Scientific Affairs (NIH/OD)

From: Nadon, Nancy (NIH/NIA)
Sent: Wednesday, November 09, 2005 4:20 PM
To: Scientific Affairs (NIH/OD)
Cc: Nadon, Nancy (NIH/NIA)
Subject: FW: NIH Requests Information on New Standards for the Care and Use of Laboratory Animals
Attachments: Nadon Lab Animal 0904-36.pdf

Maggie,

I have attached an article on maintaining aged rodents that I published in Lab Animal. One area that is not covered well in the Guide is end-of-life decisions for aged rodents in lifespan studies. I know of one investigator who is planning on submitting an R13 application for a workshop on that topic. Let me know if I can provide any other information.

N2

Nancy L. Nadon, Ph.D.
Head, Office of Biological Resources and Resource Development
National Institute on Aging

7201 Wisconsin Ave., GW 2C231
Bethesda MD 20892
Phone: 301-402-7744
FAX: 301-402-5997
nadonn@nia.nih.gov

NIA resources page http://www.nia.nih.gov/ResearchInformation/ScientificResources/

From: Wigglesworth, Carol (NIH/OD)
Sent: Wednesday, November 09, 2005 2:34 PM
To: List OLAW-L
Subject: NIH Requests Information on New Standards for the Care and Use of Laboratory Animals

The NIH is soliciting new scientifically valid information, methods or practices, published data or other advances in the humane care and use of laboratory animals in order to explore the need to update the laboratory animal welfare standards of the *Guide for the Care and Use of Laboratory Animals*. The Request for Information, NOT-OD-06-011, contains additional details and is available at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-06-011.html.

Carol Wigglesworth
Acting Director
Office of Laboratory Animal Welfare
National Institutes of Health
RKL1, Suite 360, MSC 7982
6705 Rockledge Drive
Bethesda, MD 20892-7982
301-402-5913

fax: 301-402-2803
wigglesc@od.nih.gov

Subscribe to the OLAW Listserv list to get timely information concerning OLAW activities, updates, and guidance. Send an email to LISTSERV@LIST.NIH.GOV with the following text in the message body: subscribe OLAW-L <your name> (e.g., subscribe OLAW-L Jane Doe). (The LISTSERV will retrieve your email address from the "From:" section of your email message.)

NAME: Nancy Nadon/NIH-NIA

ARTICLE/CONTENT: Maintaining Aged Rodents for Biogerentology Research

SOURCE: Nadon, N. (2004), Lab Animal, Vol. 33, No.8, pp. 36-41

Scientific Affairs (NIH/OD)

From: NJ Benevenga [njbeneve@wisc.edu]
Sent: Monday, November 14, 2005 4:04 PM
To: Scientific Affairs (NIH/OD)
Cc: jshurts@biochem.wisc.edu; Holly McEntee; welter@rarc.wisc.edu
Subject: NOT-OD-06-011
Attachments: 100_04242.JPG; 100_04262.JPG; 100_04282.JPG; 100_04292.JPG; 100_04302.JPG; 100_04332.JPG; 100_04342.JPG; 100_04352.JPG

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD NIH
6705 Rockledger 1, Suite 4184, MSC 7983
Bethesda, MD20892-7983

This is in regard to **NOT-OD-06-011**

Release Date November 9, 2005.

I have a comment for the section on, Housing for laboratory animals.

Last year our animal care committee was concerned about the use of wire mesh caging for rats. As a result of that concern I designed a shelving unit [I call it the Rat Loft] that fits inside standard wire mesh rat cages and provides a shelf that the animal can lay upon. The stainless steel Rat Loft can be washed while still in the cage as the rack of cages is washed. The advantage of its use is that the animal has a solid floor that it can lay on but yet the investigator can still use wire mesh cages to meet the needs of Nutrition research. I have a publication on the development of the Rat Loft in the August 2005 issue of Tech Talk, the Newsletter for Laboratory Animal Science Technicians. The references is

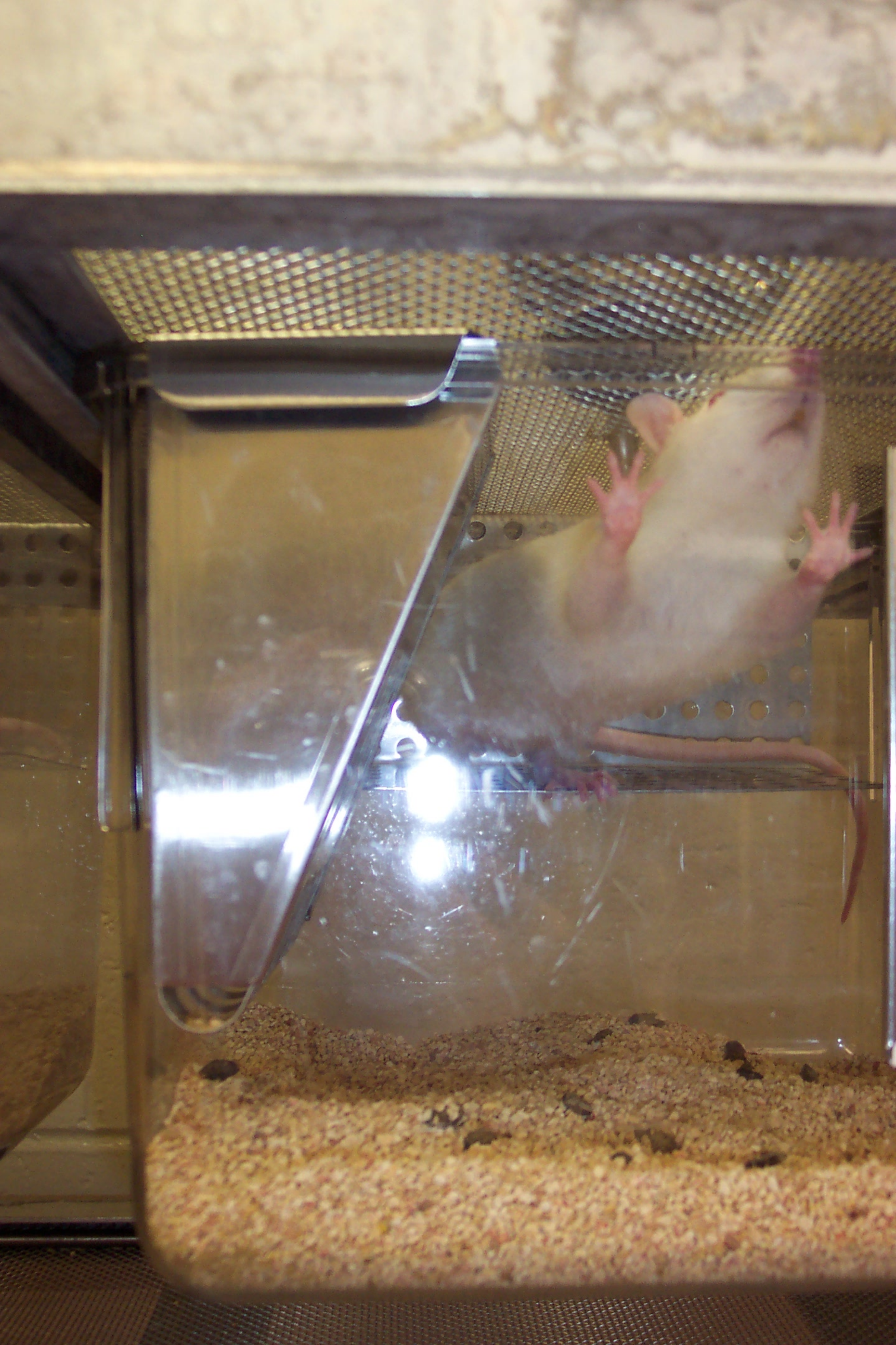
Benevenga, N. J., Mary Kaiser and Margaret Clagett-Dame 2005. Development of the Rat Loft. Tech Talk. 10/No. 4. August Pg 3.
I included three photos of the Rat Loft in use in this paper. I have attached also some photos of the Rat Loft. I hope these help so you can visualize the potential of the Rat Loft. The Department of Biochemistry here at the University of Wisconsin made 1000 of these units and have concluded that they are used by the rats and add to the environment of the rat. I hope my idea will be helpful to others who need wire mesh caging to support their research needs.
Thanks for looking, NJB

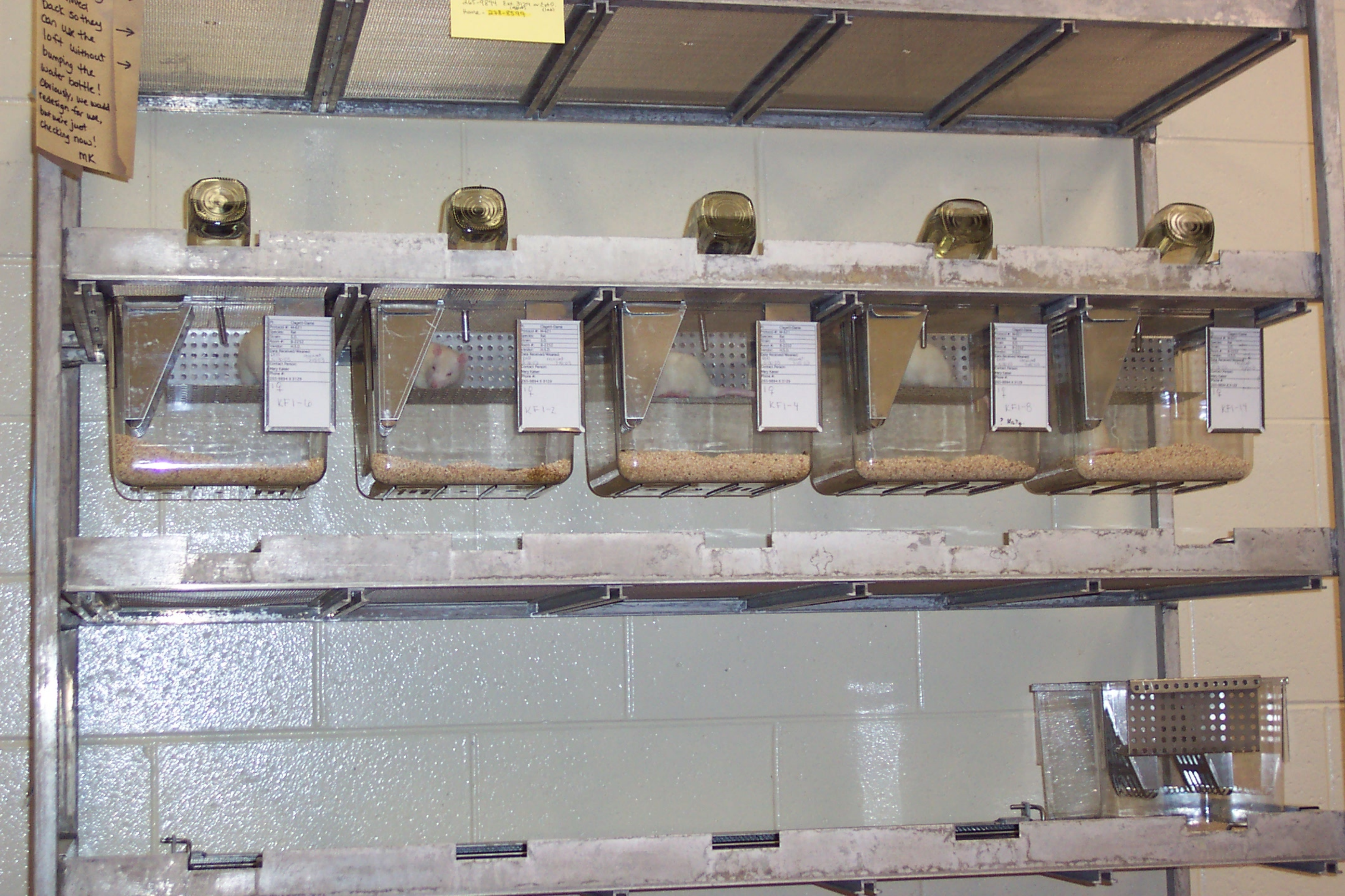
Date: Tue, 24 Aug 2004 08:20:04 -0500
To: benevenga <njbeneve@ansci.wisc.edu>
From: "Kaiser, Mary" <kaiser@biochem.wisc.edu>
Subject: Re: can you help?
X-Virus-Scanned: by amavisd-new at biochem.wisc.edu
X-OriginalArrivalTime: 24 Aug 2004 13:20:20.0957 (UTC) FILETIME=
[1B2788D0:01C489DD]

Hi Ben - I've attached some images to this reply, and I hope they come through OK - Only one of them is of rats on wire(file 100_0435.JPG), most are in shoeboxes. If you need more images of them used with wire caging, I could get them for you today, I just need a bit of time to take the digital camera downstairs and take them! So just let me know 1) If these come through OK and 2) If you need additional images! Hope this helps out! mary

P.S. I'll also toss some images onto a CD for you, so you have a more "permanent" record - I'll drop it off one of these days soon on my way out! Mary

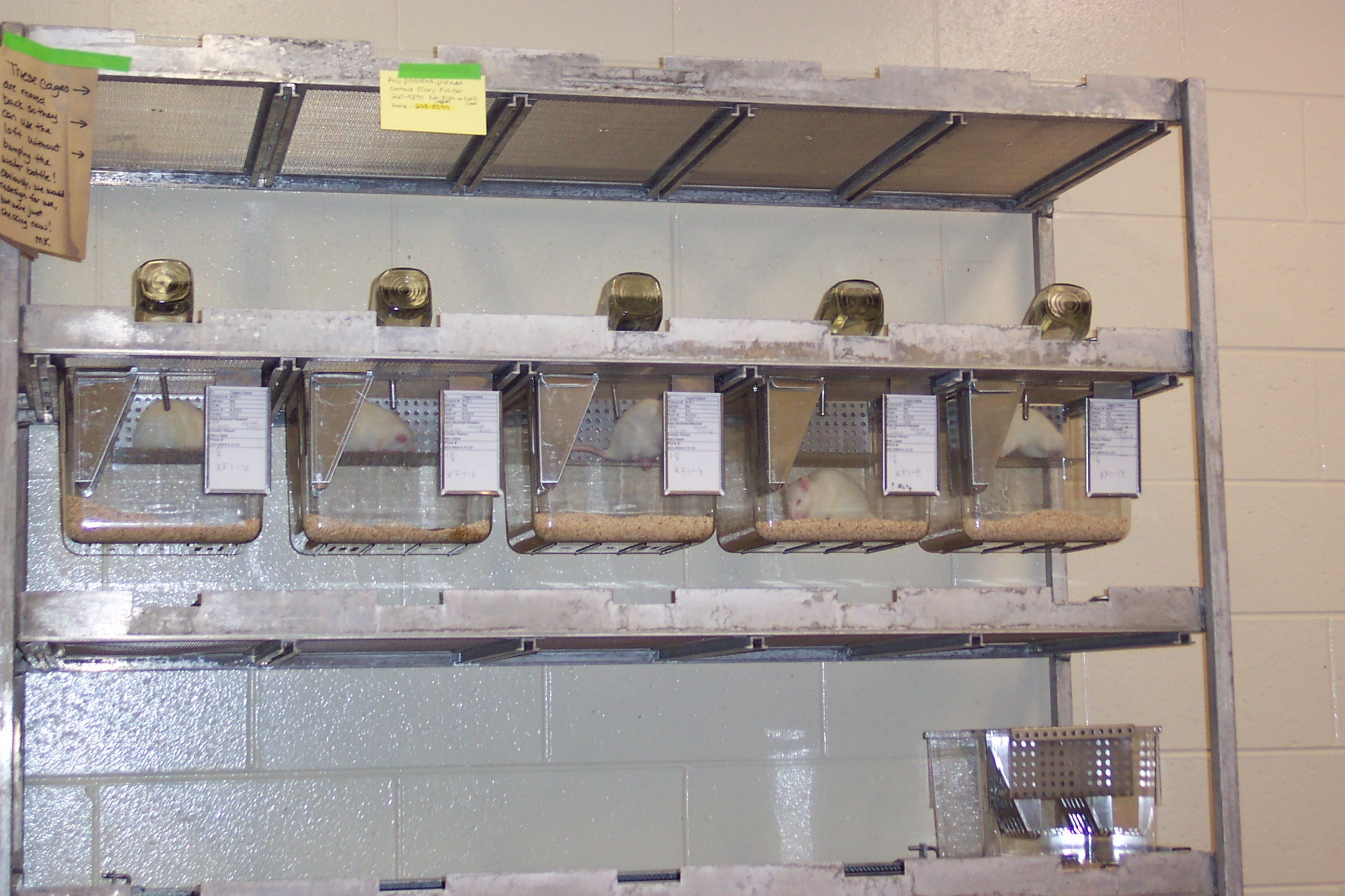
> Mary, talked to two potential producers of the "Rat Loft". I also talked to Jennifer Gottwald from WARF who is following up. In all this I realized that I no longer have the email with the pictures you took of the rats in the wire mesh cages and the shoebox cages on the lofts. Is it possible to send those or similar ones so I can use them? Ben

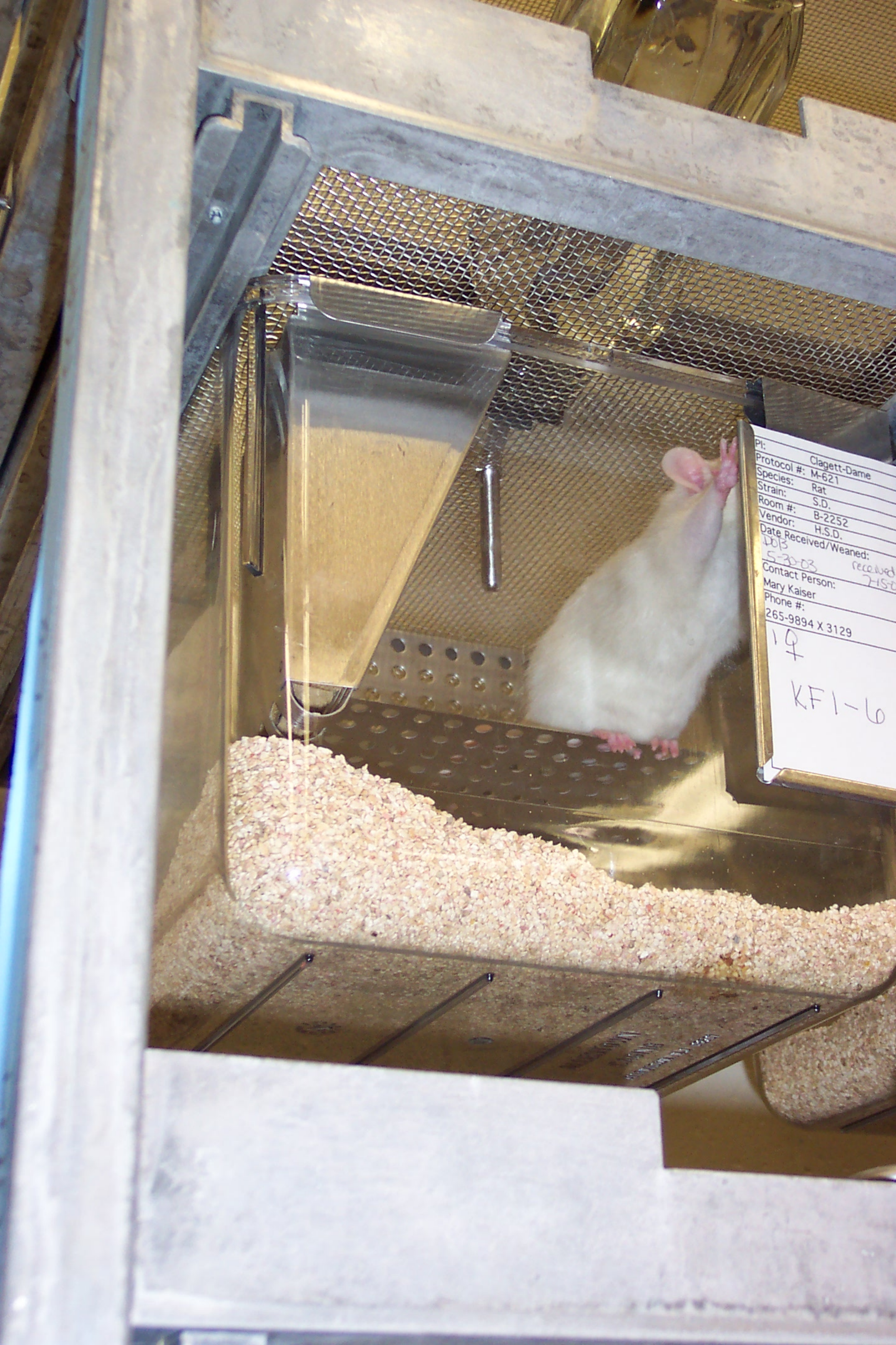


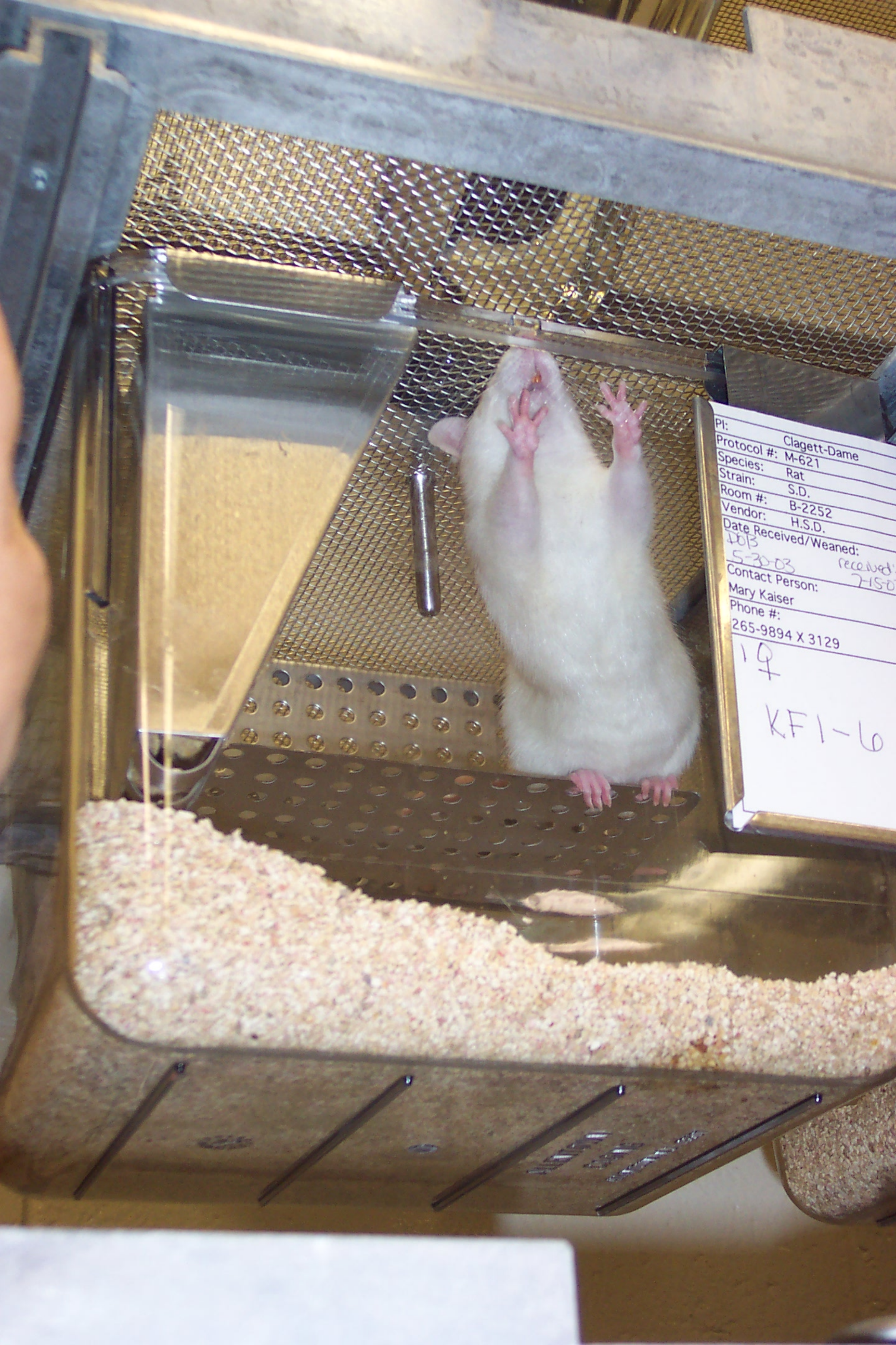

back so they
can use the
loft without
bumping the
water bottle!
Obviously, we would
redesign for use,
but we're just
checking now!
MK


PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
DOB 5-30-03 received 7-15-03
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1♀
KF1-4
PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1♀
KF1-8

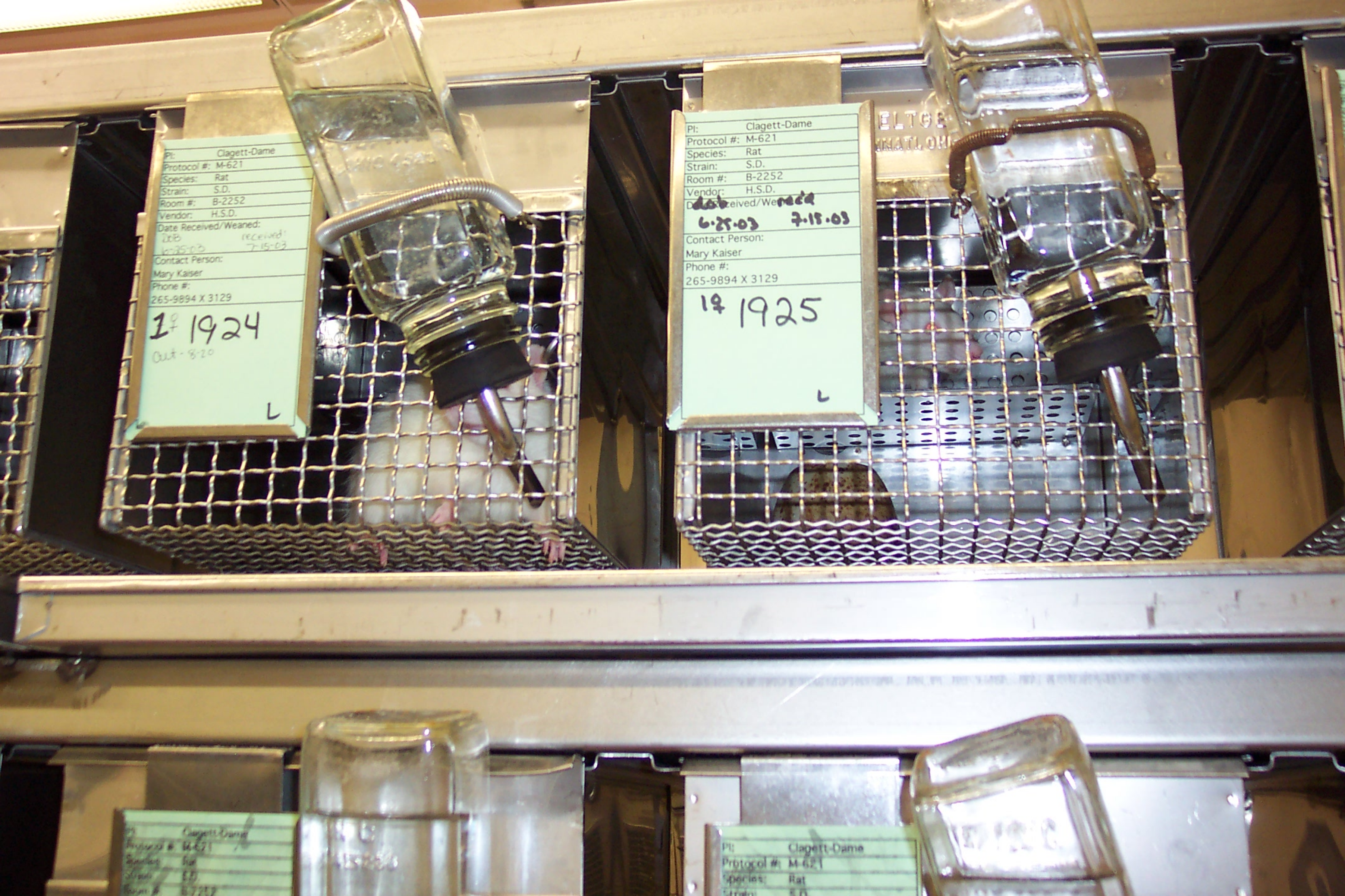

PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1♀
KF1-4
PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1♀
KF1-8


These cages are moved back so they can use the loft without bumping the water bottle! Obviously, we would redesign for real, but we're just checking now!
MK
Any problems, please
contact Mary Kaiser


PI:
Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
DOB
5-30-03
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1♀
KF1-6



PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
DOB
5-30-03
received:
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1♀
KF1-6


PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
Date Received/Weaned:
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1924
L
PI: Clagett-Dame
Protocol #: M-621
Species: Rat
Strain: S.D.
Room #: B-2252
Vendor: H.S.D.
6-25-03
7-15-03
Contact Person:
Mary Kaiser
Phone #:
265-9894 X 3129
1925
L

#3

Scientific Affairs (NIH/OD)

om:	Robert Meyer [meyer@cvm.msstate.edu]
nt:	Monday, November 14, 2005 10:14 AM
:	Scientific Affairs (NIH/OD)
Subject:	RFI No. NOT-OD-06-011
Attachments:	Meyer and Fish Lab An TBE review.pdf; Rollins_Meyer and Morrow Euthanasia.pdf; Teicher22 _Meyer.pdf

Meyer and Fish Lab An TBE revi... Rollins_Meyer and Morrow Eutha... Teicher22_Meyer.pdf (143 KB)

I am submitting 3 publications to be considered in response to the RFI No. NOT-OD-06-011 request for updated information for the Standards of Care and Use of Laboratory Animals:

Appendix A: Anesthesia, Pain and Surgery
Meyer RE, Fish R. A review of tribromoethanol anesthesia for production of genetically engineered mice and rats. Lab Animal (NY, 34(10): 47-52, 2005.

Appendix A: Animal Models and Resources
Meyer RE, Braun RD, Dewhirst MW. Anesthetic considerations for the study of murine tumors. In: Teicher BA, (ed.), Tumor Models in Cancer Research, Totowa NJ, Humana Press, 2002: (Ch. 22, pages 407-431).

Appendix A: Euthanasia
Meyer RE and Morrow WEM. Euthanasia. In: Bernard E. Rollin and G. John Benson ls): Improving the well-being of farm animals: ximizing welfare and minimizing pain and suffering, Ames IA, Blackwell Publishing, 2004: (Ch. 17, pages 351-362).

These are individually attached as pdf files.

Thank you for your consideration. Please let me know if you need addition materials from me.

Robert E. Meyer DVM, DACVA
Dept of Clinical Sciences, Campus Mailstop 9825 College of Veterinary Medicine Mississippi State, MS 39762-6100 Office 662.325.1453; Fax 662.325.4596 Pager 662.325.4224 - 019

"The great aim of education is not knowledge, but action" - Herbert Spencer

"Never confuse movement with action" - Ernest Hemingway

NAME: Robert Meyer/College of Vet. Medicine Mississippi

1. ARTICLE/CONTENT: "Anesthetic considerations for the study of murine tumors"

SOURCE: Meyer, R.E., et al, In: Teicher BA, (ed), Tumor Models in Cancer Research, Totowa NJ, Humana Press, (2002): (Ch. 22 pp 407-431).

2. ARTICLE/CONTENT: "A Review of tribromoethanol anesthesia for production of genetically engineered mice and rats."

SOURCE: Meyer, R.E., Fish, R. (2005). Lab Animal Vol 34 No. 10, pp. 47-52

3. ARTICLE/CONTENT: "Euthanasia"

SOURCE: Meyer, R.E., and Morrow WEM (2004). In: Bernard E. Rollins and G. John Benson (eds): Improving the well-being of farm animals: Maximizing welfare and minimizing pain and suffering. Ames, IA, Blackwell Publishing, Ch. 17, pp 351-362.

#6

BASSETT HEALTHCARE

November 16, 2005

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

Re: RFI No. NOT-OD-06-011

Dear Dr. Snyder,

This correspondence is in regard to the RFI: Standards for the Card and Use of Laboratory Animals, dated November 9, 2005. Please find attached several of our recent publications pertaining to environmental light contamination at night in the animal facility. This work has also been presented at Annual Meetings of the American Association for Cancer Research, the Federation of the American Societies for Experimental Biology, the American Association for Laboratory Animal Science, the CIE International Commission on Illumination, and the International Dark Skies Society.

Our laboratory, the Laboratory of Chrononeuroendocrine Oncology, is located at the Bassett Research Institute in Cooperstown, NY, and has been a NIH awardee laboratory over the past several years. The Director of our laboratory is David E. Blask, Ph.D., M.D. (e-mail: david.blask@bassett.org & dblask@usa.net). Our work demonstrated that environmental light contamination at night in rodent facilities stimulates tumor growth and metabolism in both rodent and human tumor xenograft models. We feel our work to be pertinent to the development of ongoing and improved guidelines in *The Guide* pertaining to animal room illumination, particularly during the dark phase.

With kind regards,

Robert T. Dauchy, Manager
Laboratory of Chrononeuroendocrine Oncology
Tel. #607.547.3958

NAME: Robert Meyer/College of Vet. Medicine Mississippi

1. ARTICLE/CONTENT: "Anesthetic considerations for the study of murine tumors"

SOURCE: Meyer, R.E., et al, In: Teicher BA, (ed), Tumor Models in Cancer Research, Totowa NJ, Humana Press, (2002): (Ch. 22 pp 407-431).

2. ARTICLE/CONTENT: "A Review of tribromoethanol anesthesia for production of genetically engineered mice and rats."

SOURCE: Meyer, R.E., Fish, R. (2005). Lab Animal Vol 34 No. 10, pp. 47-52

3. ARTICLE/CONTENT: "Euthanasia"

SOURCE: Meyer, R.E., and Morrow WEM (2004). In: Bernard E. Rollins and G. John Benson (eds): Improving the well-being of farm animals: Maximizing welfare and minimizing pain and suffering. Ames, IA, Blackwell Publishing, Ch. 17, pp 351-362.

#S

Scientific Affairs (NIH/OD)

From: Pritt, Stacy [stacy.pritt@covance.com]
Sent: Tuesday, November 22, 2005 4:14 PM
To: Scientific Affairs (NIH/OD)
Subject: RFI No. NOT-OD-06-011
Attachments: TrainDoc.pdf

Dear Dr. Snyder,

In response to the Request for Information on the Standards for the Care and Use of Laboratory Animals, I have attached an article I wrote in 2004 giving more specific information about laboratory animal care and use training. The specific needs for training in laboratory animal care programs have become more defined and rigorous since the 1996 edition of the *Guide* was published. This information fits in with the Technical and Professional Education category in Appendix A of the 1996 edition of the *Guide.*

Sincerely,

Stacy

Stacy Pritt, DVM, MBA
Director, Regulatory Operations
Covance Research Products, Inc.
PO Box 7200
Denver, PA 17517
(Phone) 717.336.4921 ext. 225
(Fax) 717.336.5344
stacy.pritt@covance.com

NAME:	Stacy Pritt/Covance Research Prod., Inc.
ARTICLE/CONTENT:	"Creating a comprehensive Training Documentation Program"
SOURCE:	Pritt, S., et al. (2004) Lab Animal, Vol 33 No. 4 pp 38-41

Scientific Affairs (NIH/OD)

\# 9

From: Mcglone, John [john.mcglone@ttu.edu]
'nt: Friday, December 30, 2005 10:03 AM
: Scientific Affairs (NIH/OD)
ubject: RFI No. NOT-OD-06-011

Attachments: Space needs of pigs JAS review.pdf

Space needs of pigs JAS review...

I am sending information in reference to the request for new information relative to laboratory animal needs in announcement RFI No. NOT-OD-06-011. Earlier I send some information on new information about space requirements of mice. We recently published a literature review, summary and analysis of space needs for pigs. The attached 2006 reprint will provide details that more closely support the space needs in the FASS 1999 (Ag Guide) than the ILAR 1996 laboratory animal Guide. The information in this recently-published review is certainly one substantial piece of information that is new since the 1996 ILAR Guide.

Thank you.

John J. McGlone, PhD
Professor
Texas Tech University
806-742-2805, ext. 246
john.mcglone@ttu.edu

NAME:	John McGlone/Texas Tech University
ARTICLE/CONTENT:	"Application of broken-line analysis to assess floor space requirements of nursery and grower-finisher pigs expressed on an allometric basis"
SOURCE:	Gonyou, H.W., et al. (2006) J. Animal Science Vol. 84: pp 229-235

#/C

Battelle
The Business *of* Innovation

505 King Avenue
Columbus, Ohio 43201-2693
(614) 424-6424 Fax (614) 424-5263

January 16, 2006

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184 , MSC 7983
Bethesda , MD 20892-7983

Dear Dr. Snyder,

In response to RFI No. NOT-OD-06-011, enclosed are 3 copies of an article I authored that was published in a peer-reviewed scientific journal in 2001. It is relevant to the housing of rodents. Here is the citation: Effects of Caging Type and Animal Source on the Development of Foot Lesions in Sprague Dawley Rats (*Rattus norvegicus*), Contemporary Topics in Laboratory Animal Science, Volume 40, Issue 5, September 2001.

Thank you for the opportunity to provide input.

Sincerely,

Tracy A. Peace, DVM, MS, DACLAM
Attending Veterinarian
Battelle Memorial Institute
505 King Avenue, Room 7-1-20
Columbus, OH 43201-2693
Phone (614) 424-3140
Fax (614) 458-3140
peacet@battelle.org

Enclosures

BATTELLE
75 YEARS OF INNOVATION

NAME:	Tracey Peace/Battelle Memorial Inst.
ARTICLE/CONTENT:	"Effects of Caging Type and Animal Source on the Development of Foot Lesions in Sprague Dawley Rats (Rattus norvegicus)"
SOURCE:	Peace, T.A, (2001) Contemporary Topics Vol. 40 No. 5 pp 17-21.

11

Scientific Affairs (NIH/OD)

From: Engin Ozertugrul (e-mail)
nt: Saturday, January 21, 2006 12:15 PM
: Scientific Affairs (NIH/OD)
Subject: Notice Number: NOT-OD-06-011

Attachments: Response to NIH request.doc

Response to NIH request.doc (3...

Thank you for the opportunity to comment on a possible update to the Guide. In the attachment you will find my comments and recommendations.

Note : These comments reflect my personal opinions and are not necessarily shared by any institution.

Sincerely,

Engin Ozertugrul

NAME: Engin Ozertugrul

ARTICLE/CONTENT: Recommendations to the 1996 Guide

SOURCE: Institutional policies and responsibilities

REF: Institutional policies and responsibilities

Inimical activist agenda is placing great pressure on the research enterprise. It is time to consider radical changes in Institutional policies and responsibilities.

REASONS:

Extending to early 1980's researchers did not loose the battle against increasingly demanding public moral concerns; they simply never went to war. Rising public moral issues or ethical concerns that vitally related to "pain", "fear", "anxiety", "boredom" researches were vastly rejected in the account of being 'non-scientific" or "irrelevant". However, this ideology was at a loss to respond to questions like "If they are totally disanalogous, why do research on them?" Scientists have never been formally educated or trained on animal ethics or moral issues, therefore initial response to expanding public queries was to avoid the issue altogether. In the outcome, scientist anti –animal activist propaganda were merely focused on the benefits of researches or when it is unavoidable, most of the energy and time consumed on the premises of dissimilarities of "pain", "fear", "anxiety" and etc. between animals and humans. As a result researchers were loosing the battle that they had refused to engage on the fields of animal ethics.

Mid 80's witnessed dramatic changes in animal research. Beginning in 1984, animal pain and its control had become a major topic in veterinary and laboratory animal journals, after years of silence. In June 1985 issue, Lab Animal published an article entitled 'Animal Pain: evaluation and control'. In here, contrary to orthodox practice, the authors were offering to give the benefit of doubt to animals on the usage of analgesia even if pain is uncertain.

Ironically, this remarkable leap on the scientist's ideology was not empirical as one might expect. It was solely philosophical and valuational. Researchers such brave and pioneering efforts were the first signs of moral basis of this shift, and initiated a new era in animal science. This breakthrough manifested it self in expanding articles and text books.

To many, ethics is nothing more than a statement of a personal opinion but so do the other social values which are enforced as laws when society is ready for them. Subjective value of ethics do not postulate that one can not educate professionals, beginning with the high school science student, to develop skills that promote sound analysis and effective decision making in social and public policy.

Research scientists must do their part. To implant and to establish a stable, coherent and pragmatic animal ethics is essential to prevent backsliding to old days in animal pain and pain control issues.

RECOMMENDATIONS:

Starting from large institutions, the possibility to establish full time 'in-house public relations offices' might be worthy of further consideration. This can be done by extending IACUC "non-affiliated member" enforcement further to constitute a more coherent and interactive relation with the public and the individual institutions. The duties and construction of such offices may include but not limited to:

A-Designate and train in-house spokespersons for internal and external audiences

B- Organize educational public outreach programs and opinion polls in the "defined" local area

C- Establish incident response teams

D- Have detailed plans of action in case of emergency

E- Organize management for crisis communication process

F- Informal Participation of IACUC protocol reviews

Prior to fabrication of such a unit, it is worthwhile to assign a number of values to each criterion (similar to suggested A-F) after careful consideration of applicability of the proposed items. This can be best achieved by the IACUC members that are already in force and in contact with institution's local environment.

It is critical not to hire research professionals for such internal unit (except in-house trainer) to increase public participation in the regulatory process by having them as informal participants in IACUC protocol review meetings.

Depending on the threats of extremist animal activists and their specific targets, it is understandable that the willingness of institutions to provide public information about their use of animals may vary. However, this suggested in-house public department might, if carefully organized, help to allay public fears and suspicions about what goes on behind 'closed doors' of such institutions. In public eye, this kind of reassurance may be perceived more trustworthy than AAALAC and USDA assurance because of its "seeing is believing" value. The scientist/ public communication has usually been only one way, from the scientist to the public which impairs the true two-way dialog. The existence of public representatives in research facilities enable researchers listen to the public directly. This is very important because face to face contact is the most effective way to find out what public thinks and feels about research issues. Furthermore, the quality and dimensions of scientists' message seems to be much higher than the activists' plain emotion based propaganda. The simplicity of activists' message allows powerful communication through the internet by turning animal rights movement to a global threat. It is clear that scientist' propaganda is likely to work on a local basis to establish a coherent and continuing communication with the public due to its multidimensional complex identity.

A further possibility would be the inclusion of the in- house public office and its educational activities to NIH grant application scoring system to ensure the availability of funds for eligible institutions. This can be done as analogous to NIH favor in honoring grants to AAALAC accredited facilities.

Finally, such a unit can be included in the AAALAC inspections to assure the continuing assessment of education.

In addition, AALAS training programs may institute more comprehensive ethical/moral issues and/ or case scenarios in their certification tests that may lead to more integrant, analytical and morally equipped technicians. Also, mandatory Animal Welfare/Ethics Course is crucial for animal users to ensure high quality animal care.

\# 12

January 26, 2006

Dr. Margaret Snyder,
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184, MSC 7983
Bethesda, MD 20892

Dear Dr. Snyder:

We are pleased to hear of the proposed revision to the Guide for the Care and Use of Laboratory Animals. We would like to offer our assistance in any way possible, specifically in the area of Individually Ventilated Caging (IVC's).

Over the past 39 years, we have used a combination of scientific research, third-party testing and input from our research customers in order to define the parameters we must meet and work between to ensure appropriate animal housing, untainted research and researcher safety.

Outside of the above-mentioned efforts there are no industry parameters for ventilated housing which we can refer to – nor are there common testing protocols to help define appropriately functioning IVC equipment. We feel there is a definite need not only for the creation of industry-wide scientific parameters, but for the setting of defined protocols to test and ensure that the standards are being met.

To that end, we would like to share our extensive experience in all aspects of IVC's – specifically but not limited to the areas of Cage Area, Humidity, Ammonia Levels, CO^2 Levels, Air Exchange Rates, Air Velocity, Noise Levels, Vibrational Levels, Illumination and all other cage-level environmental criteria. We would also like to share our knowledge and research relating to the macro laboratory environment specifically in the areas of allergen control, noise exposure and many other ergonomic issues.

As an example of the data we can share with you, as recently as within the past six months we have commissioned independent Allergen Capture, Acoustical and Vibrational testing of our IVC equipment. Equally as valuable, we would like to share the experience and practical knowledge we've acquired in delivering over 10,000 IVC units worldwide.

In the months ahead I hope we can be of assistance to you and a valuable contributor to your cause. I've enclosed my business card along with this letter. Please feel free to contact me at your convenience. Thank you for your time.

Sincerely,

Michael A. Coiro, Sr.
CEO

CC: John Coiro, President
Vince Pombo, Vice President Sales & Marketing
Brian Bilecki, Director of Airflow Technology

Allentown Caging Equipment Co., Inc.
165 Route 526, P.O. Box 698 Allentown, N.J. 08501-0698 1-800-762-CAGE 609-259-7951 FAX: 609-259-0449

#13

Animal Welfare Institute
Washington, D.C.

Viktor Reinhardt
address
USA
e-mail

January 23, 2206

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge 1, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

Re.: HFI **No. NOT-OD-06-011**

Dear Mrs. Snyder,

on behalf of the Animal Welfare Institute, I am taking the opportunity to submit three copies of suggestions and new information related to the first two chapters of the 1996-*Guide,* hoping that the material will be of some help for the possible update of the ***Guide.*** For your convenience, I am enclosing also a floppy with the Microsoft Word document file.

The citations used in this submission have been found in the free database on Refinement and Environmental Enrichment for All Animals kept in Laboratories:
http://www.awionline.org/lab_animals/biblio/laball.htm

I have focused on articles published between 1996-dato but have also included some older papers addressing basic husbandry issues.

With kind regards,

V. Reinhardt

Viktor Reinhardt, DVM

MONITORING THE CARE AND USE OF ANIMALS

Physical Restraint

"Many dogs, nonhuman primates (..), and other animals can be trained, through use of positive reinforcement, to present limbs or remain immobile for brief procedures." p 11

It may be appropriate to add references also for "dogs" and specify "other animals" as rats (Guhad and Hau, 1996; Huang-Brown and Guhad, 2002), rabbits (Marr et al., 1993) and goats (Lager, 1998). If no reference for "dogs" can be found, dogs should not be included here.

