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To: Dr. Michael Gottesman
Deputy Director for Intramural Research

From: Karl Pfeifer **KP**
Chair, NICHD ACUC

Date: December 17, 2014

Subject: Animal Welfare Investigation – Animal Welfare Assurance A4149-01
Case 9Y – Part 2

This report describes the NICHD response to the concerns raised by Dr. Katherine Roe of the People for the Ethical Treatment of Animals (PETA) in regard to nonhuman primate research described in NICHD ASP 14-043. The research program described in ASP 14-043 addresses the role of the social environment in social and cognitive development of juvenile rhesus monkeys and also looks at interactions between genes and the social environment. One of Dr. Roe's specific concerns was that these experiments were inappropriately designated as USDA pain/distress category C. Moreover, she maintained that the research goals could be successfully accomplished using non-animal models and also that the research was not sufficiently novel to warrant the use of animals. Essentially, Dr. Roe asked us to evaluate whether NICHD had sufficiently considered principles II, III, and IV of the *US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*. Dr. Roe's letter and supporting documents are attached.

At our meeting on September 17, 2014, we reviewed the information provided by PETA and agreed that it was appropriate and necessary to investigate the concerns described above for the following three reasons. First, we agreed on the great importance of being very careful in our oversight of nonhuman primate research. Second, as discussed in below, we have always understood that the correct assignment of the USDA pain/distress category is not entirely straightforward in regard to this research. In fact, some of the same issues raised by Dr. Roe are ones that we discussed during our original review of the protocol in April 2014. Third, we recognized that this was a particularly useful time to review this ASP. The renewed ASP included significant new procedures for animals born in 2014. Since the birthing season for 2014

was over, it was a good time to review how those new procedures worked in practice and then based on our experiences, to consider further refinements and to determine if new information might affect the USDA classification.

A subcommittee of five members directed our investigation which included the following: 3 visits to the Poolesville facility to inspect the animals and the facility and to conduct extensive talks with the research and the animal care staff; several phone and multiple email discussions with key research and animal care staff; and discussions with animal care staff at National Primate Centers. We prepared several formal questionnaires for the PI to address specific concerns raised by Dr. Roe and by ACUC members. Our facility veterinarian also responded to these queries. In addition to these completed questionnaires, we distributed the following documents to all committee members: the PETA letter with the accompanying summary report and collection of supporting letters; USDA Policy 11 – *Painful and Distressful Procedures*; NIH OACU *Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain and Distress Categories*; National Research Council Discussion – *Stress or Distress*; and OLAW FAQs describing Institutional responsibilities for scientific review. The PI’s peer-reviewed manuscripts are available through PubMed but we also directly provided each member with three key recent publications that addressed the impact of nursery rearing on long-term animal welfare (<http://www.ncbi.nlm.nih.gov/pubmed/23184974>; <http://www.ncbi.nlm.nih.gov/pubmed/22615410>; <http://www.psych.utah.edu/people/people/fogel/jdp/journals/1/journal1-05.pdf>). To supplement our understanding of the USDA requirements, I consulted by phone and email with a key staff veterinarian at that agency. Finally, we obtained and considered information from the Office of the Scientific Director regarding the scientific review of the research described in this ASP. Progress of our subcommittee was reviewed at our meeting on October 15, 2014 and the conclusions described here were obtained after extensive discussion at our meetings of November 19, 2014 and December 17, 2014.

In regard to Scientific Review and consideration of novelty and relevance to human health and the good of society (US Government Principle II): In addressing this concern, we used OLAW FAQs (<http://grants.nih.gov/grants/olaw/faqs.htm>) as our primary guide.

According to NIH Intramural policies, all intramural investigators must undergo a review of their scientific research program once every four years. In accordance with this policy, the program associated with ASP 14-043 was reviewed in November 2012 by a panel constituted by the NICHD Board of Scientific Counselors. (The Board of Scientific Counselors (or BSC) is the NICHD Intramural program’s external formally constituted advisory body.) The November 2012 review panel consisted of 4 scientists: 2 BSC representatives as chair and co-chair and 2 ad hoc reviewers who are specialists in the field. NICHD external reviewers are specifically charged to evaluate research significance and also the appropriateness and likely success of the research plan. The panel reviewed the research favorably. At their semi-annual meeting in June 2013, the NICHD BSC reviewed and endorsed the site visit report and the research program. No concerns about vertebrate animal research were raised in the site visit report or by the BSC. The signature of our Scientific Director in Section O of the ASP attests that the research program was appropriately reviewed and verifies the congruence of the research plans described in the ASP with those reviewed by the external reviewers.

