100 human population and so although one strain of mice might help the statistical quality of
101 the experimental design, it is not very representative of a population since it is only one
102 type. So, perhaps keeping it random is better in some cases. [Comments? Send to
103 amy@primatesinc.com] One group member commented: It's important to make results
104 applicable to the general population, which is difficult when subjects are inbred,
105 genetically similar animals. If you know what you're looking for and need to decrease
106 the number of variables then inbred strains are appropriate, but they are not representative
107 of a population so should not be the strategy for the initial stages of an experiment...The
108 bottom line is that it is very important to control for the variables before starting a study.
109 One must examine all of the variables and confounds including housing conditions and
110 enrichment.

111 Festing Concludes:

112 Good experimental design ensures that experiments give, as far as possible, the
113 right results with sufficient power to detect clinically or biologically important responses
114 but that are not so large that scientific resources and animals are wasted. A good
115 understanding of variation and the way that it can be controlled is essential... Random
116 variation is minimized by using animals of a narrow age and weight range, by using
117 inbred strains if the experiment involves rats or mice, by using careful experimental
118 technique, so as to minimize measurement error, and by using randomized block or other
119 more advance designs to take account of time and space variation. (p 263)

120

121 **Elvidge, H., Challis, J.R.G., Robinson, J.S., Roper, C., Thorburn, G.D. (1976) Influence**
122 **of handling and sedation on plasma cortisol in rhesus monkeys (macaca mulatta).**
123 **J Endocr. 70, 325-326.**

124 Amy summarized the article.
 125 Evidence has been around for quite some time showing that there are differences in
 126 plasma cortisol levels depending on the type of methodology used for data-collection.
 127 This article from 1976 describes differences in plasma cortisol concentration among 4
 128 groups of rhesus monkeys. The groups were trained, unanaesthetized; untrained,
 129 unanaesthetized; ketamine, anaesthetized; and phencyclidine, anaesthetized.
 130 The results are summarized in the table below. The authors did not describe what the
 131 term training meant and they also did not mention how long it took to draw the blood.
 132 The information was taken from Table 1 in the article.

133 Plasma cortisol (ng/ml)

Group	Day 1			Day 2		
	10.00 h	11.00 h	12.00 h	10.00 h	11.00 h	12.00 h
I. Trained unanaesthetized	160 ±14	211 ±10	224 ±14	182 ±14	196 ±17	232 ±15
II. Untrained, unanaesthetized	332 ±28	342 ±62	387 ±29	362 ±21	457 ±24	445 ±33
III. Ketamine, anaesthetized	210 ±11	239 ±24	327 ±19	330 ±49	382 ±56	446 ±67
IV. Phencyclidine, anaesthetized	270 ±33	334 ±28	414 ±12	374 ±69	417 ±43	454 ±60

134 The authors discuss the results:
 135 There was a significant effect ($P < 0.01$) of time on the mean cortisol concentration
 136 In Groups I, III and IV on day 1; however, in no group was the mean value at 10.00 h on
 137 day 1 significantly different ($P > 0.05$) from that at 10.00h on day 2. In the two groups of
 138 monkeys that were subjected to prolonged sedation, much greater variation in the cortisol
 139 concentration between and within individual animals was seen on day 2.
 140 These results extend those of Setchell *et al.* (1975) and show that significant
 141 differences may be found in the values for plasma cortisol between rhesus monkeys
 142 sampled under different ‘basal’ conditions. The present study shows that it is possible by
 143 long-term regular training to achieve mean cortisol values which are significantly lower
 144 than in untrained or anaesthetized animals.” (p 326)

145 *Discussion:*

146 Although the changes were not significantly higher on day 2, it is noteworthy to
147 see that all of the cortisol rose in the 4 groups. This could possibly be due to the animal
148 anticipating another draw. [Other suggestions? email amy@primatesinc.com.]The table
149 also shows considerable variation in methods. Today, there are many more methods of
150 obtaining cortisol values. Some methods follow the traditional literature and still use
151 restraint involving gloves, squeeze apparatus, or a squeeze-back mechanism in the home
152 cage. Other labs use positive reinforcement training and attempt to achieve progress in
153 the area of refinement. There are also non-plasma methods of cortisol collection such as
154 saliva, urine, hair, or feces to assess cortisol. (For more information relating to refinement
155 of methods, see The Annotated Bibliography on Refinement and Environmental
156 Enrichment for Primates kept in Laboratories:
157 http://www.awionline.org/Lab_animals/biblio/

158

159 **Balcombe, J.P., Barnard, N.D, Sandusky, C (2004) Laboratory Routines**
160 **Cause Animal Stress. Contemporary Topics in Laboratory Animal Science 43(6),**
161 **42-51**