Marr JM, Gnam EC, Calhoun J, Mader JT 1993. A non-stressful alternative to gastric gavage for oral administration of antibiotics in **rabbits**. Lab Animal 22(2), 47-49
"Generally, rabbits receive oral medication by gastric intubation, a method that frequently requires more than one technician, is time consuming, and places unnecessary stress on the animal." A very simple but effective training technique is described which ensures that rabbits voluntarily cooperate during oral drug administration. "We coated the tip of the syringe with sucrose sample. Inserting the syringe through the bars of the cage, we placed it in the animal's mouth and injected the sucrose solution slowly to allow the rabbit to taste and drink the fluid. We repeated the procedure three times a day for a total of 15 minutes per session, and within two days, 80% of the [10] animals voluntarily swallowed the fluid from the syringe. The [2] rabbits that did not seek out the syringe usually took it with only minimal encouragement. At the onset of the therapy, we substituted the antibiotic for the sucrose solution. .... We continued coating the tip of the syringe with sucrose granules throughout the therapy, apparently masking any unpleasant sensations produced by the antibiotic." Eight of the ten rabbits cooperated within two days. They "would stand with their paws on the front of the cages, protrude their faces from between the bars, and appear to beg for the syringe containing the antibiotic [documented with a photo]." This non-stress method of "giving tosufloxacin was successful in producing the desired serum and bone concentrations."

Guhad FA, Hau J 1996. Salivary IgA as a marker of social stress in **rats**. Neuroscience Letters 27, 137-140
"Three groups of adult male rats were housed under different
conditions (singly housed, paired with a female, and group housed). The animals were conditioned for the saliva collection by presenting a chocolate reward after session and saliva was collected by soaking filter paper discs (5 mm in diameter) with saliva directly in the rats' oral cavity."

Huang-Brown KM, Guhad FA 2002. Chocolate, an effective means of oral drug delivery in **rats**. Lab Animal 31(10), 34-36
"We trained the animals to smell the chocolate and develop a taste for it by holding the animal and placing the chocolate into its mouth using a blunt metal applicator (in our project we used a 14-gauge oral gavage needle). No esophageal contact, nor any placement more proximal than the oral cavity was necessary. We handled the rats gently to avoid association of chocolate with averse stimuli. To train the rats to expect the

treatment, we opened and closed the cage before chocolate administration. .. After individual administration and return of the animal to the cage, the caregiver then offered drug-free chocolate as a "reward" at the front of the cage with the blunt end of the gavage needle to condition the animals to this manner of treatment. ...Results from this technique demonstrated appropriate levels of drug absorption. .. The animals do not require individual housing, enhancing their social environment and reducing space usage. .. Housing the animals used for this study in groups of three per cage allowed for easy identification of animals as they received their chocolate pellets. ...The chocolate vehicle is ideal for timed delivery or when a drug needs to be administered at a certain time of day. .. In this study, 3 of 57 rats (5%) failed to become accustomed to the chocolate even after the training period and had to be restrained for drug administration."

Lager K 1998. Apparatus and technique for conditioning **goats** to repeated blood collection. Lab Animal 27(3), 38-42
"We also developed an effective reward-based conditioning program to promote restraint tolerance and voluntary entry into the restraint apparatus. We had previous success conditioning swine for repeated blood collection using cookies as a reward, and implemented a similar system for the goats. Using the modified 'goat crate' and our novel conditioning protocol, we were able to safely and efficiently collect any volume of blood, largely due the ready cooperation of the goats."

I would also recommend to add more recent references for "primates", such as: Friscino et al., 2003; McKinley et al., 2003; Tiefenbacher et al., 2003; Perlman et al., 2004; Down et al., 2005; Schapiro et al., 2005; Videan et al., 2005a,b.

Friscino BH, Gai CL, Kulick AA, Donnelly MJ, Rockar RA, Aderson LC, Iliff SA 2003. Positive reinforcement training as a refinement of a macaque biliary diversion model. AALAS [American Association for Laboratory Animal Science] 54th National Meeting Official Program, 101 (Abstract)
"Animals that adapted to wearing jackets were surgically implanted with a biliary diversion cannula system, a venous cannula and three subcutaneous access ports. .. The animals [three females and nine male rhesus] were trained to present the pouch and to remain stationary while the catheters were accessed. The length of time required for training was variable between individuals, but generally required three to four training sessions during a two-week period. These in-cage procedures precluded the need for chair or manual restraint of animals during sample collection. Instead, positive reinforcement was used to reward the animals with food for their cooperation during sample collection. This has also increased the efficiency of conducing metabolic studies and minimized the potential stress of sample collection for both the personnel and animals."

McKinley J, Buchanan-Smith HM, Bassett L, Morris K 2003. Training common marmosets (*Callithrix jacchus*) to cooperate during routine laboratory procedures: Ease of training and time investment. Journal of Applied Animal Welfare Science 6, 209-220
Behaviours taught were target training to allow in homecage weighing and providing urine samples from 12 pairs of marmosets. "Between 2 to 13, 10-minute training sessions established desired behaviors. .. Trained animals proved extremely reliable, and data

collection using trained animals was considerably faster than collection using current laboratory techniques."

Tiefenbacher S, Lee B, Meyer JS, Spealman RD 2003. Noninvasive technique for the repeated sampling of salivary free cortisol in awake, unrestrained squirrel monkeys. American Journal of Primatology 60, 69-75
"Individually housed adult male squirrel monkeys were trained to chew on dental rope attached to a pole, from which saliva was extracted by centrifugation and analyzed for cortisol. ... Eight of nine monkeys readily acquired the task, reliably providing adequate saliva samples for the assay. ... The described sampling technique provides a reliable and sensitive means for repeated measurement of HPA activity in unrestrained, awake squirrel monkeys."

Perlman JE, Thiele E, Whittaker MA, Lambeth SP, Schapiro SJ 2004. Training chimpanzees to accept subcutaneous injections using positive reinforcement training techniques. American Journal of Primatology 62(Supplement), 96 (Abstract)
"Positive reinforcement training techniques were used to train four socially-housed, adult chimpanzees to present their abdomen for a subcutaneous injection. .. Voluntary cooperation with the injection procedure was desired to eliminate the need for chemical restraint and to minimize stress on the subjects and caregivers. Subjects had been previously trained to present body parts for inspection, including the abdomen. For the present study, subjects were trained to 1) present the abdomen, 2) tolerate a pinch of the skin, 3) accept the subcutaneous insertion of a needle, and 4) remain stationary while the contents of the syringe were injected. Three of the four chimpanzees were reliably trained to voluntarily accept the subcutaneous injection. A mean of 98 minutes of training time was required for the animals to reliably accept penetration and injection of up to 10 cc through a 25-gauge needle. Training sessions lasted 5 to 8 minutes and 13 - 20 sessions (mean = 17) were required to achieve reliable performance."

Schapiro SJ, Perlman JE, Thiele E, Lambeth S 2005. Training nonhuman primates to perform behaviors useful in biomedical research. Lab Animal 34(5), 37-42
Training protocols are described and the time investment to achieve cooperation is presented.

Down N, Skoumbourdis E, Walsh M, Francis R, Buckmaster C, Reinhardt V 2005. Pole-and-collar training: A disucssion by the Laboratory Animal Refinement and Enrichment Forum. Animal Technology and Welfare 4, 157-161
http://www.awionline.org/Lab_animals/biblio/atw7.html
Experiences with the pole-and-collar training training are shared. "Yes, most monkeys can be trained but some cannot, or let's say they should not be trained because their personality - which is presumably conditioned through negative experiences with people - is very difficult to deal with."

Videan EN, Fritz J, Murphy J, Borman R, Smith HF, Howell S 2005a. Training captive chimpanzees to cooperate for an anesthetic injection. Lab Animal 34(5), 43-48
Training protocol is described in detail and the time investment presented.

Videan EN, Fritz J, Murphy J, Howell S, Heward CB 2005b. Does training chimpanzees to present for injection lead to reduced stress? Laboratory Primate Newsletter 44(3), 1-2 http://www.brown.edu/Research/Primate/lpn44-3.html#videan
"Subjects were 17 captive chimpanzees living at the Primate Foundation of Arizona, aged 10.6 to 34.5 years at the time of the study. The sample included 8 males and 9 females. Eleven of the subjects were trained, using positive reinforcement techniques, over 21 months (Videan et al., 2005). Individuals were trained to present an arm or leg to the cage mesh for anesthetic injection, using the verbal cues "arm" and "leg". Training procedures were transferred from the trainer to either the colony manager or the assistant colony manager, after behaviors were under stimulus control, in 5 of the trained subjects. .. When all trained individuals were pooled, trained subjects exhibited significantly lower levels of cortisol than untrained ($U=7$, $p<0.010$, *Table 1*)."

ANIMAL ENVIRONMENT, HOUSING, AND MANAGEMENT

"The availability or suitability of enrichment" .. "should be considered in planning for adequate and appropriate physical .. environment." p 21

Experience shows that the *suitability* of enrichment is generally not considered before funds are invested to purchase enrichment objects/gadgets. It may be appropriate to emphasize that the suitability [effectiveness and safety] of an enrichment option should be (a) either tested before it is implemented or (b) verified in the published literature. The published literature can be checked on the Internet in the free annotated database on Refinement and Environmental Enrichment for All Animals kept in Laboratories: http://www.awionline.org/lab_animals/biblio/laball.htm

"Animals should be housed with the goal of maximizing species-specific behaviors and minimizing stress-induced behaviors." p 22

It may be indicated to replace *species-specific* with *species-adequate* or *species-appropriate* behaviors, because we want to minimize some species-specific behaviors in confined animals — for example injurious fighting — or control some species-specific behaviors — for example copulation.

It could help readers to have the *selected publications* on enrichment strategies [page 87] updated and some important, data-supported examples mentioned in the chapter Structural Environment on page 37-38.

Callard MD, Bursten SN, Price EO 2000. Repetitive backflipping behaviour in captive roof **rats** *(Rattus rattus)* and the effect of cage enrichment. Animal Welfare 9, 139-152 "Repetitive stereotyped behaviours are often performed by both wild and domestic rodents in small laboratory cages. In this study, a behaviour resembling a backwards somersault or backflip is described and quantified in captive roof rats (ship or black rats, *Rattus rattus)*. ... Cage enrichment in the form of a wooden nest box resulted in dramatically lower rates of performance. Increased cage height resulted in delayed development of backflipping, as well as changes in the form of the behaviour. Results are consistent with the hypothesis that the development and expression of backflipping in young roof rats may be triggered by weaning and maintained by a heightened state of arousal in a relatively impoverished environment with limited opportunities for perceptual and locomotor stimulation."

Belz EE, Kennell JS, Czambel RK, Rubin RT, Rhodes ME 2003. Environmental enrichment lowers stress-responsive hormones in singly housed male and female **rats**. Pharmacology Biochemistry and Behavior 76, 481-486

"This study examined the physiological effects of environmental enrichment (EE) with Kong Toys and Nestlets on stress-responsive hormones of the hypothalamic-pituitary-adrenal (HPA) axis under basal and mild stress conditions in singly housed, jugular vein-cannulated, male and female rats. Animals of both sexes housed with EE had significantly lower baseline adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations compared to those housed without EE. ACTH responses to the mild stress of saline injection were significantly lower in female rats housed with EE. Interaction with the Kong Toys and Nestlets appears to have provided the rats with a diversion from monotonous cage life, resulting in lower HPA axis activity before and after mild stress. These results are important because low, stable baselines are essential for accurately discerning pharmacological and other influences on the HPA axis."

Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, Yaniv I, Pick CG 2004. Environmental enrichment in **mice** decreases anxiety, attenuates stress responses and enhances natural killer cell activity. European Journal of Neuroscience 20, 1341-1347
"We investigated the effect of EE on natural killer (NK) cell activity, psychological stress responses and behavioural parameters. [Groups of] male C3H mice were housed either in enriched [ladders, tunnels, running wheel] or standard conditions for 6 weeks. Behaviour was then examined by the grip-strength test, staircase and elevated plus maze, and corticosterone levels and NK cell activity were measured. Furthermore, animals exposed to the stress paradigm, achieved by electric shock with reminders, were tested for freezing time in each reminder. Corticosterone levels were also measured. The EE mice showed decreased anxiety-like behaviour and higher activity compared to standard mice, as revealed by a greater percentage of time spent in the open arms of the elevated plus maze, and a higher rate of climbing the staircase. A shorter freezing time in the stress paradigm and no corticosterone level reactivity were measured in EE mice. In addition, NK cell activity in spleens of EE mice was higher than that demonstrated in those of standard mice. Thus, EE has a beneficial effect on anxiety-like behaviour, stress response and NK cell activity. The effect on NK cell activity is promising, due to the role of NK cells in host resistance."

Coviello-Mclaughlin GM, Starr SJ 1997. Rodent enrichment devices - evaluation of preference and efficacy . Contemporary Topics in Laboratory Animal Science 36(6), 66-68
The **mice** preferred cotton nestles and cardboard rolls over wooden block and commercial toys. "When animals wearing wound clips were exposed to the preferred enrichment, premature wound clip removal decreased, suggesting a positive effect of enrichment on the psychological well-being of surgically manipulated mice."

Würbel H, Chapman R, Rutland C 1998. Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory **mice**. Applied Animal Behaviour Science 60, 69-81
Enrichment significantly reduced stereotypic wire-gnawing in pair-housed male mice by 40%, presumably as a consequence of the cover provided by the cardboard tubes. This is substantiated by observations that the tubes were used as a place to retreat upon

disturbance as well as for resting. As a consequence the animals showed more resting and less grooming in cages containing a cardboard tube-shelter.

Smith GD, Hoffman WP, Lee EM, Young JK 2000. Improving the environment of **mice** by using synthetic gauze pads . Contemporary Topics in Laboratory Animal Science 39(6), 51-53
"The mice with gauze pads preferred to rest on them. In addition, these mice showed a statistically significant reduction in food consumption, but their body weights and weight gains did not differ from those of animals without gauze pads."

McClure DE, Thomson JI 1992. Cage enrichment for **hamsters** housed in suspended wire cages. Contemporary Topics in Laboratory Animal Science 31(4), 33 (Abstract)
"Golden Syrian hamsters (n=99) were housed individually in suspended wire cages so that spilled food and excreta could be removed. After 8 days, the hamsters developed bizarre aggressive behavior which consisted of growling, hissing, aggressive posturing toward humans, destruction of water bottle rubber stoppers, and attacking objects introduced into the cage. Many developed inappetence which progressed to anorexia, depression, and unresponsiveness. ... When cotton nestlets were provided to all of the hamsters, their appetite and responsiveness improved, but the aggressive behavior remained unchanged. The nestlets were replaced by a 13-cm length of 5.5-cm-diameter polyvinyl chloride pipe (PVC). The water bottles were replaced by an automatic watering system. After adding the PVC, the aggressive behavior diminished in 3 days and was unnoticeable in 14 days. In conclusion, when these hamsters were provided with nesting material their well-being was improved as indicated by resolution of inappetence and depression. Providing the PVC apparently resolved the aggressive behavior problem by providing a means for seclusion in addition to functioning as a burrow and as a toy."

Arnold CE, Westbrook RD 1997/1998. Enrichment in group-housed laboratory golden **hamsters**. Animal Welfare Information Center (AWIC) Newsletter 8(3/4), 22-24
http://www.nal.usda.gov/awic/newsletters/v8n3/8n3arnol.htm
"Enriched hamsters [four same-sexed animals per group] showed varied behavior and less aggression toward their cagemates. The hamsters preferred jars to pipes" probably "because the jars' greater height, as compared to pipes, made it easy to look outside the cage."

Reebs SG, St-Onge P 2005. Running wheel choice by Syrian **hamsters**. Laboratory Animals 4, 442-451
"The hamsters did not express a preference when offered a choice of a running surface made of metal rods spaced 9 mm apart and a similar running surface covered with plastic mesh to prevent the possible stippage of between the rods. The hamsters did express a clear preference for larger wheels (35 versus 23 cm diameter), and for completely circular wheels over truncated ones.

Waiblinger E, König B 2004. Refinement of **gerbil** housing and husbandry in the laboratory. ATLA (Alternatives to Laboratory Animals) 32(Supplement), 163-169

http://www.worldcongress.net/2002/proceedings/B2%20Waiblinger.pdf
An artificial burrow system is described that prevents the development of stereotypic digging.

Banjanin S, Barley J, Bell L, Cunneen M, Johnston I, Quintero I, Weilemann R, Reinhardt V 2004. Environmental enrichment for **guinea pigs**: A Discussion by the Laboratory Animal Refinement & Enrichment Forum. Animal Technology and Welfare 3, 161-163
http://www.awionline.org/Lab_animals/biblio/atw5.html
"In summary, social-housing is the most species-appropriate living environment for guinea pigs. If a research protocol requires single-caging, guinea pigs should always be able to maintain visual, auditory and olfactory contact with other guinea pigs to buffer the stress of social deprivation. The provision of PVC tubing or, preferably rectangular boxes, addresses the animals' strong need for a covered shelter. Autoclaved hay or straw offers optimal environmental enrichment. This material can readily be presented in such a way that the animals have to work for its retrieval, i.e., engage in foraging activities."

Lidfors L 1997. Behavioural effects of environmental enrichment for individually caged **rabbits**. Applied Animal Behaviour Science 52, 157-169
Hay was more effective than grass-cubes, sticks, and a box [rat cage] in reducing behavioral disorders and giving individually housed male rabbits something to do. The hay was placed in empty water bottles to "make it a more lengthy task for the rabbits to pull the straws out. .... The wood [of gnawing sticks] came from peeled aspen [not from fir; cf. Brooks et al., 1993], and maybe the type of wood influences the amount of interest the rabbits show. It is a general idea at some animal facilities that rabbits need gnawing sticks to prevent getting their teeth too long."

Potter MP, Borkowski GL 1998. Apparent psychogenic polydipsia and secondary polyuria in laboratory-housed New Zealand White **rabbits**. Contemporary Topics in Laboratory Animal Science 37, 87-89
Three single-caged rabbits with psychogenic polydipsia [excessive drinking without apparent physiological reason] were given toys for cage enrichment, "and the abnormal behavior decreased in all three cases."

Berthelsen H, Hansen LT 1999. The effect of hay on the behaviour of caged **rabbits** *(Oryctolagus cuniculus)*. Animal Welfare 8, 149-157
"When hay was available [placed on top of cage], the [single-caged] rabbits ... performed significantly less bar gnawing and excessive grooming" and were less restless. "This suggests that rabbits kept in cages where hay is available are less stressed than those kept in cages where it is not." When kept in otherwise barren cages, rabbits interacted with the hay 16% of one-hour observation sessions.

Krohn TC, Ritskes-Hoitinga J, Svendsen P 1999. The effect of feeding and housing on the behaviour of the laboratory **rabbit**. Laboratory Animals 33, 101-107
"Feeding the animals at 14:00 h [wild rabbits forage primarily late in the afternoon and during the night!] reduced abnormal behaviour during the dark period compared to feeding at 08:00 h. ... While the [individually housed] rabbits in cages spent 2-5% of the

time performing abnormal behaviour like biting the bars or scratching the bottom of the cage, these activities were virtually absent in group-housed rabbits in floor pens."

Hansen LT, Berthelsen H 2000. The effect of environmental enrichment on the behaviour of caged **rabbits** *(Oryctolagus cuniculus)* . Applied Animal Behaviour Science 68, 163-178
Rabbits kept in conventional single-cages, showed more restlessness, excessive grooming, bar-gnawing and timidity than rabbits kept in cages that were provisioned with a platform and a shelter. "Only a few rabbits, particularly the females, used the box as a shelter or resting-place. On the other hand, they more often used the roof of the box as a look-out or resting-place."

Harris LD, Custer LB, Soranaka ET, Burge R, Ruble GR 2001. Evaluation of objects and food for environmental enrichment of NZW **rabbits**. Contemporary Topics in Laboratory Animal Science 40(1), 27-30
"Male and female 6-week old New Zealand White rabbits were divided into three groups: food-enriched (Bunny Stix, Bunny Blocks, or celery), non-food enriched (Jingle Ball, Kong toy, or Nylabone), and not enriched. ... Rabbits spent significantly more time interacting with the Bunny Stix than any other food item or non-food object. In addition, total activity time was significantly greater for all rabbits enriched with food versus any of the non-food items."

Johnson CA, Pallozzi WA, Geiger L, Szumiloski JL, Castiglia L, Dahl NP, Destefano JA, Pratt SJ, Hall SJ, Beare CM, Gallagher M, Klein HJ 2003. The effect of an environmental enrichment device on individually caged **rabbits** in a safety assessment facility. Contemporary Topics in Laboratory Animal Science 42(5), 27-30
"Our study supports previous findings that interaction with enrichment devices decreases over time, thus indicating the need for frequent rotation of different enrichment devices. In addition, no adverse effects of the analyzed parameters were found, indicating that stainless-steel rabbit rattles on spring clips are suitable devices for safety assessment studies, in which the introduction of new variables is often unacceptable."

De Monte M., Le Pape G 1997. Behavioural effects of cage enrichment in single-caged adult **cats**. Animal Welfare 6, 53-66
"A loss of interest in objects [tennis ball suspended 12 cm above the floor; 12 cm diameter x 40 cm long wooden log hooked against the wall] over time was observed. On the fifth day after the introduction, [single-caged] cats spent only 3 per cent of their time using the log, and 10 per cent using the ball."

Eisele P 2001. A practical dog bed for environmental enrichment for geriatric beagles, with applications for puppies and other small **dogs**. Contemporary Topics in Laboratory Animal Science 40(3), 36-38
"The dogs were initially housed in kennel runs equipped with elevated benches, but it became apparent that some of the oldest animals had difficulties jumping down from them. To improve animal safety and comfort, practical dog beds were made out of the ends of clean high-density polyethylene barrels. Synthetic fleece bed liners were used for

dogs that did not chew them or remove them from the beds. Nine of the beagles regularly were observed to use the beds."

Kilcullen-Steiner C, Mitchell A 2001. Quiet those barking **dogs**. AALAS [American Association for Laboratory Animal Science] 52st National Meeting Official Program, 103 (Abstract)
"A 'white noise' stereo system was used, along with new age music, to effectively decrease the amount and intensity of the barking dogs." [Abstract also published in *Contemporary Topics in Laboratory Animal Science* 40(4), 91, 2001]

Rukavina GM, Young JD, Grant MG 2002. Using a toy rotation scheme to enhance canine enrichment. Contemporary Topics in Laboratory Animal Science 41(4), 118 (Abstract)
"Our approach was to design a toy rotation scheme that would provide each **dog** with a different toy on a weekly basis. .. At the end of a 2-month evaluation period, only 4 out of the 9 toys (Dental Ball, Dumbbell, Havaball, and Kong) were completely successful at meeting our standards ... while also providing lasting appeal to our canine population."

Wells DL 2004. The influence of toys on the behaviour and welfare of kenneled **dogs**. Animal Welfare 13, 367-373
"Enrichment through the provision of toys may have a positive effect upon the welfare of sheltered dogs, helping to reduce boredom. ... The dogs' interest in the toys waned over time, but the speed of habituation to the Nylabone chew was slower than to" the tug rope, Boomer ball, squeaky ball, non-squeaky ball.

Graham L, Wells DL, Hepper PG 2005. The influence of visual stimulation on the behaviour of **dogs** housed in a rescue shelter. Animal Welfare 14, 143-148
"The dogs in this investigation directed relatively little attention towards the television monitors and habituated to their presence within a short period of time."

Kessel AL, Brent L 1996. Space utilization by captive-born **baboons** (*Papio* sp.) before and after provision of structural enrichment. Animal Welfare 5, 37-44
http://www.awionline.org/Lab_animals/biblio/aw5-37.htm
'The addition of the new structures [ladder, suspended 55-gallon drums] changed the space use patterns of the [group-housed] females the most, with decreases in the use of the floor, bench and wire areas."
"Infants in this study were found on the swinging barrel more than the adults, who used the non-movable structures more."

Brent L, Belik M 1997. The response of group-housed **baboons** to three enrichment toys. Laboratory Animals 31, 81-85
"Abnormal, cage-directed, inactive and self-directed behaviours all significantly decreased after the [simultaneous] provision of the toys."