As noted above, scientific merit and research significance are primarily addressed through external review. However, the IACUC also plays a role through its evaluation of the responses to Section D of the ASP. During our review of the ASP this fall, we asked the PI to provide an expanded version of Section D that more precisely clarified the purposes of his research. This new information was provided in two documents that are attached to our December minutes and can be supplied upon your request.

We carefully read the supporting letters provided by Dr. Roe and PETA. We are aware that several letters, especially those from Dr. Gluck and Dr. Hansen provide specific and detailed arguments that the research work performed under ASP 14-043 is not of sufficient significance to merit support by the NIH. We appreciate that scientists can disagree as to the merits of specific research programs. Therefore we have forwarded the PETA letters to our Scientific Director and asked him to share these with the NICHD BSC. *However, the unanimous conclusion of our committee is that the external review is the primary method for determining research merit and the likelihood that the protocol will contribute to human health and the advancement of knowledge. We further conclude that the information provided in response to our questions about Section D is consistent with the report of the external reviewers and that the publication of multiple peer-reviewed manuscripts is consistent with the report of the external reviewers. Therefore it is appropriate for the ACUC to conclude that the research plan is consistent with U.S. Government Principle II.*

In regard to the consideration of alternative species (US Government Principle III):

Evaluation of the appropriateness of the species is an important part of every ASP review. According to OLAW FAQs, this responsibility for addressing this issue lies with both the ACUC and the external reviewers. In our past experience, the crucial question is usually whether simpler model systems can be effectively used to address the research questions. In ASP 14-043 and in supporting documents provided as part of our investigation, the PI provided compelling reasons that justifies why a rodent (or other animal) model will not work. That is, only by using NHPs can the investigators address behaviors and manipulate a social environment that might model human cognitive development and psychology.

We note, however, that the main PETA objection is not that simpler model systems will suffice. Rather, PETA suggests that this research can be supplanted with human studies. We asked the PI to respond specifically to these concerns and his answers are available in our November minutes. His response includes supporting letters from medical researchers whose area of expertise is human behavior and psychology. As with the overall evaluation of the research scientific merit, we put primary emphasis on the external review results. The expertise of the NICHD BSC in human development and medicine is exceptionally strong. *For these reasons, our unanimous conclusion is that this ASP was sufficiently reviewed in regard to species appropriateness.*

Having made that conclusion, during our review, we identified changes in our procedures that will improve future review of NICHD NHP research. We recognize that currently we are relying on the lack of any stated concern by the external review panels as demonstration that the issue of species appropriateness was sufficiently evaluated. We will improve on our current system by having the issue of species appropriateness directly addressed by the external

reviewers since they are the scientists with the expertise in human development. Therefore, our ACUC chair will work with our Scientific Director to provide a worksheet to be included as part of future external reviews of NHP research. This worksheet will be modeled on one used by extramural researchers (<http://grants.nih.gov/grants/olaw/vaschecklist.pdf>) and will ask the PI to justify the species and to specifically address whether past or ongoing human research studies better address the research questions. The external reviewers can then explicitly indicate whether the PI's explanation is acceptable. The next external review for this research team is scheduled for 2016.

Similarly we will require specific external review of animal numbers in order to make full use of the expertise of the ad hoc reviewers and the BSC and try to refine the study in every possible way to minimize the use of NHPs.

In regard to USDA classification of procedures in regards to pain and distress: Based on OLAW FAQs, we considered this area of review to be primarily the responsibility of the IACUC.

To help OLAW understand our extended discussion, we provide a brief synopsis of the experiments: Each year in spring and early summer, up to 45 rhesus monkeys are born and sorted into two groups. Up to 20 infants are permanently separated from their mothers within 24 hours after birth and raised in a nursery. The other infants are raised by their mothers but are removed periodically for brief testing periods as discussed below. In 2014, for example, 14 monkeys were born on this ASP and 10 were raised in the nursery. The general scheme is described on the attached charts, *ASP Procedures*, which denotes all the experimental procedures performed under this ASP. We evaluated the pain/distress for each of these groups (mother-reared and nursery-reared) separately and with separate attention to the mothers and to the juveniles in each cohort.