162 GayeLyn reviewed the article.

163 Abstract:

164 Eighty published studies were appraised to document the potential stress associated with
165 three routine laboratory procedures commonly performed on animals: handling, blood
166 collection, and orogastric gavage. We defined handling as any non-invasive
167 manipulation occurring as part of routine husbandry, including lifting an animal and
168 cleaning or moving an animal's cage. Significant changes in physiologic parameters
169 correlated with stress (e.g., serum or plasma concentrations of corticosterone, glucose,
170 growth hormone or prolactin, heart rate, blood pressure, and behavior) were associated
171 with all three procedures in multiple species in the studies we examined. The results of
172 these studies demonstrated that animals responded with rapid, pronounced, and
173 statistically significant elevations in stress-related responses for each of the procedures,
174 although handling elicited variable alterations in immune system responses. Changes
175 from baseline or control measures typically ranged from 20%-100% or more and lasted at
176 least 30 min or longer. We interpret these findings to indicate that laboratory routines are

177 associated with stress, and that animals do not readily habituate to them. The data
178 suggest that significant fear, stress, and possibly distress are predictable consequences of
179 routine laboratory procedures, and that these phenomena have substantial scientific and
180 humane implications for the use of animals in laboratory research.

181 Discussion:

182 Since we now have the information available regarding less-stressful methods, a
183 standardization of methods would be beneficial across laboratories for both animal
184 welfare and data-accuracy purposes. Since the USDA (APHIS) is the common
185 regulatory body among research facilities, they should encourage IACUC committees to
186 collect the information pertaining to the types of methodologies used for data collection.
187 The committees should submit this information to the USDA. Evaluation of the
188 methodologies should occur. The least-stressful, most successful, and accurate way to
189 perform cortisol-collection should be selected and disseminated back to the IACUCs so
190 they can work with the institution on standardization. Standardization would be a step in
191 the right direction for both the refinement and reduction aspect of animal welfare.

192

193 **Prescott, M.J., Jennings, M. (2004). Ethical and Welfare Implications of the Acquisition**
194 **and Transport of Non-human Primates for Use in Research and Testing. ATLA 32**
195 **(supplement 1) 323-327**

196 Tom summarized the article.

197 Abstract:

198 Assessment of the ethical and welfare implications of any laboratory animal use should
199 encompass the entire life-history of the animals concerned, including their acquisition
200 and transport. This is particularly important in the case of non-human primates, because
201 the acquisition of some species involves capture from the wild, inadequate husbandry,
202 and/or lengthy, multistaged travel from the country of origin to the laboratory where they
203 are used. Thus, non-human primates endure considerable harms even before they reach
204 the laboratory. Despite this, the information necessary to increase awareness of, and to
205 assess, the potential harms of acquisition and transport is not readily available. This paper
206 highlights the ethical and welfare concerns associated with these processes and makes
207 recommendations intended to reduce their impact on welfare. The information presented

208 is collated from a recent report that analyses the UK trade in non-human primates for
209 research and testing, but many of the concerns and recommendations are applicable in an
210 international forum. The need to minimise suffering is emphasised, as is the need for
211 critical review of the necessity and justification for all nonhuman primate use, a reduction
212 in the numbers used, and the development of alternatives to replace their use.

213 The article focused on the following areas: the acquisition of wild animals for
214 research, the breeding of captive animals for research, and the transportation of animals
215 in research. The problems and solutions for breeding and transport are discussed below:

216 **Breeding of captive nonhuman primates:**

217 Problems: lack of data, inconsistent methods, poor conditions

218 Solutions: regulate, at least to the degree that participant have standardized practices, and
219 keep adequate records

220 **Transportation of research animals-**

221 Problems: stress caused by poor transport conditions, change in environments, exposure
222 to other animals, and small cages

223 Solutions: potentially improve every aspect of transportation; maintain good records
224

225 **Kessel, A., Brent, L. (2001). The rehabilitation of captive baboons. J Med Primatol**
226 **(30) 71-80.**

227 Abstract:

228 Eleven baboons who had been singly housed indoors for an average of 5 years were
229 moved to outdoor social groups in an attempt to provide a more species-typical
230 environment and reduce high levels of abnormal behavior. Nine of the baboons were
231 observed while in single housing and, over a 6-month period, while housed outdoors
232 socially to document long-term changes in behavior. Abnormal behavior decreased
233 significantly from an average of 14% of the observation time in the single cages to 3% in
234 the sixth month of social housing. Cage manipulation and self-directed behaviors also
235 significantly decreased, while social behavior, enrichment-directed behavior, and
236 locomotion increased in social housing. Baboons that had been in long-term indoor
237 single housing were able to reproduce and form stable social groups without injury. This

238 study provides evidence that even behaviorally disturbed nonhuman primates can be
239 successfully rehabilitated to live in social groups.

240 At first, this article may seem like common sense; however, Amy thought it was a very
241 important article, because she has heard various comments regarding abnormal behavior
242 from scientists. Some comments/suggestions are listed below:

- 243 • Abnormal behavior is a learned behavior and therefore it cannot be relieved
- 244 • If abnormal behavior cannot be relieved within the confines of the environment,
245 the animal is either sold to more research or euthanized (depending on the
246 severity).

247 The Animal Welfare Act says special attention should be given to those NHPs that show
248 signs of psychological distress through behavior or appearance. This article shows that
249 relief of abnormal behavior can occur by putting an animal in a naturalistic environment.
250 If the research facility cannot do this themselves, then retirement of the animal should be
251 considered as part of the 'special attention' that is required by the Animal Welfare Act.