Brent L, Stone AM 1998. Destructible toys as enrichment for captive **chimpanzees**. Journal of Applied Animal Welfare Science 1, 5-14

http://www.psyeta.org/jaaws/abv1n1.html
Nine singly caged chimpanzees were provided with eight different toys made of plastic, vinyl, or cloth one at a time or several at once. The toys remained in the cages an average of three days. "The chimpanzees varied greatly in their interest in the toys. One subject rarely contacted the toys and others used them a great deal and quickly destroyed them." It was concluded "that the provision of flexible, inexpensive toys one at a time can be an effective method of enrichment for captive chimpanzees."

Howell S, Schwandt M, Fritz J, Roeder E, Nelson C 2003. A stereo music system as environmental enrichment for captive **chimpanzees**. Lab Animal 32(10), 31-36
"Music was associated with a significant decrease in agitated/aggression and active/explore behaviors during the AM hours [feeding and cleaning time]. .. At these times, we suggest music can be beneficial as an environmental enrichment. When colony activities are relatively low, however, we suggest music may not be an effective environmental enrichment because it may result in decreased activity levels."

Videan EN, Fritz J, Schwandt ML, Smith HF, Howell S 2005. Controllability in environmental enrichment for captive **chimpanzees** (*Pan troglodytes*). Journal of Applied Animal Welfare Science 8, 117-130
The animals used destructible enrichment items more than indestructible items.

Lutz CK, Farrow RA 1996. Foraging device for singly housed longtailed **macaques** does not reduce stereotypies. Contemporary Topics in Laboratory Animal Science 35(3), 75-78
"All [10 single-housed] subjects manipulated the foraging boards, but stereotyped behaviors and activity levels were not significantly affected by the presence of the boards." Subjects "used" the boards approximately 2 minutes per 30 minute-observation sessions. "No reduction in board usage was observed over time of day or on repeated presentation, indicating that there was no novelty effect or reduction in motivation."

Schapiro SJ, Suarez SA, Porter LM, Bloomsmith MA 1996. The effects of different types of feeding enhancements on the behaviour of single-caged, yearling rhesus **macaques**. Animal Welfare 5, 129-138
http://www.awionline.org/Lab_animals/biblio/aw5-129.htm
"Enrichment use" in minutes/observation hour was as follows: Turf mats 25.8 minutes; Acrylic puzzles 22.1 minutes; Produce 17.4 minutes; Frozen juice 14.6 minutes. ...We feel that a feeding enrichment program similar to the one that we used [for single-housed subjects], that provides some combination of stimulating devices and foods that are novel and require processing, can have a very positive impact on the behaviour of captive primates. We have used a similar feeding enrichment program for older, pair-housed and group-housed rhesus with less success."

Platt DM, Novak MA 1997. Videostimulation as enrichment for captive rhesus monkeys (***Macaca*** *mulatta*). Applied Animal Behaviour Science 52, 139-155
The animals spent substantially more time watching selected videotapes than manipulating the joystick; females were more interested in both than males.

Reinhardt V 1997. The Wisconsin Gnawing Stick. Animal Welfare Information Center (AWIC) Newsletter 7(3-4), 11-12
http://www.nal.usda.gov/awic/newsletters/v7n3/7n3reinh.htm
The sticks consist of branch segments cut of dead red oak trees. They are used by caged macaques about 5% of the time - more by young animals, less by adult animals - for gnawing, manipulating and playing. "All caged rhesus **macaques** (more than 700 animals) and all caged stumptailed macaques (approximately 36 animals) have continual access to gnawing sticks since that time [1989]. ... Long-term exposure to the sticks has resulted in no recognizable health hazards."

Bertrand F, Seguin Y, Chauvier F, Blanquié JP 1999. Influence of two different kinds of foraging devices on feeding behaviour of rhesus **macaques** *(Macaca mulatta)*. Folia Primatologica 70, 207 (Abstract)
A foraging device fitted on the ceiling of the cage (H), and a foraging device fitted on the front of the cage (V) and filled with pellets were tested in 12 individually housed animals. "The animals moved the pellets from the reserve to a hopper. ... We found that the amount of waste food was up to 17 times lower in the V foraging device than in the control feeder and that the feeding time was much longer with the foraging device than with the control feeder. Over 90% of the food was eaten within the first 15 minutes with the control feeder, whereas it took 60 or 75 minutes to reach this percentage using the foraging device, whether it was a V or an H one. Each puzzle required specific skills. Whichever the feeding device, the subjects ate their whole daily ration and their weight remained stable."

Harris HG, Edwards AJ 2004. Mirrors as environmental enrichment for **African green monkeys**. American Journal of Primatology 63, 459-467
"Stainless steel circular mirrors were employed in an enrichment plan for 105 singly housed male African green monkeys. We observed 25 randomly selected males to measure mirror use and to assess the mirrors' effectiveness as an enrichment item. We conducted additional mirror-use surveys on all 105 males using fingerprint accumulation as an indicator (rated on a scale of 0 to 4). Use was defined as either being in contact with the mirror (contact use (CU)) or looking directly into the mirror without contact (non-contact use (NC)). Mirror-use data were collected 10 months after the initial introduction of the mirrors and again at 16 months. The two time points were compared by paired t-tests. No significant difference in use was found between the two data collection points. On average, the monkeys used the mirrors 5.2% of the total time intervals recorded (approximately 3 min/hr). Results from the five fingerprint-accumulation surveys showed that 102 of 105 males (97%) had CU with their mirrors over the survey points. Based on the sustained use of the mirrors over a 6-month period, we concluded that the mirrors were an effective enrichment tool that the vast majority of our monkeys routinely used. Habituation did not appear to occur even a year after the mirrors were introduced."

Seier JV, Loza J, Benjamin L 2004. Housing and stereotyped behaviour: Some observations from an indoor colony of **vervet monkeys** (*Chlorocebus aethiops*). Folia Primatologica 75(Supplement 1), 332
Adult females displaying stereotypies in single cages were exposed sequentially to a foraging log and an exercise cage, as well as cages of varying complexity and dimensions.

In another study females and males housed single in the bottom row, and females and males housed singly in the top row of the animal room were exposed sequentially to a foraging log and an exercise cage. The results of the first study showed that "females spent most time in stereotypies when in unenriched single cages. This was significantly reduced by the provision of either an exercise cage or a foraging log. No stereotyped behaviour was observed in the largest most enriched cages."

de Rosa C, Vitale A, Puopolo M 2003. The puzzle-feeder as feeding enrichment for common **marmosets** (*Callithrix jacchus*): a pilot study. Laboratory Animals 37, 100-107
"The use of a puzzle-feeder, as feeding enrichment, was investigated in three families of captive common marmosets (*Callithrix jacchus*). The study was carried out as a simultaneous choice test between two cages: one contained the puzzle-feeder, the other contained the usual food dishes, but otherwise both were arranged similarly. The monkeys were allowed to choose whether to feed from the usual dishes, or from the puzzle-feeder which required more effort. They were observed for two sessions in which they were differently motivated to feed. The enriched cage was always visited first, the marmosets managed to extract food from the puzzle-feeder, and spent more time eating from the puzzle-feeder when less hungry."

Majolo B, Buchanan-Smith HM, Bell J 2003. Response to novel objects and foraging tasks by common **marmosets** (*Callithrix jacchus*) female pairs. Lab Animal 32(3), 32-38
The presence of novel objects "may be beneficial for the psychological well-being of [isosexual female pairs] captive common marmosets, especially for monkeys with high baseline levels of stress." Such objects "reduce boredom through increases in exploratory behavior, decrease the occurrence of stress-related behavior, and do not affect aggression within the pair."

"The environment in which animals are maintained should be appropriate to the species." p 22

It should be noted upfront that a living environment without access to the vertical/arboreal dimension of space — via elevated structures — is **not** appropriate to any nonhuman primate species found in research facilities. All species of nonhuman primates are biologically adapted to an arboreal or semi-arboreal life style, all species show a vertical flight response, and all species sleep at 'safe' locations well above the ground.

PHYSICAL ENVIRONMENT

Housing

"Acceptable primary enclosures .. allow for the normal .. behavioral needs of the animals." p 23

If this statement is earnest, it should be made clear that a primary enclosure is not acceptable when a social animal is not allowed to live with another or with several other compatible conspecifics. This applies not only to primates but to all social animal species.

National Research Council 1998. The Psychological Well-Being of Nonhuman Primates . National Academy Press, Washington, DC
"Social interactions are considered to be one of the most important factors influencing the psychological well-being of most nonhuman primates. ... Knowing that most primates benefit from social interactions, it should be obvious that they can be harmed by a lack of social interaction [p. 16]. ... The common practice of housing rhesus monkeys singly calls for special attention [p. 99] ... Every effort should be made to house these [singly caged] animals socially (in groups or pairs), but when this is not possible, the need for single housing should be documented by investigators and approved by the IACUC."

"Solid-bottom caging, with bedding, is therefore recommended [because rodents prefer it over wire flooring]." p 24

It would be indicated to stipulate that solid-bottom caging without bedding should not be used unless there are specific scientific reasons to do so. Evidence indicates that a mere recommendation is not enough to encourage facilities to move away from wire bottom cages:

Stark DM 2001. Wire-bottom versus solid-bottom rodent caging issues important to scientists and laboratory animal science specialists. Contemporary Topics in Laboratory Animal Science 40(6), 11-14
"This article reviews the results of a recent survey of 12 United States-based pharmaceutical and contract toxicology laboratories. ... The 1999 survey showed that more than 80% of the rodents in surveyed toxicology facilities were housed in wire-bottom cages. ... Considerable short-term and long-term costs to programs would be associated with a change from wire-bottom to solid-bottom caging."

Recent evidence shows that rodents not only avoid wire floors but that unbedded flooring is also a stressor for them.

Krohn TC, Hansen AK, Dragsted N 2003. Telemetry as a method for measuring the impact of housing conditions on rats' welfare. Animal Welfare 12, 53-62

"The study revealed significant differences in systolic and diastolic blood pressure, heart rate and body temperature between rats housed in the tree conditions, indicating that both grid floors and plastic floors are more stressful for the animals than bedding. The observed differences did not diminish over the two-week observation period."

"Successful management of outdoor housing relies on consideration of ...." p 24

This statement is self-evident, but it could be useful if turned into a recommendation with bullets #2-5 being elaborated on the basis of a few data-supported references.

Space Recommendations

"For cats, a raised resting surface should be included in the cage." p 25 "Raised resting surfaces or perches are also often desirable for dogs and nonhuman primates." p 25-26

A "raised resting surface" should be included not only for cats but also for nonhuman primates. A resting surface is "always" desirable, i.e., a **biological necessity** for nonhuman primates and should, therefore, be basic furniture of any primary enclosure for nonhuman primates. To my knowledge there is no primate species used for research that is not biologically adapted to spend most of the 24-h day well above ground level, but if there is one, it may be exempt from this requirement.

Roonwal ML,Mohnot SM 1977. Primates of South Asia - Ecology, Sociobiology, and Behavior. Harvard University Press, Cambridge, MA
"***Macaca arctoides*** lives in dense forests and near cultivated land and villages. ... It is fairly terrestrial but spends a great deal of time in tress, which it ascends for the sake of food or safety and in which it sleeps.... Ten to 45 minutes before darkness sets in they [the macaques] are near or in the trees where they sleep."
"In Malay and Borneo it [**long-tailed macaque**] generally prefers to move among the trees rather than walk on the ground and largely feeds in the canopy. When frightened, it runs away through the treetops."
Bonnet macaques spend the night in sleeping trees. "A few selected places in the range were used as core areas where the macaques spent much time, were more relaxed, and had few aggressive actions. These areas were marked by the presence of many tall trees, including the roosting trees." In bonnet macaques "weaning occurs when the infant is 8-12 months old."
In Malaya, **pig-tailed macaques** "remain in the highest trees after dark and during the early morning and late evening."
Lion-tailed macaque "moves to the top of high trees and remains motionless whenever an observer arrives: it very rarely comes down to the ground in an observer's presence. ... It mainly stays in trees when feeding and resting. "

Lindburg DG 1971. The **rhesus monkey** in North India: an ecological and behavioral study. In Primate Behavior: Developments in Field and Laboratory Research, Volume 2 Rosenblum LA (ed), 1-106. Academic Press, New York, NY
Animals spent the night in trees. "When on the ground, the typical response to a shrill bark [alarm vocalization] was mass flight to the nearest tree. ... After climbing a few meters above ground, they then paused to look around for the source of danger and then moved to higher perches."

Wheatley BP 1980. Feeding and ranging of East Bornean ***Macaca fascicularis***. In The Macaques: Studies in Ecology, Behavior and Evolution Lindburg DG (ed), 215-246. Van Nostrand Reinhold, New York, NY

Photographic documentation of a typical sleeping tree. "The study troop spent more than 97 percent of their time in the trees."

Smith K, St. Claire M, Byrum R, Harbaugh S, Harbaugh J, Erwin J 2003. Use of space, cage features, and manipulable objects by laboratory primates: individual differences and species variability. American Journal of Primatology 60(Supplement), 76-77 (Abstract) "Rhesus (74%), longtailed (71%), vervets (94%), and patas (82%) significantly exceeded the expected rate of perch use [during the day] (25%), while pigtailed (28%) did not differ from expectation."

DeVore I, Hall KRL 1965. **Baboon** ecology. In Primate Behavior - Field Studies of Monkeys and Apes DeVore I (ed), 20-52. Holt, Rinehart and Winston, New York, NY
Photograph documenting a group of baboons who "has taken refuge from a lioness by climbing into the trees. These trees are smaller than those used for sleeping. ... The danger of predators sets limits on baboon day ranges and home range. Refuge sites - tress, cliffs, 'koope' - limit baboon range as much as available food and water. A group's day range is limited by the necessity of returning to a safe sleeping site at night. ... The absence of trees in some areas may deny baboons access to rich food sources when food items in general are scarce."

Hamilton WJ 1982. **Baboon** sleeping site preferences and relationships to primate grouping patterns. American Journal of Primatology 3, 41-53
"Baboons select nocturnal roosts with characteristics which suggest that choices of alternatives are based primarily upon their degree of security from predation. Sites chosen, in decreasing order of preference, are steep cliff faces, emerging trees, closed canopy forest trees and open woodland trees. Free-ranging baboons have never been reported to sleep on the ground."

Reynolds V, Reynolds A 1965. **Chimpanzees** of the Budongo Forest. In Primate Behavior - Field Studies of Monkeys and Apes DeVore I (ed), 368-424. Holt, Rinehart and Winston, New York, NY
"At a very rough estimate, chimpanzees in the Budongo Forest spent an average of from 50 to 75 percent of the daylight hours in trees."

Di Bitetti MS, Vidal EML, Baldovino MC, Benesovsky V 2000. Sleeping site preferences in tufted **capuchin monkeys** *(Cebus apella nigritus)*. American Journal of Primatology 50, 257-274
"The sleeping trees share a set of characteristics not found in other trees. ... Our results and those from other studies suggest that predation avoidance is a predominant factor driving sleeping site preferences."

Morrissey G 1994. Optimal foraging in the captive-bred common **marmoset**, *Callithrix jacchus*. In Welfare and Science, Proceedings of the Fifth FELASA Symposium Bunyan J (ed), 337-342. Royal Society of Medicine Press, London, UK
"The dominant pair, when given the choice, preferred to forage from the high-level box [filled with deep litter containing raisins], allowing the other group members to forage mainly at ground level. .. When feeding at floor level the marmosets took a raisin and

retreated to the branches to each it. .. By foraging at high level they will, unlike the other [low ranking] group members, avoid predation."

National Research Council 1998. The Psychological Well-Being of Nonhuman Primates . National Academy Press, Washington, DC
http://books.nap.edu/books/0309052335/html/index.html
"**Under natural conditions, many primates spend much of their lives aboveground and escape upward to avoid terrestrial threats. Therefore, these animals might perceive the presence of humans above them as particularly threatening ... Even macaques, which some describe as semiterrestrial, spend most of the day in elevated locations and seek the refuge of trees at night** ...Optimal use of available cage space might well depend more on the placement of perches, platforms, moving and stationary supports, and refuges than on cage size itself ."

"Some species of nonhuman primates use the vertical dimension of the cage to a greater extent than the floor. For them, the ability to perch and to have adequate veritcal space to keep the whole body above the cage floor can improve their well-being." p 26,27

There is no species of nonhuman primates used for research that does not use the vertical dimension to a greater extend than the ground in its biological natural habitat. They all retreat to elevated locations during the night, and they all retreat to elevated locations during alarming situations (see above references). When they are kept in cages, they often are forced to spend the night on the ground and retreat in a corner of the back of the cage during alarming situations because the vertical dimension lacks structures that could be used as resting and retreat places. This situation of not "appropriate to the species" [p 22], does not "allow for ... behavioral needs" [p 23] and it does not enhance animal "well-being" [p 21 & 37].

When they have a choice, primates will rest well above the ground, because the arboreal dimension of space is biologically safer for them than the ground.

Bennett CL, Davis RT 1989. Long term animal studies. In Housing, Care and Psychological Well-being of Captive and Laboratory Primates Segal EF (ed), 213-234. Noyes Publications, Park Ridge, NJ
"In the interim holding facility the [guenons] animals spent [only] 2% of their time on the ground, 83% in the mid levels, and 15% climbing across the roof."

Buchanan-Smith HM 1991. A field study on the red-bellied tamarin, *Saguinus l. labiatus,* in Boliva. International Journal of Primatology 12, 259-276
Tamarins spent 90% of their time in the upper half of their 186 cm-high cages when observations were made from a hide.

Reinhardt V 1992. Space utilization by captive rhesus macaques. Animal Technology 43, 11-17
http://www.awionline.org/Lab_animals/biblio/at.htm
"The area covered by the floor was 3 times larger than that covered by elevated structures; nonetheless the animals were located significantly more often (89.8% of 108 scan samples) on elevated structures than on the floor (8.6% of 108 scan samples). ... The higher an animal's rank position, the more pronounced was its habit to utilize high-level (>130 cm above floor) structures of the pen, while low ranking animals had to be content with low-level structures (40 cm above floor) and the floor. .. All members of the group would inevitably take to elevated sites whenever they heard or saw fear-inducing personnel. ... The animals huddled together with regularity on high-level structures but never on low-level structures or on the floor. ... It was concluded that [group-housed] laboratory rhesus macaques prefer the vertical dimension over the horizontal dimension as primary living space."

Goff C, Howell SM, Fritz J, Nankivell B 1994. Space use and proximity of captive chimpanzees (*Pan troglodytes*) mother/offspring pairs. Zoo Biology 13, 61-68
"Results confirmed the importance of vertical cage dimension and suggested the provision of horizontal substrates above the enclosure floor is important."

Buchanan-Smith HM, Shand C, Morris K 2002. Cage use and feeding height preferences of captive common marmosets (*Callithrix j. jacchus*) in two-tier cages. Journal of Applied Animal Welfare Science 5, 139-149
"Marmosets spent significantly more time at the top-positioned bowl than at the bottom-positioned bowl. .. Lower tier monkeys spent less time at the bottom bowl and more at the top bowl than upper tier monkeys. ... This suggests .. that lower tier marmosets are more reluctant to spend time on the floor. ... Marmosets spent substantially more time stationary in the top half of the cage than in the bottom half (79% vs. 21%)."

Kravic MA, McDonald K 2003. Environmental enrichment of nonhuman primates with PVC pipe constructs. AALAS [American Association for Laboratory Animal Science] 54th National Meeting Official Program, 138-139 (Abstract)
Benefits of PVC perches placed at different heights and swings are listed. Rhesus macaques kept in a double vertical cage, spent more time perching in the top space than the bottom ($p<0.05$).

Buchanan-Smith HM, Shand C, Morris K 2002. Cage use and feeding height preferences of captive common marmosets (*Callithrix j. jacchus*) in two-tier cages. Journal of Applied Animal Welfare Science 5, 139-149
"Marmosets spent significantly more time at the top-positioned bowl than at the bottom-positioned bowl. .. Lower tier monkeys spent less time at the bottom bowl and more at the top bowl than upper tier monkeys. .. This suggests .. that lower tier marmosets are more reluctant to spend time on the floor. .. Marmosets spent substantially more time stationary in the top half of the cage than in the bottom half (79% vs. 21%)."

"Acceptable primary enclosures allow for the normal ... behavioral needs ... normal movement and postural adjustments ... conspecific social interaction." p 23

A primary enclosure for nonhuman primates that is not furnished with at least one elevated resting surface is not acceptable because it does not allow the captive animal(s) to express their behavioral needs to (a) show vertical flight responses during alarming situations and (b) retreat to the "safe arboreal" dimension during periods of rest, especially during the night.

"Low resting surfaces that do not [*sic*] allow the space under them to be comfortably occupied by the animal should be counted as part of the floor space" p 25,26 However, "at a minimum, an animal must have enough space to turn around and to express normal postural adjustments, must have ... unobstructed area to move and rest in" p 25

Primates housed in cages with minimum heights do not have enough space to turn around and express normal postural adjustments, and they do not have unobstructed area to move and rest when a resting surface is installed at a too low level of the cage:

Reinhardt V 2003. Legal loophole for subminimal floor area for caged macaques. Journal of Applied Animal Welfare Science 6, 53 -56
http://www.awionline.org/Lab_animals/biblio/jaaws9.html
"Perches, ledges, swings, or other suspended fixtures have to be installed in such a way that they do *not* block part of the minimum floor space that is needed by an animal to make species-typical postural adjustments with freedom of movement. .. The placement of the perch does not allow the space underneath it to be comfortably occupied. It blocks part of the legal minimum floor area that is necessary for normal postural adjustments with freedom of movement (**Figure 1**)."

If the clause "Low resting surfaces that do not allow the space under them to be comfortably occupied by the animal should be counted as part of the floor space." **will be re-used in the new edition of the *Guide* some explanation would be warranted. The same clause is also incorporated in the AWR — §3.80a,2,xi — and is puzzling many readers. This *Guide* could finally correct this error or offer some clarification.**

"Space allocations [height, p 28 for nonhuman primates] should be re-evaluated to provide for enrichment of the primary enclosure" p 27

In order allow for the proper placement of an elevated resting surface — e.g., perch or swing, listed in the Animal Welfare Regulations §3.81 under (b) Environmental Enrichment — that does not hinder the nonhuman primate to turn around freely, express normal postural adjustments and move freely on the floor of the cage, the minimum height stipulations for nonhuman primate cages have to be "re-evaluated" [page 27] and the minimum height increased. It would be fair to offer some guidance here.

Reinhardt V, Liss C, Stevens C 1996. Space requirement stipulations for caged nonhuman primates in the United States: A critical review. Animal Welfare 5, 361-372 http://www.awionline.org/Lab_animals/biblio/aw4space.htm
"Space requirements for non-human primates are not adequate unless they stipulate that sufficient height be provided to accommodate properly placed elevated structures. .. US legal-sized cages do not provide sufficient height to permit the installation of an elevated structure in such a way that it blocks neither space *below* nor *above* it for the expression of species-characteristic terrestrial and arboreal postures and activities (**Figure 1**)."

"An animal's space needs are complex, and consideration of only the animal's body weight or surface area is insufficient." p 25 "Some species benefit more from wall space .. shelters ..., or cage complexity." p 25

Some species not only *benefit* from shelters, but they need shelters, nest boxes or nest material as 'safe' retreats during alarming situations and comfortable places for undisturbed resting. For them a species-appropriate shelter [rats, guinea pigs], nest box [hamsters] and/or nesting material [mice] should be a basic standard furniture in similar way as a high resting surface should be a standard furniture for nonhuman primates.