Re-evaluation of mothers participating in mother rearing experiments: Our review focused on a significant new procedure for this laboratory: at 3-4 months of age (when animals are beginning to self wean), juveniles are removed from their mothers and singly housed for 25 hours for behavioral testing. This procedure is modeled on testing done at the California National Primate Research Center (see Capitanio JP et al., 2006, *Nursery rearing and biobehavioral organization*. In: Gene P Sackett et al. (eds.) Nursery rearing of nonhuman primates in the 21st Century. Springer Science + Business Media Inc., NY, pp. 191-214.), one of the groups we consulted during our investigation.

Please note that this long separation follows 4 briefer separations (up to 1.5 hours). (See the *ASP Procedures* attachment for details). Thus there is prior adaptation training for both mothers and infants.

Consistent with information from other Primate Centers, our experience is that stress to the mothers appears to be minimal and discrete. Mothers do sometimes call for their infants, especially when they first notice human caretakers entering their area. However, this vocalization has always been limited in scope and the mothers continue to interact with their cohorts and they feed and groom normally.

We considered the possible use of drug therapy such as Valium to reduce potential stress to the mothers. Our veterinary staff assured us that this was not appropriate for our protocol. That is, given the lack of symptoms associated with these separations, even the very low risk associated with use of Valium could not be medically justified.

Instead, in collaboration with the veterinary staff, the research team has proposed several refinements to the ASP to ensure minimal stress to the mothers. These refinements will be formalized in an amendment to the ASP that must be approved by the ACUC prior to beginning these experiments next summer. Specifically, the amendment will define a mechanism for multiple behavioral observations using a checklist that describes behaviors that indicate distress to the mother. If these behaviors are noted, the experiment will be terminated by early reunion of the mother and infant. *Based on the laboratory's experience this past summer and given the ASP amendment to include clear experimental endpoints, our unanimous conclusion is that in regard to the mothers, this separation procedure is appropriately labeled as column C.*

As mentioned above, the 25-hour separation follows 4 shorter separations. For the safety of the research staff, each of these separations requires sedation of the mother. Thus the 1-1.5 hour separation period includes only about 30 minutes where the mother is aware of the separation of her infant. There are no behaviors, such as continuous vocalizations, that suggest distress. *Our unanimous conclusion is that in regard to the mothers, these separation procedures are appropriately labeled as column C*

Re-evaluation of infants participating in mother rearing experiments: Our review focused first on the 25-hour separation that was a new procedure to this ASP. We reviewed this procedure carefully with the research staff and inspected the standard operating procedures and also the rooms and equipment for this experiment. We discussed veterinary and research records for this summer's experiments.

We do presume that this procedure does result in some stress or discomfort to the infants. Their eager reunion with their mothers at the end of the 25-hour test demonstrates that the infants prefer to be with their mothers. Accepting this fact, our obligations as an ACUC are twofold. First, we need to determine whether the stress is sufficient so that it would be more accurate to refer to it as distress and therefore to re-label the experiment as USDA column E. Second, and regardless of the USDA classification, we are obligated to seek ways to refine the experiment so that we cause the minimal stress and discomfort that is consistent with obtaining data necessary to address the experimental question.

Consistent with reports from other primate centers performing similar studies, there is some stress for the separated infants. Specifically, our research and veterinary staff noted one infant (of 4 tested) that particularly showed significant agitation when he came into contact with humans during his time in the test. (This contact with humans would occur as he was moved back and forth from his home cage space to the testing procedure area, during the two 5 minute periods when saliva samples were collected, and also during one behavioral test where his reaction to a human visitor was recorded for 5 minutes.) On the other hand, when this infant was removed from human contact, he calmed down. (We know this because of the cameras in the testing rooms). Overall, our veterinarian and primatologists concluded that this animal should still be classified as a column C but his reactions gave us information about the sorts of negative response we might encounter and allowed the researchers to develop refinements to limit

discomfort and stress in future studies. Therefore the following refinements will be incorporated into the ASP before resumption of the experiments next spring: First, researchers will make changes to the home cage environment to provide the sorts of enrichment that have been already demonstrated to comfort nursery reared animals. Second, researchers will install cameras in the room that acts as the home base of the animals. This will allow us the ability to monitor animals for the full 25-hour period and not just during the several hours they are being actively tested. Third, the amendment will establish behavioral criteria that will act as endpoints requiring premature termination of the experimental procedures by unification of the infant with the mother. *In regard to the infants, the USDA classification of this procedure will depend upon the specific endpoints described in the amendment. We will look at those endpoints very critically before assigning USDA category. However, given the experiences this summer and the stated goal of the researchers to terminate the procedure (if necessary) before the animals are distressed, we think it is likely that this will remain a column C classification.*