Townsend P 1997. Use of in-cage shelters by laboratory **rats**. Animal Welfare 6, 95-103
Rats with access to an appropriate shelter are more explorative and less timid than those in barren cages.

Patterson-Kane EG 2003. Shelter enrichment for **rats**. Contemporary Topics in Laboratory Animal Science 42(2), 46-48
"Nest boxes are a simple and effective form of environmental enrichment. Rats accept a wide range of nest-box types but have the strongest...preference for enclosed, opaque, thermoplastic boxes. ... Tubes have proven a relatively ineffective enrichment for rats. ... Nesting paper may substitute for nest boxes to some extend, but nest boxes are preferred to nesting paper when the two are offered separately."

Saad M, Sharp J, Azar T, Lawson D 2004. **Rat** preferences for commercially available "simulated burrows". AALAS [American Association for Laboratory Animal Science] 55th National Meeting Official Program, 137 (Abstract)
Rats preferred to spend their time during the light phase in Rodent Retreats compared to Rat Shacks or on open bedding.

Van de Weerd HA, van Loo PLP, van Zutphen LFM, Koolhaas JM, Baumans V 1997. Preferences for nesting material as environmental enrichment for laboratory **mice**. Laboratory Animals 31, 133-143
http://www.library.uu.nl/digiarchief/dip/diss/01801846/c3.pdf
"All [group-housed] mice showed a clear preference for cages with [paper] tissues or [paper] towels as compared to paper strips or no nesting material, and for cages with cotton string or wood-wool as compared to wood shavings or no nesting material. Paper-derived materials were preferred over wood-derived materials, although the results also suggest that the nature (paper or wood) of the nesting material is less important than its structure, which determines the nestability of the material." Both sexes built nests and there was no sex difference in preference for nesting materials. ... "10-20% of the time budget was spent on manipulation of the nesting material during day or night. ... Nesting material may be a relatively simple method to contribute to the well-being of laboratory mice."

Van de Weerd HA, van Loo PLP, van Zutphen LFM, Koolhaas JM, Baumans V 1998. Strength of preference for nesting material as environmental enrichment for laboratory **mice**. Applied Animal Behaviour Science 55, 369-382
http://www.library.uu.nl/digiarchief/dip/diss/01801846/c5.pdf
"On average, the 47 mice tested spent significantly more time in the cage with the nesting material [paper towel or tissue] (more than 69% of their total time, whereas less than 25% of their time in the cage with the nest box [perforated metal or clear perspex box]. In the second experiment the preferred nesting material (tissues) was placed in a cage with a grid floor (previously found to be avoided) and the nest box (perforated metal) was placed in another cage, connected to the first, with a solid floor covered with sawdust bedding material. In this experiment, 24 female mice were tested and on average spent more than 67% of their time in the cage with the nesting material, despite the presence of a grid floor. Thus, it was concluded that providing a cage with nesting material (in addition to bedding) may be essential for the well-being of laboratory mice."

Van Loo PLP, Blom HJM, Meijer MK, Baumans V 2005. Assessment of the use of two commercially available environmental enrichments by laboratory mice by preference testing. Laboratory Animals 39, 58-67
All three strains of **mice** showed a significant preference for the paper box. The paper box was much lighter [20 g] than the plastic box [95 g]. This allowed the mice to move the paper box around, manipulate it and change the position of the entrance within the cage. The plastic box seemingly was too heavy for such maneuvering and, hence, never changed its place. The mice also gnawed the paper box, occasionally nibbled an extra hole in the side, or shredded part of the box, using the shreds to strengthen their nest. They could not do this with the plastic box. All groups of mice slept inside the paper box but they never slept in the plastic box. If they chose to sleep in the cage that contained the plastic box, they did so in the sawdust outside the box. When tissue paper was provided, the mice dragged the material into the paper box and built a nest, but they never combined this nesting material with the plastic box.

Sherwin CM 1997. Observations on the prevalence of nest-building in non-breeding TO strain **mice** and their use of two nesting materials. Laboratory Animals 31, 125-132
"Within 2-3 min of the nesting materials being placed in the cages, many mice had pulled the paper towel from the pot into the main cage, investigated, chewed and manipulated the sheet. .. Thirty-six of the mice constructed nests during the first dark phase after the materials had been placed in the cage - the remaining three mice constructed nests during the following 48 h.... The most frequently constructed nest was build under the feeder and comprised a mixture of both the [cellulose] fibre and the paper. ... Two [of 39] mice constructed their nest entirely of paper. ... Providing paper towels is an inexpensive and practical means of environmental enrichment for non-breeding, laboratory mice. .. The function of non-maternal nests may be directly related to welfare [e.g. thermoregulation, seclusion] which is negated in the absence of suitable nesting materials. ... Providing a pre-formed nest-box as a form of environmental enrichment may be inappropriate" because mice are not highly motivated to use them for sleeping. "It seems that manipulable material [e.g., paper] is preferred to a rigid pre-formed shelter/nesting area [e.g., empty pots, tubes]."

It should be pointed out that the central floor area of rodent cages is of little value unless it is equipped with some structure, e.g., shelter, vertical wall(s), serving as cover and/or wall-protection. The classical *open-field test* testifies that being exposed to an *open,* i.e., unprotected area induces fear and anxiety, hence distress, in rodents.

Anzaldo AJ, Harrison PC, Riskowski GL, Sebek LA, Maghirang R, Stricklin WR, Gonyou HW 1994. Increasing welfare of laboratory rats with the help of spatially enhanced cages. Animal Welfare Information Center (AWIC) Newsletter 5(3), 1-2 & 5 http://www.nal.usda.gov/awic/newsletters/v5n3/5n3anzal.htm
Rats tend to 'shy away' from the center of barren cages. Instead they prefer to spend most their time in contact with surrounding walls of the cage, seldom using the floor space available in the center. A cage "equipped with a set of L-shaped partitions for tactile retreat and additional wall contact" was designed to address this behavioral characteristic." The animals preferred such a cage over a much bigger cage which allowed them to move in three dimensions [platforms], thereby better using the volume of the cage. The rats chose security over extra floor space.

White WJ, Balk MW, Lang CM 1989. Use of cage space by guinea pigs. Laboratory Animals 23, 208-214
Guinea pigs do not evenly use the space of a barren cage, which contains neither bedding nor any structure. The animals spent most of the time at the periphery, close the walls of such a cage rather than in the center [which offers no cover whatsoever]. The findings were used to draw the following conclusion: "The findings of the present study suggest that the current guidelines [AWA and Guide] for guineapig housing based on area allocation per guineapig, cannot be supported by behavioural characteristics of these animals or careful quantification of their patterns of cage space use."

"Some animals, such as various species of nonhuman primates, might need additional individual space when group-housed to reduce the level of aggression." p 26

Aggression can be a serious husbandry problem not only in nonhuman primates but also in male mice, hamsters and rabbits housed in social settings. It would be useful to point out that animals housed in groups or pairs not only *do* need additional individual, i.e., *social space* — for "social adjustments" (USDA 2002. Animal Welfare Regulations Revised as of January 1, 2002. U.S. Government Printing Office, Washington; page 129) — but also species-appropriate visual barriers to reduce the level of aggression.

Armstrong KR, Clark TR, Peterson MR 1998. Use of cornhusk nesting material to reduce aggression in caged **mice**. Contemporary Topics in Laboratory Animal Science 37(4), 64-66
The provision of cornhusk reduced aggressive interactions by offering subordinate animals cover and escape routes.

Gwinn LA, Krauthauser CL, Kerr JS 1999. Impact of home cage alterations on aggression in **mice**. Abstracts of the AALAS [American Association for Laboratory Animal Science] Meeting, 35 (Abstract)
PVC straight pipes, plumbing elbows and T pipes, and shreddible nesting squares were evaluated. "Nesting squares appear to be the most effective enrichment object for reducing the incidence of aggression in group-housed male mice."

Van Loo PLP, Kruitwagen CLJJ, Koolhaas JM, Van de Weerd HA, Van Zutphen LFM, Baumans V 2002. Influence of cage enrichment on aggressive behaviour and physiological parameters in male **mice**. Applied Animal Behaviour Science 76, 65-81
"From welfare perspective group housing of mice is preferred over individual housing. Group housing of male laboratory mice, however, often leads to problems due to excessive aggressive behaviour. ... Overall, nesting material reduced aggressive behaviour, while a shelter increased aggressive behaviour compared to control housing. This effect was also reflected in the number of wounds counted. Furthermore, during shelter housing mice gained less body weight, drank less and showed higher corticosterone levels, while in housing conditions with nesting material, mice ate less. We conclude that providing male mice with nesting material reduces aggression between male mice, and may, thus, be promoted as being beneficial to their physical health and psychological well-being."

Van Loo PLP, Van Zutphen LFM, Baumans V 2003. Male management: coping with aggression problems in male laboratory **mice**. Laboratory Animals 37( ), 300-313
"We review results from the literature and our own research with regard to coping with excessive aggressive behaviour in male laboratory mice. Based on this review practical recommendations concerning the housing and care of male laboratory mice are formulated. In short, it is recommended to avoid individual housing, to transfer odour

cues from the nesting area during cage cleaning and to apply nesting material as environmental enrichment. Furthermore, group size should be optimized to three animals per cage."

Reinhardt V, Reinhardt A 1991. Impact of a privacy panel on the behavior of caged female **rhesus monkeys** living in pairs. Journal of Experimental Animal Science 34, 55-58
http://www.awionline.org/Lab_animals/biblio/es34-5~1.htm
"Paired partners spent significantly more time in close proximity when the privacy panel was provided. At the same time, they were more engaged in affiliative interactions while the incidence of agonistic interactions tended to decrease."

Westergaard GC, Izard MK, Drake JD, Suomi SJ, Higley JD 1999. **Rhesus macaque** *(Macaca mulatta)* group formation and housing: Wounding and reproduction in a specific pathogen free (SPF) colony. American Journal of Primatology 49, 339-347
"When forming new rhesus macaque breeding groups, divided corrals that provide for social and visual separation of individuals lead to lower rates of traumatic wounding than do undivided corrals."

McCormack K, Megna NL 2001. The effects of privacy walls on aggression in a captive group of **rhesus macaques** *(Macaca mulatta)*. American Journal of Primatology 54(Supplement 1), 50-51 (Abstract)
"Preliminary results suggest that non-contact aggression (vocalizations, fear grimaces, chases, and threats) is significantly reduced after the introduction of the privacy walls ($p<.05$). However, a change in contact aggression was not observed with the introduction of the walls."

Maninger N, Kim JH, Ruppenthal GC 1998. The presence of visual barriers decreases agonism in group housed **pigtail macaques** *(Macaca nemestrina)*. American Journal of Primatology 45, 193-194 (Abstract)
"Instances of bite, grab and chase were found to be significantly greater [among members of harem groups of 23 pig-tailed macaques] when visual barriers were absent compared to when they were present."

Illumination

"In general, lighting should ... provide sufficient illumination ... to allow good housekeeping practices, adequate inspection of animals — including the bottom-most caged in racks." p 34

Reality shows that this requirement is not met in animals kept in multi-tier caging systems.

Weihe WH, Schidlow J, Strittmatter J 1969. The effect of light intensity on the breeding and development of rats and golden hamsters. International Journal of Biometeorology 13, 69-79
"It was noticed that animals subjected to an illumination of 2000 lux were tame and playful with handling, while those at lower light intensities resisted handling and tried to bite when vaginal smear was taken. ... The weight of some important organs, such as adrenals and testes, and also the breeding performance, showed a significant relationship to light intensity which was not seen in the hamster. ... For practical purposes it can be inferred, that, to obtain uniform results, rooms for rat breeding need to be more equally illuminated. ... The different light intensities from 1 to 5,000 lx, that we have found in animal rooms, may have some effect on the responses of animals to experimental procedures."

Ott JN 1974. The importance of laboratory lighting as an experimental variable. In Environmental Variables in Animal Experimentation Magalhaes H (ed), 39-57. Bucknell University, Lewisburg, PA
The importance of light and illumination as extraneous variable is discussed."My suggestion is that the cage conditions are too crowded in our present racks, and there should be the same lighting for all cages in the bottom shelf as well as the top shelf. Then you won't have to rotate them, because they should be subjected to the same light, I believe, throughout all of the experiments."

Bellhorn RW 1980. Lighting in the animal environment. Laboratory Animal Science 30, 440-450
"What we basically have done to date is to provide lighting suitable to our needs and assumed it was all right for the animal." [p. 441] Light intensities in stacked cages vary substantially.

Clough G 1982. Environmental effects on animals used in biomedical research. Biological Reviews 57, 487-523
"The intensity of light in animal cages is likely to be the most variable environmental factor in the average animal room."

Reasinger DJ, Rogers JR 2001. Ideas of improving living conditions of non-human primates by improving cage design. Contemporary Topics in Laboratory Animal Science 40(4), 89 (Abstract)
"It is difficult to observe animals in the bottom cages due to insufficient lighting.

Flashlights can increase visualization in this situation. New cage specifications are designed to admit light through a bar opening in the upper half of the rear cages."

Reinhardt V, Reinhardt A 2000. The lower row monkey cage: An overlooked variable in biomedical research. Journal of Applied Animal Welfare Science 3, 141-149
http://www.awionline.org/Lab_animals/biblio/jaaws1.htm
"In the traditional double-tier system, monkeys of the bottom row are forced to live in the crepuscular shade area of the upper row (**Figure 1**). .. Because cage illumination often is poor, a flashlight is needed to identify and inspect the cage occupants correctly ... as well as to properly illuminate the cage interior ... and the drop pan. Inadequate animal care and insufficient cage hygiene often result."

Schapiro SJ, Bloomsmith M 2001. Lower-row caging in a two-tiered housing system does not affect the behaviour of young, singly housed rhesus macaques. Animal Welfare 10, 387-394
"Although lower-row cages are significantly darker than upper-row cages at our facility, the data from the present study demonstrate that the diminished lighting and other supposed disadvantages experienced by lower-row-housed monkeys have few behavioural consequences."

"Rotating cage position relative to the light source .. can be used to reduce inappropriate light stimulation of animals." p 35

An animal caged on the top shelf (a) lives in an environment that is much higher and (b) receives different illumination than one caged on the bottom shelf. Rotating cage position rotates these two variables — distance from light source and distance from floor — between the subjects, but it does not address the real problem of minimizing or eliminating them.

BEHAVIORAL MANAGEMENT

Social Environment

"Consideration should be given to an animal's social needs." p 37

There is scientific evidence that not only social animals — such as primates and rats — but also animals who tend to be more solitary and intolerant of other conspecifics — such as hamsters, male mice, and rabbits — have *social needs* as demonstrated by the fact that they also prefer companionship over social isolation:

Arnold CE, Estep DQ 1990. Effects of housing on social preference and behaviour in male golden **hamsters** *(Mesocericetus auratus)*. Applied Animal Behaviour Science 27, 253-261
One strange male was introduced into a group of 4 littermates in a barren 5-chamber cage without preliminaries, and left "with the stimulus animals for about 46 h. ... The hamsters showed an overall preference for being with conspecifics and better growth when housed in same-sexed groups [of 5 males; rather than singly], thus supporting the conclusion that hamsters do not prefer being housed individually."

Arnold CE, Gillaspy S 1994. Assessing laboratory life for Golden Hamsters: Social preference, caging selection, and human interaction. Lab Animal 23(2), 34-37
Female **hamsters** preferred social contact with other females to solitary housing. "Since these animal prefer contact with conspecifics, and since group-housed hamsters are easier for humans to handle [less aggressive] than singly housed hamsters, perhaps pair-housing would be a suitable alternative."

Van Loo PLP, de Groot AC, Van Zuthpen BFM, Baumans V 2001. Do **male mice** prefer or avoid each other's company? Influence of hierarchy, kinship, and familiarity. Journal of Applied Animal Welfare Science 4, 91-103
"Experiments that allowed male mice with different histories to choose either an inhabited or an empty cage have shown that the mice preferred the proximity [separated by wire mesh or Perspex wall with holes] of another male over individual housing."

Van Loo PLP, Van de Weerd HA, Van Zutphen LFM, Baumans V 2004. Preference for social contact versus environmental enrichment in male laboratory mice. Laboratory Animals 38, 178-188
"Results indicated that when other conditions were similar, **male mice** preferred to sleep in close proximity to their familiar cage mate. Furthermore, the need to engage in active social behaviour increased with age. Tissues were used to a large extent for sleeping and sleep-related behaviour. It is concluded that single housing in order to avoid aggression between male mice is a solution with evident negative consequences for the animals. When individual housing is inevitable due to excessive aggressive behaviour, the presence of nesting material could partly compensate for the deprivation of social contact."

Chu L, Garner JP, Mench JA 2002. Pair-housing **rabbits** in standard laboratory cages: The relative importance of social enrichment. Contemporary Topics in Laboratory Animal Science 41(4), 114 (Abstract)
"We then conducted a preference test during which rabbits were required to push through weighted doors in order to gain access to various resources. ... Rabbits were willing to push more weight and spent more time with food and conspecifics than enrichment [nestbox, tunnel]. Together, these studies highlight the importance of social contact for laboratory rabbits."

Patterson-Kane EG, Hunt M, Harper D 2002. **Rats** demand social contact. Animal Welfare 11, 327-332
"Most of the rats in this experiment showed a persistent demand for social contact but not for physical cage improvements. These data suggest that social enrichment should be given the highest priority as a source of environmental enrichment for laboratory rats."

Pérez C, Canal JR, Dominguez E, Campillo JE, Guillén M 1997. Individual housing influences certain biochemical parameters in the **rat**. Laboratory Animals 31, 357-361
Individual as opposed to group-housing of female rats provoked variations in certain biochemical parameters [glucose, triglycerides, food intake]. It was concluded that this circumstance could make scientific data unreliable or even dubious.

Dettmer E, Fragaszy D 2000. Determining the value of social companionship to captive tufted capuchin **monkeys** *(Cebus apella)*. Journal of Applied Animal Welfare Science 3, 393-304
"To measure the need for social companionship, subjects [6 males, 1 female] were asked to choose between two commodities: food and social companionship. The only time subjects showed a food preference was when they were provided with a social companion but deprived of food for at least 12 hr prior to testing trials. .. Tufted capuchin monkeys value social companionship as they value food: It is a necessity, not a luxury."

"A social companion might buffer the effect of a stressful situation." p 37

It may be indicated to include more supportive references for primates, but also add supportive references for rodents:

Davitz JR, Mason DJ 1955. Socially facilitated reduction of a fear response in **rats**. Journal of Comparative and Physiological Psychology 48, 149-151
The presence of a conspecific mediates fear responses to a stressful situation.

Latané B 1969. Gregariousness and fear in laboratory **rats**. Journal of Experimental Social Psychology 5, 61-69
In a novel open-field environment rats showed less signs of fear [number of fecal boluses excreted] when tested in pairs versus alone. The presence of a caged companion was less effective than a free-moving companion in reducing fear.

Taylor GT 1981. Fear and affiliation in domesticated male **rats**. Journal of Comparative and Physiological Psychology 95, 685-693
"Unfamiliar conspecifics were just as effective in allaying fear as familiar animals. Even the individually reared rats, unused to other rats, were less fearful with conspecifics than when they were stressed alone. These findings simply attest to the strength of the capability of conspecifics to reduce fear."

Sharp JL, Zammit TG, Azar TA, Lawson DM 2002. Stress-like responses to common procedures in male rats housed alone or with other **rats**. Contemporary Topics in Laboratory Animal Science 41(4), 8-14
"Heart rate (HR), mean arterial blood pressure (MAP), and movement in the cage were collected by using radiotelemetry for 24 h. ... Rats housed four per cage showed significantly lower HR and MAP in response to acute husbandry and experimental procedures than rats housed alone, and the HR and MAP of rats housed in pairs were not consistently lower than those of rats housed alone. Procedure-induced arousal behaviors were observed in all housing groups after the acute husbandry and experimental procedures, but rats housed four per cage returned to sleeping behavior more quickly than did rats in the other housing groups. In light of these results, we concluded that ... common procedures induce noteworthy stress-like responses in male rats, and that the magnitude and duration of these responses are reduced by group housing."

Sharp JL, Zammit T, Azar TA, Lawson DM 2003. Stress-like responses to common procedures in individually and group-housed female **rats**. Contemporary Topics in Laboratory Animal Science 42(1), 9-18
"When rats were subjected to acute husbandry and experimental procedures, HRs increased 80 to 180 beats per min (bpm) above a baseline of 300 to 325 bpm and were significantly ($P < 0.05$) increased for periods of 30 to 90 min after the procedures. MAP showed increases that were proportionately the same as those in HR. Group housing often, but not always, reduced these cardiovascular responses. Procedure-induced arousal behaviors occurred in all housing groups after the acute husbandry and experimental procedures, but the occurrence of these behaviors was less frequent and of shorter

duration in group-housed rats than rats housed alone. .. We conclude that common procedures induce significant stress-like responses in female rats, and the magnitude and duration of these responses are reduced by group housing."

Kaiser S, Kirtzeck M, Hornschuh G, Sachser N 2003. Sex specific difference in social support - a study in female **guinea pigs**. Physiology and Behavior 79, 297-303
"In female guinea pigs social support can be provided by social partners. In contrast to males, however, not only the bonding partner is able to reduce the female's stress responses, but also a familiar conspecific, though in a less effective way."

Mason WA 1960. Socially mediated reduction in emotional responses of young **rhesus monkeys**. Journal of Abnormal and Social Psychology 60, 100-110
"Previous observations that social stimuli may function as a source of security and a means of mitigating emotional distress in young primates are fully supported by the present results."

Gonzalez CA, Coe CL, Levine S 1982. Cortisol responses under different housing conditions in female **squirrel monkeys**. Psychoneuroendocrinology 7, 209-216
Plasma levels of cortisol "were significantly lower in pair-housed females than in those living in a social group or individually. The increment in cortisol levels after stress (handling and ether anesthesia) also was smaller in females housed in pairs." Dominant and subordinate partners of female pairs did not differ in their plasma cortisol levels.

Coelho AM, Carey KD, Shade RE 1991. Assessing the effects of social environment on blood pressure and heart rates of **baboons**. American Journal of Primatology 23, 257-267
In the social companion condition, a subject was able to have visual, tactile, and auditory interactions with his companion through the wire mesh walls of the specially designed cages. "When animals were housed with social companions their blood pressures were consistently lower than when they were either housed individually or with social strangers. ... Measurements of cardiovascular physiology obtained under social housing may more closely model normal physiology than .. individual housing."

"A social companion might ... reduce behavioral abnormality." p 37

This statement is supported by primatological references only. It applies also to rodents and rabbits:

Lidfors L 1997. Behavioural effects of environmental enrichment for individually caged **rabbits**. Applied Animal Behaviour Science 52, 157-169
Hay was more effective than grass-cubes, sticks, and a box [rat cage] in reducing behavioral disorders and giving individually housed male rabbits something to do.

Potter MP, Borkowski GL 1998. Apparent psychogenic polydipsia and secondary polyuria in laboratory-housed New Zealand White **rabbits**. Contemporary Topics in Laboratory Animal Science 37, 87-89
Three single-caged rabbits with psychogenic polydipsia [excessive drinking without apparent physiological reason] were given toys for cage enrichment, "and the abnormal behavior decreased in all three cases."

Held SDE, Turner RJ, Wootton RJ 2001. The behavioural repertoire of non-breeding group-housed female laboratory **rabbits** *(Oryctolagus cuniculus)*. Animal Welfare 10, 437-443
"Trichophagy and stereotypic behaviors observed in singly caged rabbits were not observed in group-housed does."

Chu L, Garner JP, Mench JA 2004. A behavioral comparison of New Zealand White **rabbits** (*Oryctolagus cuniculus*) housed individually or in pairs in conventional laboratory cages. Applied Animal Behaviour Science 85, 121-139
"We compared the behavior of female New Zealand White rabbits (*Oryctolagus cuniculus*) housed either individually (N=4) in cages measuring 61 cm×76 cm×41 cm or in non-littermate pairs (four pairs) in double-wide cages measuring 122 cm×76 cm×41 cm. ... Over the 5 months, individually housed rabbits showed an increase in the proportion of the total behavioral time budget spent engaged in abnormal behaviors (digging, floor chewing, bar biting), from 0.25 to 1.77%, while pairs remained unchanged at 0.95% . ... Paired rabbits engaged in more locomotor behavior ($F_{1,6}$=16.49; $P<0.0066$) than individual rabbits (average proportions of time budget: 2.71 and 0.70% for paired and individual rabbits, respectively), which may be important because caged rabbits are susceptible to osteoporosis and other bone abnormalities due to the restricted ability to move."

It may be indicated to up-date the primate references with more recent information:

Hartner MK, Hall J., Penderghest J, White E, Watson S, Clark L 2000. A novel approach to group-housing male cynomolgus macaques in a pharmaceutical environment. Contemporary Topics in Laboratory Animal Science 39(4), 67 (Abstract)
"Twenty percent of our primates are maintained in a single-housed environment. Of those single-housed animals, 40% exhibited moderate to marked degrees of self-directed

activity; i. e., hairpulling. By contrast, none of the pair or group-housed animals exhibited these behaviors. Our goal was to provide increased socialization in a group of juvenile cynomolgus male macaques. Through a stepwise process, we transitioned these animals from a single cage environment to pair housing, and finally into a large enrichment unit, where they have been successfully maintained for over one year. We firmly believe that these primates are now more receptive to handling and training, and will therefore be better animal models, as noted by a marked decrease in vocalization and self-directed behavior during pole/collar capture and chair restraint procedures."

Weed JL, Wagner PO, Byrum R, Parrish S, Knezevich M, Powell DA 2003. Treatment of persistent self-injurious behavior in rhesus monkeys through socialization: A preliminary report. Contemporary Topics in Laboratory Animal Science 42(5), 21-23
Six individually caged males who engaged in persistent self-injurious behavior (SIB) were vasectomized and subsequently paired with females. The incidence of SIB was "markedly reduced for all male monkeys after social pairing." One male engaged in severe SIB after 32 months of pair-housing when he was temporarily removed from his partner for a procedure.

Alexander S, Fontenot MB 2003. Isosexual social group formation for environmental enrichment in adult male *Macaca mulatta*. AALAS [American Association for Laboratory Animal Science] 54th National Meeting Official Program, 141 (Abstract)
Isosexual groups [averag group size: 4.2 animals] of 80, previously single-caged 4-10 years old male rhesus macaques were formed [group formation protocol is not outlined]. "Thirty-one [38.8%] of these animals had at least one prior incidence of SIB [self-injurious biting]. ... During the year prior to group formation, the clinical history of the subjects included a 20% of diarrhea, 1.0% incidence of wound infection and 12.5% incidence of severe SIB requiring pharmacological intervention and wound care. Animals with severe SIB were treated pharmacologically for 2-11 months prior to group formation. All of these cases were removed from treatment prior to group formation. Over the 4-month period post formations <5.0% of the animals were removed for treatment of minor fight wounds. Less than 2.0% of the animals were removed for clinical purposed (e.g., diarrhea, dehydration). No occurrence of severe SIB was noted. We concluded that the formation of isosexual social groups is a suitable alternative to individual housing of adult male rhesus monkeys and may decrease the occurrence of SIB in a susceptible population."

Bourgeois SR, Brent L 2005. Modifying the behaviour of singly caged baboons: evaluating the effectiveness of four enrichment techniques. Animal Welfare 14, 71-81
Seven singly caged adolescent [mean age: 4.2 years] male baboons were studied. "Analysis of baseline behaviour verified substantial durations of abnormal behaviour [9.8/30- min observations (33% of time)]. We tested the effectiveness of ... positive reinforcement training (PRT), food enrichment [fruits, frozen fruit/juice, foraging devices], non-food enrichment [toys], and social enrichment (pair/trio). ...The social enrichment condition resulted in the most positive behavioural changes, including ...near elimination of abnormal behaviours [0.7/30-min observation (2% of time)]. Significant reduction in total abnormal behaviour levels were also found for other types of enrichment, but only social enrichment and PRT were effective in reducing whole-body

stereotypies. ... Animate enrichment (human or conspecific stimulation), as opposed to inanimate enrichment, provides optimal means of behaviour modification for singly caged baboons."

"When they must be housed alone, other forms of enrichment should be provided to compensate for the absence of other animals." p 38

It would be fair to emphasize that *other forms of enrichment* should be provided that have been proven to be safe and useful beyond novelty effects.

National Research Council 1998. The Psychological Well-Being of Nonhuman Primates. National Academy Press, Washington, DC
http://pompeii.nap.edu/books/0309052335/html/index.html
"Enrichment methods that have not been subjected to empirical testing should be viewed simply as invalidated ideas, regardless of how well intended they might be. Without appropriate measurement and verification, we might do more harm than good in our efforts to improve animal conditions." [p 114].

As part of the BEHAVIORAL MANAGEMENT [p 36] of the Social Environment [p 37] some recommendations would be helpful regarding the management of aggression in pair- and group-housed animals:

Armstrong KR, Clark TR, Peterson MR 1998. Use of cornhusk nesting material to reduce aggression in caged **mice**. Contemporary Topics in Laboratory Animal Science 37(4), 64-66
The provision of cornhusk reduced aggressive interactions by offering subordinate animals cover and escape routes.

Gwinn LA, Krauthauser CL, Kerr JS 1999. Impact of home cage alterations on aggression in **mice**. Abstracts of the AALAS [American Association for Laboratory Animal Science] Meeting, 35 (Abstract)
PVC straight pipes, plumbing elbows and T pipes, and shreddable nesting squares were evaluated. "Nesting squares appear to be the most effective enrichment object for reducing the incidence of aggression in group-housed male mice."

Reinhardt V, Reinhardt A 1991. Impact of a privacy panel on the behavior of caged female **rhesus monkeys** living in pairs. Journal of Experimental Animal Science 34, 55-58
http://www.awionline.org/Lab_animals/biblio/es34-5~1.htm
"Paired partners spent significantly more time in close proximity when the privacy panel was provided. At the same time, they were more engaged in affiliative interactions while the incidence of agonistic interactions tended to decrease."

Neveu H, Deputte BL 1996. Influence of availability of perches on the behavioral well-being of captive, group-living **mangabeys**. American Journal of Primatology 38, 175-185
"A total deprivation of perches yielded an increase in aggressive behaviors and locomotion, and a decrease in cohesiveness. Placing perches progressively in the experimental cage restored the level of all the variables to levels found in the control cage

[with five perches]. ...Therefore, perches constitute a necessary feature of an adequate environment for mangabeys."

Maninger N, Kim JH, Ruppenthal GC 1998. The presence of visual barriers decreases antagonism in group housed **pigtail macaques** *(Macaca nemestrina)*. American Journal of Primatology 45, 193-194 (Abstract)
"Instances of bite, grab and chase were found to be significantly greater [among members of harem groups of 23 pig-tailed macaques] when visual barriers were absent compared to when they were present."

Nakamichi M, Asanuma K 1998. Behavioral effects of perches on group-housed adult female **Japanese monkeys**. Perceptual and Motor Skills 87, 707-714
"When [4 adult female] monkeys were housed in a cage which contained eight wooden perches to increase usable space, the rate of agonistic interactions as well as the rates of spatial proximity and social grooming decreased in comparison with those evident when they were housed in a cage [identical dimension and resting bench] without such perches."

Westergaard GC, Izard MK, Drake JD, Suomi SJ, Higley JD 1999. **Rhesus macaque** *(Macaca mulatta)* group formation and housing: Wounding and reproduction in a specific pathogen free (SPF) colony. American Journal of Primatology 49, 339-347
"When forming new rhesus macaque breeding groups, divided corrals that provide for social and visual separation of individuals lead to lower rates of traumatic wounding than do undivided corrals."

McCormack K, Megna NL 2001. The effects of privacy walls on aggression in a captive group of **rhesus macaques** *(Macaca mulatta)*. American Journal of Primatology 54(Supplement 1), 50-51 (Abstract)
"Preliminary results suggest that non-contact aggression (vocalizations, fear grimaces, chases, and threats) is significantly reduced after the introduction of the privacy walls ($p<.05$). However, a change in contact aggression was not observed with the introduction of the walls."

Felts WP, Johns TJ, Sauceda R 2002. Novel and economical structural enrichment for a unique colony of group-housed **macaques**: Success and failures. Contemporary Topics in Laboratory Animal Science 41(4), 120 (Abstract)
"Different levels of perching and visual barriers were installed. ... These economically structural changes increase the activity in the units and decreased the amount of injuries caused by fighting."

Activity

"An animals' motor activity, including use of the verical dimension, should be considered in evaluation of suitable housing." p 38

This important stipulation should make it clear that primary enclosures of nonhuman primates must be furnished with elevated resting surfaces that are placed in such a way that the caged subject(s) can turn around freely on the cage floor — if necessary using the space underneath the resting surface — and sit on the resting surface in species-typical manner without touching the ceiling of the enclosure.

BEHAVIORAL MANAGEMENT p 36-38

This chapter would benefit if it would also address the possibility of feeding enrichment to promote species-adequate behaviors and mitigate behavioral pathologies:

Lidfors L 1997. Behavioural effects of environmental enrichment for individually caged **rabbits**. Applied Animal Behaviour Science 52, 157-169
Hay was more effective than grass-cubes, sticks, and a box [rat cage] in reducing behavioral disorders and giving individually housed male rabbits something to do. The hay was placed in empty water bottles to "make it a more lengthy task for the rabbits to pull the straws out."

Berthelsen H, Hansen LT 1999. The effect of hay on the behaviour of caged **rabbits** *(Oryctolagus cuniculus)*. Animal Welfare 8, 149-157
"When hay was available [placed on top of cage], the [single-caged] rabbits ... performed significantly less bar gnawing and excessive grooming" and were less restless. "This suggests that rabbits kept in cages where hay is available are less stressed than those kept in cages where it is not." When kept in otherwise barren cages, rabbits interacted with the hay 16% of one-hour observation sessions.

Roberts RL, Roytburd LA, Newman JD 1999. Puzzle feeders and gum feeders as environmental enrichment for common **marmosets**. Contemporary Topics in Laboratory Animal Science 38(5), 27-31
"The results of this study indicate that gum feeders and Puzzle-Feeders™ loaded with waxmoth larvae are useful for reducing the rates of pacing and inactivity" in single-housed and in pair-housed marmosets.

Florence G, Riondet L 2001. Long-term effects of a food puzzle on the behaviour of **rhesus monkeys**. Folia Primatologica 72, 118-119 (Abstract)
Five adult males were tested during a 17 week period. Access to a food puzzle "yielded an overall reduction in, or even disappearance of, the stereotyped locomotion, the stereotyped self-directed behaviours and the saluting behaviour that had been observed" when the animals had access to ordinary food dispensers.

Bayne K, Mainzer H, Dexter SL, Campbell G, Yamada F, Suomi SJ 1991. The reduction of abnormal behaviors in individually housed **rhesus monkeys** *(Macaca mulatta)* with a foraging/grooming board. American Journal of Primatology 23, 23-35
All of the single-housed "animals foraged from the board to the point that a significant reduction in the level of abnormal behavior [5%] was noted. Most animals also groomed the fleece covering the board." Subjects spent on average 12.1 minutes foraging from the board per 30 minute-observation sessions. Prior to enrichment, individuals spent on average 25% of their time engrossed in abnormal behaviors.

Lam K, Rupniak NMJ, Iversen SD 1991. Use of a grooming and foraging substrate to reduce cage stereotypies in **macaques**. Journal of Medical Primatology 20, 104-109
http://www.awionline.org/Lab_animals/biblio/jmp20-1.htm
"Animals exhibited idiosyncratic repertoires of stereotyped behaviour, including repetitive pacing, swaying circling, bouncing, cage charging, and rocking. These activities occupied on average 11% of baseline observation periods" prior to the introduction of the enrichment gadget. Animals who received the fleece [cushion] alone engaged in grooming. Monkeys given fleece sprinkled with morsels of food did not groom the fleece, but foraged for long periods (up to 27 min/h). Stereotyped behaviours were reduced by up to 73% by use of the fleece pad both alone and with foraging crumbles."

Brent L, Long KE 1995. The behavioral response of individually caged **baboons** to feeding enrichment and the standard diet: A preliminary report. Contemporary Topics in Laboratory Animal Science 34(2), 65-69
PVC pipe with finger holes, filled with a mixture of peanut butter and seeds. The mean amount of feeder use was 51 minutes per 60 minute observation sessions. "Increasing foraging opportunities in this study reduced abnormal behaviors from 16.4% of the data points in the baseline condition to 4.9% and 5.7% in the chow [normal feeding condition] and feeder condition, respectively."

HUSBANDRY

Food

"Feeders should be designed and placed to allow easy access to food and to minimize contamination with urine and feces." p 39

This recommendation contrasts with successful feeding enrichment strategies designed to make it more difficult for the animals to access their daily food ration. Animals who apply skillful foraging techniques to retrieve their daily food ration eat all the food they obtain rather than drop part of it thereby increasing the chances of contamination with urine and feces.

Wrightson D, Dickson C 1999. Diet restriction through hopper design. Animal Technology 50, 45-46
Group-housed **rats** were induced to 'work' for their food by soldering metal plates over their food hoppers, so that only 3% of the original area remains available. The animals "fed for longer periods and rested less during the night, but there were no adverse clinical effects and no problems with rats' muzzles, gums, teeth or forepaws. The rats were not aggressive to one another or to humans, and were more confident when handled. No changes were observed in the rats' social hierarchy and there were no increases in fighting with restricted hoppers, as up to three rats could feed at a time. ... It was felt that this method of food restriction was preferable to giving less food [to avoid obesity]. ... Rather than rapidly eating a reduced ration and feeling hungry for long periods, the rats worked harder for their food, which enabled them to burn more calories and eat throughout the day. This reduces the incidence of obesity and its associated disorders and also encourages more 'natural' behaviour patterns, both of which improve welfare."

Van Berkum LE 2000. Use of a feeder insert to reduce obesity in **rats**. AALAS [American Association for Laboratory Animal Science] 51st National Meeting Official Program, 125 (Abstract)
"By inserting a modified stainless steel plate into the feeder, area of exposed food is reduced, and may result in increased exploratory activity, which may lead to decrease in body weights and food consumption values (all while continuing to provide ad libitum access to food). ... Although body weights and food consumption were not significantly different for weeks 1-9, weeks 10-14 showed a trend towards lower body weights and food consumption in the treated groups. ... The study will be continued."

Johnson SR, Patterson-Kane EG, Niel L 2004. Foraging enrichment for laboratory **rats**. Animal Welfare 13, 305-312
"The limited-access hopper had a tendency to reduce food consumption, but the time spent feeding increased."

Markowitz H 1979. Environmental enrichment and behavioral engineering for captive primates. In Captivity and Behavior Erwin J, Maple T, Mitchell G (eds), 217-238. Van Nostrand Reinhold, New York, NY
Food dispensing apparatuses were developed and successfully implemented as feeding

enrichment options for group-housed **gibbons**, siamangs and diana monkeys. "The problem of excess food lying around and decaying on the floor had been reduced to a minimum."

Reinhardt V 1993. Enticing nonhuman primates to forage for their standard biscuit ration. Zoo Biology 12, 307-312
http://www.awionline.org/Lab_animals/biblio/zb12-30.htm
Ordinary feeder-boxes were converted into food puzzles by remounting them onto the mesh of the front of the cages, away from original access holes. The total amount of time [pair-housed] adult male **rhesus macaques** engaged in gathering the standard biscuit ration was 141 times higher at food puzzles [42.2 min] than at feeder-boxes [0.3 min].

Reinhardt V 1993. Using the mesh ceiling as a food puzzle to encourage foraging behaviour in caged **rhesus macaques** (*Macaca mulatta*). Animal Welfare 2, 165-172
http://www.awionline.org/Lab_animals/biblio/aw3mesh.htm
"Daily commercial dry food rations consisting of 33 bar-shaped or 16 star-shaped biscuits per animal were placed on the mesh ceiling of the cages instead of in the feed-boxes. This induced an 80-fold increase and 289-fold increase, respectively, in foraging time" in the pair-housed males.

Reinhardt V 1993. Evaluation of an inexpensive custom-made food puzzle used as primary feeder for pair-housed **rhesus macaques**. Laboratory Primate Newsletter 32(3), 7-8
http://www.brown.edu/Research/Primate/lpn32-3.html#food
"Working for their standard food rather than collecting it from freely accessible food boxes did not impair the [pair-housed] animals' body weight maintenance, suggesting that their general health was not impaired by the new feeding technique."

Reinhardt V 1994. Caged **rhesus macaques** voluntarily work for ordinary food. Primates 35, 95-98
http://www.awionline.org/Lab_animals/biblio/primat~1.htm
"When feeding from the food box, the animals were rather careless and dropped many biscuits on the floor. As a consequence they consumed only 52% of the biscuits retrieved, leaving 48% as leftover. When feeding from the food puzzle, the animals were more concentrated. They focused their dexterity on the retrieval of one biscuit at a time which they immediately consumed in 98% of cases and dropped on the floor in only 2% of cases."

Murchison MA 1994. Primary forage feeder for singly-caged [**pig-tailed**] macaques. Laboratory Primate Newsletter 33(1), 7-8
http://www.brown.edu/Research/Primate/lpn33-1.html#mark
Perforated feeder box requires the single-housed subject to use the fingers to maneuver biscuits to access holes at different levels. "Apparently the animals consumed nearly all the food retrieved from the forage feeders, leaving less on the cage floor to become contaminated. The animals spent significantly more time foraging with the forage feeder than the standard feeder."

Murchison MA 1995. Forage feeder box for single [**pig-tailed macaque**] animal cages. Laboratory Primate Newsletter 34(1), 1-2
http://www.brown.edu/Research/Primate/lpn34-1.html#forage
Standard feeder with small access holes rather than one big access hole. Time spent foraging during the first hour after biscuit distribution increased from 51 seconds when 40 biscuits were presented in the standard feeder [one large access hole] to 400 seconds when 40 biscuits were presented in the forage feeder [four small access holes]. "There were no differences between the standard and forage feeders in number of biscuits fed and consumed." More biscuits fell on the cage floor and beneath the cage on the floor of the room in the standard feeder situation than in the forage feeder situation.

These feeding enrichment strategies aim at making the animals work for their food, which some species [most species have not been tested] do voluntarily in captivity when they can chose to do so.

Neuringer AJ 1969. Animals respond for food in the presence of free food. Science 166, 399-401
"Pigeons pecked a response disk to gain access to grain rewards while identical grain was freely available ... Similarly, rats pressed a lever for food pellets while free pellets were present. ... The act of producing food can serve as its own motivation and, therefore, as its own reward."
Carder B, Berkowitz K 1970. Rats' preference for earned in comparison with free food. Science 167, 1273-1274
"When work demands are not too high, **rats** prefer earned food to free food."

Markowitz H 1979. Environmental enrichment and behavioral engineering for captive primates. In Captivity and Behavior Erwin J, Maple T, Mitchell G (eds), 217-238. Van Nostrand Reinhold, New York, NY
Food dispensing apparatuses were developed and successfully implemented as feeding enrichment options for group-housed **gibbons**, siamangs and diana monkeys. "Frequently, often with free food in their hands, they [gibbons] attempted to get the lights and levers to respond" and missed the opportunity to 'produce' food.

Line SW, Markowitz H, Morgan KN, Strong S 1989. Evaluation of attempts to enrich the environment of single-caged non-human primates. In Animal Care and Use in Behavioral Research: Regulation, Issues, and Applications Driscoll JW (ed), 103-117. Animal Welfare Information Center National Agricultural Library, Beltsville, MD
Rhesus macaques removed monkey biscuits from a puzzle feeder "despite the fact that the same kind of food was available free-choice at the twice-daily feedings." [Abstract of this work has been published in: *American Association for Laboratory Animal Science Bulletin* 30(4): 17, 1991; Line SW; An environmental enhancement plan for a large primate colony.]

Reinhardt V 1994. Caged **rhesus macaques** voluntarily work for ordinary food. Primates 35, 95-98
http://www.awionline.org/Lab_animals/biblio/primat~1.htm
Individuals spent on average 32 sec retrieving biscuits from the ordinary food box, and 673 sec retrieving biscuits from the food puzzle. "It was inferred that the animals voluntarily worked for ordinary food, with the expression of foraging activities serving as its own reward."

de Rosa C, Vitale A, Puopolo M 2003. The puzzle-feeder as feeding enrichment for common **marmosets** (*Callithrix jacchus*): a pilot study. Laboratory Animals 37, 100-107
"The use of a puzzle-feeder, as feeding enrichment, was investigated in three families of captive common marmosets (*Callithrix jacchus*). The study was carried out as a simultaneous choice test between two cages: one contained the puzzle-feeder, the other contained the usual food dishes, but otherwise both were arranged similarly. The monkeys were allowed to choose whether to feed from the usual dishes, or from the puzzle-feeder which required more effort. They were observed for two sessions in which they were differently motivated to feed. The enriched cage was always visited first, the marmosets managed to extract food from the puzzle-feeder, and spent more time eating from the puzzle-feeder when less hungry."

Inglis IR, Forkmann B, Lazarus J 1997. Free food or earned food? A **review** and fuzzy model of contrafreeloading. Animal Behaviour 53, 1171-1191
Unless they are quite hungry, animals of many species prefer to work for food rather than eat freely available food, a phenomenon known as contrafreeloading. "Animals will work (e.g. lever press) for 'earned' food even though identical 'free' food can easily be obtained from a nearby dish. ... Animals work for earned food in order to update their estimate of a currently sub-optimal food source because, in the longer term, it may unpredictably become the optimal place to feed. Contrafreeloading is therefore a behaviour that, under natural conditions, is adaptive."

Bedding

"The veterinarian or facility manager, in consultation with investigators, should select the most appropriate bedding material." p 41

Reader may find it helpful to get some data-based advice on this issue directly from the *Guide*.

Port CD, Kaltenbach JP 1969. The effect of corncob bedding on reproductivity and leucine incorporation in mice. Laboratory Animal Care [Laboratory Animal Science] 19, 46-49
Preweaning mortality was increased when the mice were housed on corncob bedding (22%) when compared with pine sawdust bedding (13%).

Mulder JB 1975. Bedding preferences of pregnant laboratory-reared **mice**. Behavior Research Methods and Instrumentation 7, 21-22
Pregnant mice invariably preferred aspen bedding over nine other commercially available bedding materials.

Odynets A, Simonova O, Kozhuhov A 1991. Beddings for laboratory [**mice**] animals: criteria of biological evaluation. Laboratornye Zhyvotnye 1, 70-76
Aspen bedding was the favorite of five bedding materials.

Blom HJM, van Tintelen G, van Vorstenbosch CJAHV 1996. Preferences of **mice** and **rats** for types of bedding material. Laboratory Animals 30, 234-244
"The results seem to indicate that size and manipulability are among the main determinants of the appreciation of bedding particles by laboratory mice and rats, and larger particles are preferred. .. In the test system with two test cages, [aspen] wood chips were preferred over sawdust and wire mesh. ... Shredded filter paper was so attractive to female laboratory mice that it masked differential preferences for wood chips, sawdust and wire mesh floor."