As described above, mother-reared infants are also separated for four shorter periods of 1-1.5 hours. These separations allow for behavioral testing of the infants and for collection of biological samples (mothers and infants). During each separation the mother is sedated or emerging from sedation for the first half while the infant is sedated or emerging from sedation for the second half. Sedation in each case is used as a chemical restraint to allow safer handling of the animals and is not alleviate pain. *The experience of the research staff – verified by the veterinary staff – indicates that these short-term separations are appropriately considered USDA column C.*

We also note that the PI has already amended this ASP in regard to these shorter separations to remove the following three procedures: EEG analyses on neonates, one blood draw, one CSF tap. A second amendment to remove all CSF taps is now being prepared. These changes were possible because sufficient data had been collected this summer obtained to answer the experimental questions. During our annual reviews of this ASP, we will continue to work with the PI to identify areas for further refinement.

Re-evaluation of the nursery rearing. Perhaps the main issue raised by the PETA report concerns the possibility that animals raised in the nursery are experiencing distress. In support of this idea, the PETA report cites literature that notably includes manuscripts from this PI and describing data obtained from previous iterations of this ASP. The PETA arguments are straightforward and can be summarized as follows: “This study purports to investigate the effect of social deprivation. If the experimenters are truly succeeding in creating this deprivation and altering behavioral outcomes in the process, is it not correct to label the study as causing more than transient distress and pain?” This is a reasonable question and an important one.

The PETA report implicitly and explicitly compares the current ASP to early studies from Harlow et al. at the University of Wisconsin. In these early studies, infants were separated at birth and then raised under truly deprived conditions with minimal environmental stimulus and resulting in severe behavior deficits. This is not the case with this current ASP. Rather, as described in our OLAW memo of October 2014, infants are raised with an intense environmental enrichment program. The purpose of this study is not to cause distress but to isolate the effects of the social environment on infant development. Our program includes regularly rotated toys, handling sessions with human caretakers, and a regular rotation of various food items. Infants are

within visual, olfactory, and tactile contact with one another and receive no less than 2 hours per day of physical social interaction. Infants receive surrogate cloth mothers for the first 4-8 months of life (peers are weaned from surrogates at 4 months and surrogate-reared infants keep them until they leave the nursery). We have adapted the surrogate mother to include a fleece-lined pouch that provides a hiding place and additional comfort for the infant. Additionally, nursery infants undergo a battery of cognitive and social tasks/observations, which gives them numerous daily interactions with human caregivers, occurring between 25-50% of their waking hours (and often more). Our animal care staff routinely observes nursery animals. ACUC members inspect the nursery twice each year. The ACUC also regularly reviews SOPs for nursery care. It is relevant to note that our triennial AAALAC inspection has always occurred during the late spring or early summer when the nursery is in use and that our inspectors routinely commend the NICHD NHP facility with emphasis on the richness of our enrichment program. It is therefore our conclusion that the nursery care in this facility fulfills all the measurable requirements of the *Guide for the Care and Use of Laboratory Animals* (8th edition).

Of course, the effectiveness of an enrichment program must be determined not just by tallying the inputs but also by observing the output, i.e. the animal behavior. Clearly the researchers intend to impact behavior, otherwise this research program would be pointless. In fact, multiple publications now document differences between nursery-reared and mother-reared infants in the following areas: cognitive development, anxiety in novel situation, alcohol preference, position in the social hierarchy, and stereotypic behaviors. It would be disingenuous not to note that in each case, nursery rearing moves the average behavior of the monkey toward something that humans would consider less desirable. But it is equally important to note that these are all population effects. That is, nursery rearing is not inducing a novel behavior but is increasing the frequency of a behavior that is already often observed in a normal (i.e. mother-reared) population. Moreover, other factors, for example genetic background or innate sensitivity to cortisol, also increase the frequencies of these behaviors, sometimes even more so than nursery rearing.