Van de Weerd HA, van den Broek FAR, Baumans V 1996. Preference for different types of flooring in two **rat** strains. Applied Animal Behaviour Science 46, 251-261
"The rats showed a significant preference for the cages with wood shavings and paper bedding, both consisting of large particles. ... The cages with sawdust and wire mesh floor were relatively avoided. Rats slept in the cages with large-particles bedding, but used the other cages for active behaviour such as eating and defecating; furthermore, many rats preferred different cages [with different substrates] during day and night. It is suggested that different behavioural activities may require different cage floor covering. .... Possibly the widely used concept of housing laboratory rats on one type of cage flooring should be abandoned and replaced by a cage concept with different types of flooring to enable the rats to express a more complete behavioural repertoire."

Ras T, Van de Ven M, Patterson-Kane EG, Nelson K 2002. **Rats**' preferences for corn versus wood-based bedding and nesting materials . Laboratory Animals 36, 420-425
"Corn by-products can be used as bedding and nesting products. Corn-cob bedding resists

ammonia build-up and corn-husk nesting material resists dampness. It is not clear whether these advantages are at the expense of animal comfort. Corn cob was compared to aspen chip bedding, and corn husk to paper strip nesting material. Data from 20 rats with differential early bedding experience suggested that they prefer aspen chip, but are also biased towards the bedding they were raised on. Data from 10 rats with no prior nesting material experience suggested that paper strip was preferred over cornhusk. Thus, corn-cob products are not recommended except in situations where air quality and/or flooding are significant problems."

Krohn TC, Hansen AK, Dragsted N 2003. Telemetry as a method for measuring the impact of housing conditions on **rats**' welfare. Animal Welfare 12, 53-62
"The study revealed significant differences in systolic and diastolic blood pressure, heart rate and body temperature between rats housed in the tree conditions, indicating that both grid floors and plastic floors are more stressful for the animals than bedding. The observed differences did not diminish over the two-week observation period. "

Pettijohn TF, Barkes BM 1978. Surface choice and behavior in adult Mongolian **gerbils**. The Psychological Record 28, 299-303
Both males and females clearly chose to be most frequently on the sand, follwed by the wood chip bedding material."

Hawthorne AJ, Loveridge GG, Horrocks LJ 1997. The behaviour of domestic **cats** in response to a variety of surface-textures. In Proceedings on the 2nd International Conference on Environmental Enrichment Holst B (ed), 84-94. Copenhagen Zoo, Frederiksberg, DK
Cats prefer polyester fleece to cotton-looped towel, woven rush-matting and corrugated cardboard as bedding material.

Eisele P 2001. A practical dog bed for environmental enrichment for geriatric beagles, with applications for puppies and other small **dogs**. Contemporary Topics in Laboratory Animal Science 40(3), 36-38
"The dogs were initially housed in kennel runs equipped with elevated benches, but it became apparent that some of the oldest animals had difficulties jumping down from them. To improve animal safety and comfort, practical dog beds were made out of the ends of clean high-density polyethylene barrels. Synthetic fleece bed liners were used for dogs that did not chew them or remove them from the beds. Nine of the beagles regularly were observed to use the beds."

Ludes E, Anderson JR 1996. Comparison of the behaviour of captive white-faced **capuchin monkeys** (*Cebus capucinus*) in the presence of four kinds of deep litter. Applied Animal Behaviour Science 49, 293-303
The group-housed capuchins were given the choice of four types of litter evenly spread out on the floor of the enclosure: woodchips, dried ground corncob, woodwool and garden peat. Peat was associated mostly with locomotion and social contacts, while woodwool was the preferred litter for foraging and play. The ground corncob was avoided by the monkeys.

Sanitation

Cage cleaning is typically associated with serious aggression in group-housed male mice. It would be helpful to offer some guidance on how the problem can best be addressed:

Ambrose N, Morton DB 2000. The use of cage enrichment to reduce male mouse aggression. Journal of Applied Animal Welfare Science 3, 117-125
"Even a simple enrichment aid such as a glass water bottle can significantly reduce postcage-cleaning aggression compared with mice kept in a barren cage."

Van Loo PLP, Kruitwagen CLJJ, Van Zutphen LFM 2000. Modulation of aggression in male mice: Influence of cage cleaning regime and scent marks. Animal Welfare 9, 281-295
"Group housing of male laboratory mice often leads to welfare problems due to aggressive behaviour. ... Aggression peaks after disturbances such as cage cleaning. ... Our results indicated that neither kinship nor distribution of urine marks affected aggression. Olfactory cues from nesting and bedding material, however, affected aggression to a marked degree: transfer of nesting material reduced aggression significantly, while transfer of sawdust containing urine and faeces seemed to intensify aggression. ... We conclude that the transfer of nesting material will reduce aggression, or at least slow down its development, and thus aid the reduction of social tension due to cage cleaning."

Van Loo PLP, Van der Meer E, Kruitwagen CLJJ, Koolhaas JM, Van Zutphen LFM, Baumans V 2004. Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains. Laboratory Animals 38, 169-177
"Long-term provision of nesting material and its transfer during cage cleaning was found to influence several stress-related physiological parameters. Mice housed in cages enriched with nesting material had lower urine corticosterone levels and heavier thymuses, and they consumed less food and water than standard-housed mice. ... We conclude that the long-term provision of nesting material, including the transfer of nesting material during cage cleaning, reduces stress and thereby enhances the welfare of laboratory mice."

#-14

UNIVERSITY OF PENNSYLVANIA MEDICAL CENTER

University of Pennsylvania School of Medicine
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Paul H. Edelstein, M.D.
Director
Clinical Microbiology
Professor of Pathology
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January 31, 2006

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184 , MSC 7983
Bethesda , MD 20892-7983

Re: RFI No. NOT-OD-06-011

Dear Dr. Snyder:

I am writing to request that a review of the surgical site fur clipping recommendations be undertaken in preparation for the next version of the NIH Guide for the Care and Use of Laboratory Animals. The current recommendations for surgical site fur clipping are of unproven value, are based on faulty scientific data, have the potential for animal harm, and may be onerous. Based on data presented in this letter surgical site fur clipping is unnecessary, at least for clean surgery. Based on all these factors, the review committee should write a more circumspect recommendation regarding fur clipping, making it optional in clean surgery at low risk of infection.

Until recently, I performed anterior neck dissection to expose the trachea of guinea pigs without prior fur clipping. Over a period of 25 years I performed this procedure on approximately 6,000 guinea pigs (male, 250-300 g). The tracheal exposure was required to instill *Legionella pneumophila* bacteria into the trachea using a needle and syringe, resulting in an animal model of Legionnaires' disease. Animals are observed and treated with and without antibiotics over a two to 14 day period post-infection. Over that time period the wound infection rate was approximately 0.1%, including animals not treated with antibiotics. Aseptic technique is used, including extensive skin and fur disinfection with 10% povidone iodine, with three separate applications over a five to ten minute period; no fur clipping is used. The procedure is performed in a laminar flow hood, using sterile instruments, while gowned, gloved and masked. Almost all of the infections occurred in the first few years that I performed this procedure, probably related to inexperience. The majority of infections involved the area posterior to the trachea, most likely from esophageal injury during insertion of the needle into the trachea. Wound infections due to hair contamination would be expected to be anterior to the trachea, not posterior to it. I recall only one surgical infection in the last 18 years of performing this procedure. Necropsies are routinely performed on animals in my protocols, and as part of that the superficial and deep neck tissues are examined for evidence of infection, making it very unlikely that any clinically significant infections were missed.

Because of this very low infection rate for this procedure, I was surprised by the change in recommendations for fur clipping in the current guide, which were not in the 1985 guidelines. The 1985 NIH Guide for the Care and Use of Laboratory Animals makes no mention of clipping fur prior to surgery, but does require aseptic technique. The 1985 guide further specifies that less stringent procedures may be used for minor surgery, defined as that not entering a body cavity or having the potential for permanent disability. The 1996 guide changed recommendations slightly in that it uses clipping fur as an example of aseptic surgery. The exact wording is "Aseptic technique includes preparation of the patient, **such as** (my emphasis) hair removal and disinfection of the operative site..." The 1996 guide also specifies that the guiding principle of aseptic technique is to reduce contamination of the operative site: "Aseptic technique is used to reduce microbial contamination to the lowest possible practical level." As with the 1986 guide, less stringent technique is required for minor surgery. The 1996 guide gives no rationale for the inclusion of hair removal in its guidelines.

I contacted two member of the committee that wrote the 1996 guidelines, Dr. Kathryn Bayne (AAALAC) and Dr. John VandeBerg (SW Foundation for Biomedical Research, San Antonio). Only Dr. Bayne could give me a rationale, citing the work of Bradfield, et. al. (Lab Animal Sci 1992;42:572-8) as the support for this change. I believe that Dr. Bayne and the rest of the committee overinterpreted the Bradfield paper, and as a result made a recommendation without firm scientific backing. Bradfield subjected rats to craniotomies or laparotomies using aseptic technique, including clipping the fur. The experimental group had their wounds painted with 10^8 cfu of either *Pseudomonas aeruginosa* or *Staphylococcus aureus* before wound closure, whereas the controls had no such application of bacteria. Rats were then subjected to behavioral tests before and for four days post-surgery, such as ability to maneuver a maze and response time to very loud noise. In addition, a variety of biochemical tests were performed on the rats, and finally skin biopsies of the surgical sites was performed four days post-procedure. Rats inoculated with bacteria had more difficulty using a maze, responding to loud noises, and turning a wheel than the uninfected animals; all but maze activity normalized by about day two post-surgery. Various biochemical differences were shown between the groups. Not surprisingly, infected animals had bacteriologic evidence of wound infection with the inoculated bacteria, as well as histologic evidence of wound inflammation. Of note, none of the control animals had wound colonization with *P. aeruginosa* or *S. aureus*. The authors claimed that the wound infections were "subclinical" , meaning that they could only be detected by histologic evidence of inflammation and had no clinical signs of infection. The authors then concluded that this experiment showed that aseptic technique was required for rat surgery. It is important to note that this was not a study of clipping fur versus not clipping fur. A subsequent letter to the editor by Speth (Lab Anim Sci 1996;46:5-7) raised issues of improper statistical analysis of the data, and the inapplicability of the use of wound inoculation with bacteria as a surrogate for non-aseptic surgery, some of which was contested by Bradfield. Dr. Van Hoosier, the journal editor remarked in his response to this letter that the conclusions of the study were perhaps too broad, and that the journal needed better oversight of statistical testing used in papers published in the journal. The editor stated that a better study of the importance of wound antisepsis was to be found in the study of Festing, et al (Lab Animals 1994;28:212). The Festing study compared subclavian vein catheter infection rates and complications in two groups of rats, both of which underwent aseptic surgery and had clipped fur. The experimental group underwent surgery without the use of surgical drapes, and had non-sterile catheters inserted in the veins. Post-operatively, the control group had aseptic technique used for catheter care and use, whereas no aseptic technique was used in the catheter care of the experimental animals. After 20 days, the experimental group had a higher catheter infection rate and mortality rate than did the controls. The authors of this study concluded that long term venous catheterization of rats requires the use of surgical drapes during catheter insertion, and aseptic catheter care. Again, this was not a study of clipping fur. Neither study shows that clipping animal fur prevents wound infections. The Bradfield study proved little other than that animals with wound infections behave differently from those who do have infections, and that a good way to cause wound infections is to inoculate high numbers of pathogenic bacteria into the wounds. These two studies provide no scientific evidence that supports a recommendation that fur clipping be used.

Hair removal has not been shown to reduce surgical wound infections in humans, and its removal by any method may increase the infection rate (cited in CDC Guideline for Prevention of Surgical Site Infection, 1999; Infect Control Hosp Epidemiol 1999;20:247-278). A number of human studies have shown that hair removal is not necessary to prevent surgical wound infection, including in complex neurosurgical procedures (Acta Neurochir 2001;143:533-537; Otolaryngol Head Neck Surg 2003;128:43-47; Arq Neuropsiquiatr 2004;62:103-107; J Neurosurg 2002;97:1476-1478). The U.S. Centers for Disease Control 1999 Guideline for Prevention of Surgical Site Infection recommends "Do not remove hair preoperatively unless the hair at or around the incision site will interfere with the operation. Category IA". Category IA evidence is "Strongly recommended for implementation and supported by well-designed experimental, clinical,or epidemiological studies".

While proper controlled studies have not been performed in animals, there are theoretical reasons why clipping animal fur prior to surgery could increase the infection rate similar to what has been observed in human studies. If this is true then both animal welfare and experimental results could be compromised. It is entirely possible that loose clipped fur could contaminate the wound to a greater degree than would non-clipped fur. Once clipped, fur is difficult to remove except by washing, and in addition circulates in the room air. Adding an additional step of washing the neck after clipping the fur could potentially compromise the effectiveness of the skin disinfectant by causing dilution of the disinfectant. In addition, fur dust circulating in the room could land on the disinfected surgical site, resulting in its recontamination.

It is possible that fur clipping could reduce my surgical infection rate below 0.1%, but if so this would be impossible to prove without a controlled study. Although my data are uncontrolled, the exceptionally low observed infection rate makes a controlled study unlikely to be useful. This is because tens of thousands of animals would need to be performed to show a benefit. To detect a 75% reduction in the wound infection rate from 0.1% to 0.025% would require a study with 10,000 animals in each group (α_2=0.05, 1-β=0.80). Since this is an infection model that causes severe pneumonia, any behavioral or biochemical abnormalities attributed to "subclinical" wound infection are not germane, and therefore their study would not clarify this point.

A critical point that needs emphasis here is that the surgical procedure that I perform is unlikely to result in wound infection, as it lacks the characteristics of wounds that get infected. In humans these characteristics include prolonged surgery, tissue injury through ischemia or use of an electrocautery, inadequate skin disinfection, operation on a hollow viscus or mucosal site, operation of an infected site, extensive hemorrhage at the operative site, and leaving artificial material in the wound (catheter or drain) (Cruse, Chapter 18 In: Howard RJ, Simmons RL, Surgical Infectious Diseases, 2nd ed. Appleton and Lange, 1988). There may be a role for animal fur clipping in wounds at risk for wound infection, but certainly not for clean minor short duration surgery. Proper disinfection and surgical technique excellence are additional factors that could be weighed. The failure of the current animal care and use guide to make this distinction is unfortunately interpreted by regulatory agencies as requiring fur clipping for all surgical procedures.

In addition to potentially harming animals, fur clipping has potential human hazards. Clipping fur causes gross air contamination with animal dander, a major risk for those with dander allergies, and a major risk for the development of future allergies in animal handlers.

I respectfully ask that the future edition of the guide be written to acknowledge that animal fur clipping is optional especially for short duration simple clean surgery, and that it is of unproven benefit in the prevention of surgical wound infections. The recommendation should be worded to allow flexibility in application of this specific guideline by local IACUCs and by USDA inspectors.

Sincerely yours,

Paul H. Edelstein

Paul H. Edelstein, M.D.

Enclosed: Copies of Acta Neurochir 2001;143:533-537; Otolaryngol Head Neck Surg 2003;128:43-47; Arq Neuropsiquiatr 2004;62:103-107; J Neurosurg 2002;97:1476-1478; CDC Guideline for Prevention of Surgical Site Infection, 1999; Lab Anim Sci 1996;46:5-7; Lab Animal Sci 1992;42:572-8

NAME: Paul Edelstein, M.D./Univ. of PA Med. Ctr

1. ARTICLE/CONTENT: Guideline for Prevention of Surgical Site Infection

SOURCE: Vol. 20 No. 4, 1999

2. ARTICLE/CONTENT: The Effect of Hair on Infection after Cranial Surgery

SOURCE: Acta Neurochir 2001

3. ARTICLE/CONTENT: Implantation of deep brain stimulation electrodes in unshaved patients

SOURCE: J Neurosurg./Vol. 97, Dec. 2002

4. ARTICLE/CONTENT: Craniotomy without tricotomy

SOURCE: Arq Neuropsiquiatr 2004

5. ARTICLE/CONTENT: A role of hair shaving in skull base surgery

SOURCE: 2003 American Academy of Otolaryngology

6. ARTICLE/CONTENT: Behavioral and Physiological Effects of Inapparent Wound Infection in Rats

SOURCE: Lab Animal Science, 1996

#-31

From: Ken Boschert, DVM [ken@dcm.wustl.edu]
nt: Wednesday, February 08, 2006 4:26 PM
. Scientific Affairs (NIH/OD)
ubject: Notice Number: NOT-OD-06-011

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184 , MSC 7983
Bethesda , MD 20892-7983

February 8, 2006

To whom it may concern:

While new information about various lab animal species, procedures, equipment, etc., is generated each year, much less is known about what their ultimate impacts are on research animals. For example, I've observed completely opposite reactions to various recent recommended procedures and practices simply based on the genetic diversity of mice alone.

Since the previous version of the Guide is "performance-based", clearly that is the best general approach (period). From that foundation of principles and guidelines, I believe professional judgement is adequate for now and that it would be counterproductive to throw any new ideas or concepts into the current setup. Better to focus on whether existing Guide recommendations are being followed.

What evidence, other than the simple passage of time, does anyone have that the current Guide is outdated or no longer applies? Yes, there are some new references, but these ~~ould be collected and provided as an update on some website to supplement the current de. Bottom line is there have been very few significant changes in laws or regulations the past 20 years and thus, any global changes in the Guide based on new and relatively unproven techniques or equipment are simply not warranted.

And please don't change the Guide just for the sake of change. The job of applying and enforcing the Guide, AWA, GLP, CDC, etc. etc., is already difficult and expensive for many institutions. Adding various new regs/guides, etc., only adds to the stress of running animal facilities and maintaining collegial relationships with investigators and compliance among all personnel. New regs/guides may stretch the budgets and capacities of various people and equipment already under enough pressure or force some research to never see the light of day if new regulations price it out of consideration. Grant funding is not exactly what it was just a few years ago. Changes without signficant justification are not desireable.

In my 22 years of professional experience, animal welfare as a whole is generally well-covered with the current Guide and everyone's time and efforts would be better served implementing those performance standards and current PHS principles rather than adapting to whatever is new and unproven. Please don't punish the lab animal working community unless there are significant reasons for improving the lot of research animals as well as the people working with them.

Speaking as a citizen with >50% of his paycheck funding all sorts of government efforts, I find it curious to observe the general public's solicitation for their reasons to determine your agenda. If changes were obvious or necessary, would you really have to ask? I suggest it is a wiser use of tax money to let those instinctive, bureaucratic urges rest a while longer, preferably sometime much closer to your and/or my retirement. Thank you for your consideration and good luck with your deliberations.

Sincerely,

n Boschert, DVM

--
Ken Boschert, DVM <ken@dcm.wustl.edu>
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#22

Scientific Affairs (NIH/OD)

From: Dale.Martin@sanofi-aventis.com
Sent: Wednesday, February 08, 2006 1:56 PM
To: Scientific Affairs (NIH/OD)
Subject: Input for Guide revision
Attachments: ILAR Chapter 3.doc; ILAR Chapter 4.doc; ILAR Guide General Comments.doc

Dear NIH-

The Guide needs to be revised. Below are some brief comments and additional references for the introduction and two Chapters of the Guide. Given more time, I could come up with a much more comprehensive list of references or many more examples where the science and welfare of animal research has progressed way beyond what the 1996 Guide.

Kind Regards,

Dale Martin, DVM, PhD, ACLAM, ECLAM, ACVPM
Regional Director, US Laboratory Animal Science and Welfare
sanofi-aventis

Past President, ACLAM

Currently President, Council on Accreditation, AAALAC International

ILAR Chapter 3

General Comments- Although surgery and post-surgical care is mentioned in detail, other post-procedural care is not prominently mentioned. In general, oversight by the IACUC and Veterinary Staff in all areas of post-procedural care is where there is room for major improvements in many animal care and use programs. Post-procedural care outside the central animal facility is where the greatest need is. Little attention is also given to maintenance of adequate medical records (many times to document post-procedural care). With the massive increase in rodents and Investigators (in many cases) delegated the responsibility for most post-procedural care, more guidance and principals should be given in this area.

The term "transgenic mice" throughout document should be replace with something more generic like "genetically engineered animals" The creation of animals with abnormal phenotypes which could predispose them to overt disease needs more attention in Chapter 1 & 2 as well as Chapter 3 Veterinary Care.

Specific Comments-

Page 56

- add bullet for Adequate Medical Records (Note new reference on ACLAM Recommendations for Medical Records)
- Certified (see ACLAM, add ECLAM and JACLAM add ECLAM and JACLAM info in appendix)
- End of paragraph at bottom of page- add adequate oversight post-procedure care (beyond post-surgical care) and the necessity of maintaining adequate medical records for this.

Page 57

- recommend delete the second sentence. Very, very few still use class B dealers for dogs and cats. Stating that all transactions involving animal procurement are conducted in a lawful manner is enough.
- Replace "transgenic mice" throughout document with something more generic like "genetically engineered animals" Animals now can be transgenic, knock-in, knock-out, or genetically altered in many ways.
- Update latest transportation documents/resources. Transportation requirements should include all appropriate international references.

Page 58

- update references for primate quarantine and stabilization periods.

Page 59

- Most of the references for examples given on page 59 are from the 1960s. Eliminate some/all of these examples. Use newer examples with recent references.
- Surveillance, Diagnosis, Treatment, and Control of Disease -- at least in AAALAC, International. The mantra was- every animal must be viewed every day. Although, this paragraph refers to not observing animals every day (when it is not practical) then gives the example of herds in an outdoor setting. This could also be said of some rodent colonies that number >100,000 in some universities.

Page 60

- The statement "Methods of disease prevention, diagnosis, and therapy should be those currently accepted in veterinary practice." Is outdated, and may only apply to a very small percent of a research institute's population. Since >90% of most research animals are rodents. (and treatment of most rodents ....euthanasia or serologies/PCRs not resembling practice. Moreover, most of the "large" animals are not dogs any more!
- Examples of subclinical rodent issues needs to be updated.
- Delete information on MAP/RAP/HAP testing...unless anyone knows anyone that has done these tests in the last 5 years! Updated alternatives are commonplace.

Page 60 Bottom (SURGERY) General comments. Much detail provided, however, since the majority of surgery now is performed on rodents, more information on rodent specific surgery (i.e. use of bead sterilizers, instrument tip surgical procedures) could be included. Also could refer much of large animal surgical procedures to Ag Guide.

Page 61/62

- Point of discussion—
  - Would castration be still considered a minor surgical procedure, especially if it was not done on a very young animal? Besides the "invasiveness" of the procedure, it also produces a change in physiologic function. (maybe this is a guy thing and I am overly sensitive to topic?!)
  - Hair removal in rodents prior to surgery. --- Is there really evidence that this improves outcomes in rodents?
  - Use of alcohol as a disinfectant prior to surgery.--- Some have argued and presented data that this is OK. Are there any definitive references on this now?
- Include reference for newer Gas Sterilization processes (i.e. Hydrogen Peroxide Plasma Sterilization) becoming very popular. Much less toxic than Ethylene Oxide. Byproducts CO2 and water. Does not need to be vented. Sits on tabletop.

Page 64-65 Pain, Analgesia and Anesthesia. The principals remain the same. Update references where available.

Page 65-66 Euthanasia- principals still the same. Update AVMA Panel on Euthanasia.

Below are some general references in this area that are new since the last Guide.

2005 ACLAM Position Statement on Medical Records for Animals Used in Research, Testing and Training. I think this was published in in AALAS Journal and/or AVMA.....I could not find the reference

Report of the American College of Laboratory Animal Medicine on Adequate Veterinary Care in Research, Testing, and Teaching. 1996. (Adopted September 1996) ACLAM, 200 Summerwinds Drive, Cary, NC 27511. http://www.aclam.org/pub_adquate_care.html

A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 21(1):15-23. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van de Vorstenbosch C (European Federation of Pharmaceutical Industries Association and European Centre for the Validation of Alternative Methods). 2001.