In addition to these manuscripts generated by the researchers, we also considered data and conclusions generated by our animal care staff. Animal caretakers evaluate each animal twice daily for physical and for psychological health. Health records therefore include information about such issues as appetite, hair loss, lethargy, or any behavior that might be evidence of stress or distress or an inability of the NHP to adapt to its social environment. In addition to these twice-daily checks, a NICDH veterinarian performs weekly evaluations of each animal. These daily and weekly evaluations are used to prescribe additional environmental enrichments and/or alterations to promote the psychological health of each animal on the protocol. Finally, in addition to these health checks, trained specialists on our staff perform formal behavioral analyses twice yearly on each animal. These analyses form the bases for independently evaluating the effectiveness of our environmental enrichment program for each animal. We recognize that each NHP is an individual and our staff develops an enrichment and housing program that is appropriate and beneficial for that animal. The ACUC reviews the veterinary care and behavioral care as part of its semi-annual review when we visit the facility in Poolesville and also by organizing additional meetings in Bethesda where the entire ACUC meets with key personnel to review the environmental enrichment SOPs and the overall success of behavioral management for NICHD NHPs.

To address the issue of USDA classification, we put primary emphasis on understanding whether the behavioral changes interfered with normal biology or social function of the animals. We focused especially on two specific behaviors associated with adult animals that had been

nursery-reared – they tend to be lower in social hierarchy and they are much more likely to exhibit stereotypic behaviors. These behaviors are especially important for three reasons: 1) the differences between nursery- and mother-reared animals are statistically significant; 2) these behaviors did not always need to be induced by specific experimental conditions but can be apparent upon simple observation; and 3) these behaviors might conceivably interfere with normal life in the colony.

Although nursery-reared animals have lower status on average, they are not behaving differently than non-nursery reared animals of the same status. Thus whatever the impact of social status on animal welfare/happiness, there is no net change in the collective colony because of nursery rearing.

The frequency of stereotypic behaviors among nursery-reared animals is clearly increased, especially in stressful situations (e.g. during observation by a human visitor). However, it is important to note that the severity of the behaviors does not increase. There is not even anecdotal evidence that nursery-reared animals show self-injury behaviors or that stereotypy interferes with daily activities such as social interactions, infant-rearing, foraging, or grooming. Our observation is that these stereotypic behaviors are more accurately viewed as effective mechanisms for coping with increased anxiety than as pathological and preventing normal social relations. In fact, nursery-reared animals are able to interact normally with mother-reared peers and their reproductive health and ability to form family units is good and is comparable to mother-reared animals. *Altogether based on our analysis of the research literature and our Program's own inspection and observation of the animals while in the nursery and after reunion with their cohorts, we unanimously conclude that nursery rearing has been appropriately labeled as column C.*

Summary

--We thank PETA for their interest in the welfare of the NHPs at the NICHD and appreciate the reasoned and passionate report that instigated this investigation.

--As detailed in this report, our investigation has led to several important refinements that will protect and improve animal welfare. 1) Already, we have approved an amendment to the ASP to remove several procedures including neonatal EEG analyses, CSF taps, and one blood draw. 2) New ASP Amendments will define distress behaviors so that the 25 hour behavioral assessment performed on mother-reared infants will not have even a potential to cause distress to mothers or infants without premature termination of the behavioral assessments. 3) Changes in our external review process will make better use of the expertise of external, unaffiliated scientists. Thus we will obtain direct feedback on species appropriateness and animal numbers so that we can work aggressively to refine NHP experiments.

-- Finally, we want to emphasize that we do not consider the issues addressed in this report to be fully settled. Rather, as new data is generated regarding nursery reared NHPs and also as new standards for animal welfare emerge, we will continually re-evaluate both the USDA classification and also the enrichment program for NHPs in the nursery and otherwise. We have unanimously agreed that we will again review this ASP next October (after the completion of the

nursery study for 2015) to re-evaluate the USDA pain categorization and to consider new refinements. Our committee believes that the ethics of an animal study is never a settled issue but one that must be constantly reconsidered by evaluation of both the merits of the study and of the animal care.

We recognize that our conclusions regarding the USDA classification will likely not be satisfactory to Dr. Roe and to her PETA colleagues. We appreciate that PETA's guiding philosophy is that animal research can essentially never be justified. In contrast, however, the US Public Health Service considers that animals can be of great importance to biomedical research. We have concluded that the assigned USDA pain designations accurately and fairly portray the care and actual welfare of the NHPs on this protocol. We understand that reasonable people might disagree with our conclusions. However, we are confident that we have addressed this question appropriately, according to the Guidelines of the PHS and the USDA, and to the best of our ability. We took this issue to heart, spent considerable time and effort, and seriously considered the question, often arguing the PETA position in our debates. Our conclusions were not predetermined but followed full consideration and debate. Certainly, regardless of the USDA classification, we will continue to seek refinements to these experiments to minimize the numbers of animals used on this study and to reduce stress and discomfort.