Council Directive 94/55/ED of 21 November 1994 on the approximation of the laws of the Member States with regard to the transport of dangerous goods by road. Official Journal L 319, 28/10/1996 p 0001 et seq.

Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (NRC 2003). http://www.nap.edu/catalog/10732.html

Guidelines for the prevention and treatment of B Virus infection in exposed persons.
Clinical Infectious Diseases, 1995, 20:421-439.

Report of the AVMA Panel on Euthanasia;
JAVMA, volume 218, no. 5, pages 669-696, March 1, 2001 (et seq.). AVMA, 930 N. Meacham Rd., Schaumburg, IL 60196. 800/248-2862.
http://www.avma.org/resources/euthanasia.pdf

Euthanasia of Experimental Animals. European Commission. DGXI, 1995. (Adopted May 1999)

*Note: references 33 through 44 are **FELASA Guidelines**. Visit http://www.felasa.org or the specific page links listed below.*

FELASA Guidelines: **Health monitoring of rodent and rabbit colonies in breeding and experimental units** http://www.lal.org.uk/pdffiles/LAfel2.PDF

FELASA Guidelines: **Health monitoring of breeding colonies and experimental units of cats, dogs and pigs**
http://www.lal.org.uk/pdffiles/LAfel1.PDF

FELASA Guidelines: **FELASA recommendations on the education and training of persons working with laboratory animals: Categories A and C** http://www.lal.org.uk/pdffiles/lafel7.pdf

FELASA Guidelines: **Health monitoring of non-human primate colonies**
http://www.lal.org.uk/pdffiles/LAfel5.pdf

FELASA Guidelines: **Pain and distress in laboratory rodents and lagomorphs. Laboratory Animals** (1994) 28: 97-112.

Chapter 4

General Comments-
Most of the Chapter contains information that is useful when institutions want to build an animal facility. More could be added in areas where significant issues arise in animal care and use programs.

The top of Page 72 states *"if needed, measures should be taken to minimize occupational hazards related to exposure to animals."* Much more should be included. Note the Guide now has sub-sections on "Noise Control"and "Storage Areas", but no section on Occupational Exposure to Hazards. Complete sections could be added to discuss principals in the following areas--

- Animal Allergen Control- Engineering controls to decrease exposure to animal allergens. (i.e. use of ventilated racks, hard-ducting exhausts from rodent rooms, down-draft tables, dump stations in cage-wash areas etc.)
- Hazardous Use Facilities- BSL 2-4 facilities/principals. The BMBL has much of the information, however, with the increase in BSL 2-4 work, and select agents, some of the building principals should be included in the Guide. The discussion of potential need for HVAC HEPA filtration should be discussed.Engineering controls to limit other Hazards from animals to include—exposure of animals to radiolabelled compounds, cytotoxic compounds.

Waste Management- There are many new realities in local, state and country legislation that require increased attention to waste management. Some general principals, information and references in Chapter 4 would be appropriate.

Security. Very important part of any animal care and use program, but not mentioned in any detail is Security. Perhaps the discussion on a comprehensive security program should be in Chapter 1. (or the introduction). Never the less, facility specific guidance should be enhanced in Chapter 4.

Page 72
Functional Areas- 5th bullet. Add Bioimaging to the list of activities that should be located near animal housing units.

Some International References

Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. Official Journal L117, 08/05/1990 p 0015-0027.

Council Directive on the Introduction of Measures to Encourage Improvement in the Safety and Health of Workers at Work (Directive 89/391/EEC), 1989.

Council Directive on the Protection of Workers from Risks Related to Exposure to Biological Agents at Work (Directive 90/679/EEC), 1990.

Guide General Comments- Intro Chapter 1,2

Although, on areas not specifically assigned. Some quick thoughts.....

Introduction

Page 2- It might be feasible/desirable to use International Principles (1985 CIOMS) in addition to--- or in place of US Government Principals. The list of guiding principals are almost identical. Council for International Organizations of Medical Sciences

The Council for International Organizations of Medical Sciences (CIOMS), www.cioms.ch, an international nongovernmental organization, published the "International Guiding Principles for Biomedical Research Involving Animals" in 1985, which has provided basic guidelines for many countries.

Page 2- only state that "animal facilities should be operated within Guide, all applicable local, state and country regulatory requirements." (The list of requirements could be in the appendices. US and International)

Chapter 1. Institutional Responsibilities
Security/Crisis Management--A section should be added to include developing programs to protect the institution from activits/terroists. This could include strategies for HR, Security, Communications, Public Policy, EHS. Also guidelines/tips/references on how to prevents infiltrations, provide adequate physical protection etc. would be useful.

IACUC could be changed to a generic term like Animal Oversight Committee. (then list examples....IACUC, Ethical Committee etc.)

More emphasis on post-procedural oversight should be included. Many of the animal health and welfare issues occur outside of the central animal facility and relate to procedures performed which may/may not be consistent with approved protocols. An additional paragraph could provide more guidance on oversight by the Committee and attending veterinarian for Post-procedure care and oversight. The oversight should go beyond post-surgical monitoring....which is covered fairly well---(which the notable exception of the maintenance of adequate records).

More emphasis/information on exposure to animal allergens, strategies to limit allergen exposure etc. should be included.

Replace terminology "transgenics" with something more generic (i.e. genetically modified animals).

Food and Fluid Restriction—should align with new Neuroscience Red Book.

Veterinary Care should be consistent with 1996 ACLAM reference "Adequate Veterinary Care."
Report of the American College of Laboratory Animal Medicine on Adequate Veterinary Care in Research, Testing, and Teaching. 1996.
http://www.aclam.org/pub_adquate_care.html

Can add CMAR to list of examples of AALAS certifications.

Chapter 2

Page 24- states that the purpose in utilizing ventilated caging is to minimize the spread of infectious disease. It should also be mentioned that this also decreases allergen load. For some institutions, this is the primary reason they went to ventilated racks.

Behavioral management recommendations - should be consistent/align with new Neuroscience Red book. Same comment for calorie restriction. Primate recommendations should be consistent with 1998 reference.

Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (NRC 2003). http://www.nap.edu/catalog/10732.html

The Psychological Well-Being of Nonhuman Primates.
National Academy Press, Washington, DC, 1998. National Academy Press, 2101 Constitution Ave., NW, Lockbox 285, Washington, DC 20055. 800/624-6242.
http://pompeii.nap.edu/books/0309052335/html/index.html

Page 44
Assessing the Effectiveness of Sanitation-
Cite new methods.

Page 46-47
Principals should be consistent with 2005 ACLAM reference on Recommendations for Medical Records.

NOTE NEW BMBL will be out in late 2005 or early 2006
Biosafety in microbiological and biomedical laboratories.
DHHS Pub. No. (CDC) 93-8395, May 1999. Division of Safety, NIH, Bldg. 31, Rm. 1C02, Bethesda, MD 20892. 301/496-2801.
www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

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DEPARTMENT OF COMPARATIVE MEDICINE • QUAD 7, BUILDING 330, STANFORD, CA 94305-5410
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Margaret Snyder
Director, Office Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge 1, Suite 4184, MSC 7983
Bethesda, MD 20893-7983

Feb 9, 2006

Dear Ms. Snyder,

Please find enclosed three copies each of six different articles on the care, housing and diseases of laboratory *Xenopus* I am submitting in response to RF No. NOT-OD-06-011.

Attached is my business card should you need to contact me.

Sincerely,

Sherril Green, DVM, PhD, Diplomat ACVIM
Director Clinical Services, Veterinary Service Center
Stanford University School of Medicine
Stanford, CA 95401

Enc: six articles on laboratory *Xenopus*

NAME:	Sherril Green, Stanford University School of Medicine
ARTICLE/CONTENT:	Postoperative Analgesics in South African Clawed Frogs after Surgical Harvest of Oocytes
SOURCE:	Comparative Medicine, Vol 53, No. 3, 2003
ARTICLE/CONTENT:	Cryptosporidiosis Associated with Emaciation and Proliferative Gastritis in a Laboratory-Reared South African Clawed Frog
SOURCE:	Comparative Medicine, Vol. 53 No. 1, 2003
ARTICLE/CONTENT:	Disease Attributed to Mycobacterium chelonae in South African Clawed Frogs
SOURCE:	Comparative Medicine Vo. 50, No. 6, 2000
ARTICLE/CONTENT:	Identification and management of an outbreak of Flavobacterium meningosepticum infection in a colony of South African clawed frogs
SOURCE:	JAVMA, Vol 214, No. 12 June 15, 1999
ARTICLE/CONTENT:	Factors Affecting Oogenesis in the South African Clawed Frog
SOURCE:	Comparative Medicine, Vol 52, No. 4, 2002
ARTICLE/CONTENT:	Thermal Shock in a colony of South African Clawed frogs
SOURCE:	The veterinary Record, March 15, 2003

#29

Scientific Affairs (NIH/OD)

From: Merel Ritskes-Hoitinga [e-mail]
Sent: Sunday, February 12, 2006 3:37 AM
To: Scientific Affairs (NIH/OD)
Subject: FRI No. NOT-OD-06-011
Attachments: Address.doc; NIH-guide.doc

Dear Dr. Margaret Snyder,

Attached please find my personal response, giving references in response to your request for updating the 1996 Guide.

In case you would need paper copies of these mentioned references, please let me know.

The organisation FELASA (www.felasa.org) will also send a separate response, this will be sent to you by the secretary Javier Guillen,

Kind regards,

Merel Ritskes-Hoitinga
Professor in Laboratory Animal Science

Merel Ritskes-Hoitinga
Prof. in Laboratory Animal Science
President FELASA www.felasa.org
231 Centraal Dierenlaboratorium (CDL)
Universitair Medisch Centrum (UMC) St Radboud
PO Box 9101
NL-6500 HB Nijmegen
The Netherlands
Tel. +31(0)24 36 13 557
Fax +31(0)24 36 16 375
M.Ritskes@cdl.umcn.nl

NOT-OD-06-011

Please find input of new scientific information of relevance for the NIH guide. In case you wish paper copies of these articles, please let me know.

New scientific information regarding housing/structural environment:

Providing male rats of inbred strains with nesting houses of the proper size, shows that nest building behaviour is still practised by laboratory rats, reduces aggression and improves reproduction succes:

Jegstrup IM, Vestergaard R, Ritskes-Hoitinga M. Nest building behaviour in male rats in three inbred strains: BDIX/Orl Ico, BN/HsdCpb and Lewis/Mol. Animal Welfare 2005, 14, 149-156.

Jegstrup I-M, Ottesen JL & Ritskes Hoitinga J (2002) Behaviour and welfare benefits from enriching rat cages: Recommendations for housing based on natural behaviour of the rat. Federation of European Laboratory Animal Science Associations, 8th FELASA Symposium, June 17th-20th 2002, Aachen, Germany

Merel Ritskes-Hoitinga, Line Bjoerndal Gravesen & Inger Marie Jegstrup. Refinement benefits animal welfare and quality of science. To be published as from 1 March 2006 on the website of the National Centre for the Replacement, Refinement and Reduction of animals in Research (NC3Rs), www.nc3rs.org.uk/felasa.

Refinement of handling:

Animal technician Camilla has developed an alternative method for fixation of rats, which is more comfortable for the person doing the job and therefore for the animal. Also, the head of the rat is under a cloth, thereby making the animal calmer.

Rasmussen C, Ritskes-Hoitinga J. An alternative method for rat fixation when giving subcutaneous, intramuscular and intraperitoneal injections (Camilla's method). Scandinavian Journal for Laboratory Animal Science 1999;26(3): 156-159.

Acidification of drinking water:

The influence of the pH on bacteriological quality and (reduction in) water intake is dicussed in:

Ritskes-Hoitinga J, Meijers M & van Herck H: Bacteriological quality and intake of acidified drinking water in Wistar rats is pH-dependent. Scandinavian Journal for Laboratory Animal Science (1998), 25(3), 124-128.

Transport stress and acclimatisation:

Van Ruiven R, Meijer GW, van Zutphen LFM & Ritskes-Hoitinga J: Adaptation period of laboratory animals after transport: a review. Scandinavian Journal for Laboratory Animal Science (1996) 23: 185-190.

Van Ruiven R, Meijer GW, Wiersma A, Baumans V, van Zutphen LFM & Ritskes-

Hoitinga J: The influence of transportation stress on selected nutritional parameters to establish the necessary minimum period for adaptation in rat feeding studies. Laboratory Animals (1998) 32: 446-456.

Phenotyping:

An inventory of reports sent to the Danish inspectorate have indicated that about 1/3 of the genetically modified strains may have welfare problems:

Thon R, Lassen J, Hansen AK, Jegstrup IM, Ritskes-Hoitinga J. Welfare evaluation of genetically modified mice in Denmark. An inventory study of the reports from 1998 to the Animal Experiments Inspectorate. Scandinavian Journal for Laboratory Animal Science 2002, 29(1) 45-55.

A literature review on which phenotyping schemes are available:

Jegstrup I, Thon R, Hansen AK & Ritskes-Hoitinga M. Characterization of transgenic mice – a comparison of protocols for welfare evaluation and phenotype characterization of mice with a suggestion on a future certificate of instruction. Laboratory Animals 2003, 37, 1-9.

FOOD

Nutrient Requirements

Because there is a clear relationship between dietary P concentration and the occurrence of nephrocalcinosis in rabbits, the recommended minimum dietary P level for rabbits ought to be turned into the maximum allowed level:

Ritskes-Hoitinga J, Grooten HN, Wienk KJ, Peters M, Lemmens AG, Beynen AC. Lowering dietary phosphorus concentrations reduces kidney calcification, but does not adversely affect growth, mineral metabolism, and bone development in growing rabbits. Brit. J. Nutr. 2004, 91(3), 367-376.

Ritskes-Hoitinga M, Skott O, Uhrenholt TR, Nissen I, Lemmens I, Beynen AC: Nephrocalcinosis in rabbits – a case study. Scandinavian J. for Laboratory Animal Science 2004, 31, 143-148.

Diet and proper experimental design:

It is essential that a good choice of experimental diets and – design is performed in order to obtain good health of the animals, and to obtain standardised, reliable and reproducible results.

Ritskes-Hoitinga J, Jilge B. Felasa quick reference paper on laboratory animal feeding and nutrition. www.felasa.org/working/nutrition.rtf. 2001

Ritskes-Hoitinga J, Chwalibog A. Nutrient Requirements, experimental design and feeding schedules in animal experimentation. In: Handbook of Laboratory Animal

Science, CRC Press (2nd. Edition). Editors: Jann Hau and Gerald van Hoosier. (2003).

Ritskes-Hoitinga J. Nutrition in laboratory mice. In: The Handbook of Experimental Animals, The laboratory mouse. Chapter 28. Editor H. Hedrich. Academic Press. 2004.

Dietary regimes and welfare

When feeding animals, it is essential to take into account species-specific characteristics (especially when feeding restrictedly) in order to obtain reliable results and maintain good welfare.

Ritskes-Hoitinga J, Strubbe J. Nutrition and animal welfare in: The welfare of laboratory animals. Editor Eila Kaliste. Kluwer Academic publishers. 2004, pp. 51-80.

Ritskes-Hoitinga J, Schledermann C. A pilot study into the effects of various dietary restriction schedules in rabbits. Scandinavian Journal for Laboratory Animal Science 1999;26(2): 66-74.

Krohn TC, Ritskes-Hoitinga J & Svendsen P: The effects of feeding and housing on the behaviour of the laboratory rabbit. Laboratory Animals (1999), 33, 101-107.

#30

Scientific Affairs (NIH/OD)

From: janelle.townsend@dpi.nsw.gov.au
Sent: Sunday, February 19, 2006 7:37 PM
To: Scientific Affairs (NIH/OD)
Cc: peter.johnson@agric.nsw.gov.au; m.rose@unsw.edu.au; lynette.chave@agric.nsw.gov.au
Subject: RFI No. NOT-OD-06-011
Attachments: Edited Draft Guidelines for the housing of guinea pigs in scientific institutions.doc

Dear Dr Snyder,

In response to the NIH call for information on guidelines for the care and housing of laboratory animals, forwarded to Assoc Professor Margaret Rose, Chair of the NSW Animal Research Review Panel (ARRP), the following information may be of interest and assistance in reviewing your guidelines.

- Housing Rabbits in Scientific Institutions
- Care and Housing for Dogs in Scientific Institutions
- Housing of Rats in Scientific Institutions

(These three guidelines are available from the Animal Ethics Infolink:
http://www.animalethics.org.au/reader/animal-care)

- Housing of Guinea Pigs in Scientific Institutions

(Please note this document is in draft and is currently under peer review prior to being circulated for public critique)

Assoc Professor Rose requested that we pass on her best regards. If you need any assistance in accessing any of the material or would like it in any other format, please let us know and we would be happy to assist in whatever way we can.

Yours sincerely,

Janelle Townsend
Clerical Officer
NSW Department of Primary Industries
Animal Welfare Inspectorial Office
PO Box 100
BEECROFT NSW 2119
Ph: (02) 9872 0570
Fax: (02) 9871 6938

NAME:	Janell Townsend/Animal Welfare Insp. Office.
ARTICLE/CONTENT:	Guidelines for the Housing of Guinea Pigs in Scientific Institutions, Guideline 21 - February 2006
SOURCE:	Guideline 21 - February 2006

#31

Scientific Affairs (NIH/OD)

From: GUILLEN, Javier [jguillen@unav.es]
Sent: Wednesday, February 22, 2006 4:19 AM
To: Scientific Affairs (NIH/OD)
Subject: RFI No. NOT-OD-06-011
Attachments: NIH-Letter.pdf; Accreditation_LAS_EdU_TRNG.pdf; CategoriesA-C.pdf; CategoryB.pdf; CategoryD.pdf; DiagnosticLabs.pdf; HM-CatDogPig.pdf; HM-NHP.pdf; HM-RodentRabbit.pdf; Nutrition.pdf

Dear Sirs,
With regard to RFI No. NOT-OD-06-011: Standards for the Care and Use of Laboratory Animals (GUIDE revision).
The Federation of European Laboratory Animal Science Associations (FELASA) acknowledges the NIH initiative related to exploring the need to update the laboratory animal welfare standards of the *Guide for the Care and Use of Laboratory Animals (Guide)*.
You will find enclosed several documents produced by FELASA that are well considered standards not only in Europe but also in other parts of the world. Most of these documents may be downloaded from www.felasa.org. Other Recommendations about Ethical Committees and Standarization of Enrichment will be available soon.
FELASA is also open for further collaboration that may result in a deeper internationalization of all standards for the care and use of laboratory animals.<>
Yours sincerely,

Javier Guillen
Hon. Secretary
FELASA

Javier Guillen, DVM
Director
Animal Services Unit
Universidad de Navarra
Pamplona (Spain)
tel.: 34 948 194700 (CIMA)
34 948 425653 (CIFA)
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34 948 425652 (CIFA)
jguillen@unav.es

<>

Dear Sirs,

With regard to RFI No. NOT-OD-06-011: Standards for the Care and Use of Laboratory Animals (GUIDE revision).

The Federation of European Laboratory Animal Science Associations (FELASA) acknowledges the NIH initiative related to exploring the need to update the laboratory animal welfare standards of the *Guide for the Care and Use of Laboratory Animals (Guide)*.

You will find enclosed several documents produced by FELASA that are well considered standards not only in Europe but also in other parts of the world. Most of these documents may be downloaded from www.felasa.org. Other Recommendations about Ethical Committees and Standarization of Enrichment will be available soon.

FELASA is also open for further collaboration that may result in a deeper internationalization of all standards for the care and use of laboratory animals.

Yours sincerely,

Javier Guillen
Secretary
FELASA

Javier Guillen, DVM
Director
Animal Services Unit
Universidad de Navarra
Pamplona (Spain)
tel.: 34 948 194700 (CIMA)
34 948 425653 (CIFA)
fax: 34 948 194718 (CIMA)
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jguillen@unav.es

Attached documents:

FELASA recommendations for the accreditation of laboratory animal science education and training

FELASA recommendations on the education and training of persons working with laboratory animals: Category A and C

FELASA recommendations for the education and training of persons carrying out animal experiments: Category B

Education of specialists in laboratory animal science (Category D)

Health monitoring of breeding colonies and experimental units of cats, dogs and pigs

Health monitoring of non-human primate colonies

FELASA recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units

FELASA quick reference guide on nutrition

Accreditation of laboratory animal diagnostic laboratories

Other articles:

FELASA recommendations for the health monitoring of experimental units of calves, sheep and goats (Laboratory Animals 34: 329-350, 2000)

Pain and distress in laboratory rodents and lagomorphs (Laboratory Animals 28: 97-112, 1994)

Sanitary aspects of handling nonhuman primates during transport (Laboratory Animals 31: 298-302, 1997)

NAME:	Javier Guillen/Animal Services Unit
1. ARTICLE/CONTENT:	Recommendation for the accreditation of Lab animal science education and training
SOURCE:	Working Party Report, 2002
2. ARTICLE/CONTENT:	Recommendation on the education and training of persons working with lab animals
SOURCE:	Working Party Report, 2002
3. ARTICLE/CONTENT:	Recommendation on the education and training of persons carrying out animal experiments
SOURCE:	Working Party Report, 2002
4. ARTICLE/CONTENT:	Guidelines for education of specialists in laboratory animal science
SOURCE:	Working Party Report, 2002
5. ARTICLE/CONTENT:	Guidance paper for the accreditation of lab animal diag. laboratories
SOURCE:	Working Party Report, 2002
6. ARTICLE/CONTENT:	Recommendation for the health monitoring of breeding colonies and experimental units
SOURCE:	Working Party Report, 2002
7. ARTICLE/CONTENT:	Health monitoring of non-human primate colonies
SOURCE:	Working Party Report, 2002
8. ARTICLE/CONTENT:	Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units
SOURCE:	Working Party Report, 2002

#39

Felasa – Quick reference paper on laboratory animal feeding and nutrition

Text compiled: June 2000/ updates November 2000 and February 2001
By Prof. Dr. Merel Ritskes-Hoitinga (Mritskes@health.sdu.dk), Biomedical Laboratory, Odense University; and Prof. Dr. Burghart Jilge (burghart.jilge@ze.uni-ulm.de), Tierforschungszentrum, Ulm University.

Contents

Introduction

Practical experience from teaching in laboratory animal science courses has shown that students (and their supervisors) are often not conscious (enough) about the influence of diet and dietary composition on the health of the animals and experimental results. This sometimes leads to the execution of experiments in which the diets used are such, that the results do not have any meaning and cannot be published. This is inappropriate use of laboratory animals.

This overview will hopefully add to the understanding of the importance of laboratory animal nutrition, avoiding doing experiments using inappropriate diets and thus unnecessary use of laboratory animals. Thereby this short overview is expected to contribute to the refinement of animal experiments, one of the important goals of Felasa.

I. Diet and experimental results

a. nutrient requirements

In order to provide each species with the proper nutrient levels of essential nutrients, nutrient requirements must be fulfilled (National Research Council documents describe nutrient requirements for each species). Nutrient Requirements of minipigs are currently under investigation (Ritskes-Hoitinga & Bollen 1997, 1998a). By providing each animal with their species specific essential nutrients in the proper amounts, diseases can be prevented as well as unwanted interference with experimental results. In case essential nutrient requirements are not fulfilled, unreliable conclusions may be obtained (Ritskes-Hoitinga et al. 1996, Ritskes-Hoitinga 2000).

b. nutrient requirements in different (transgenic) strains
Different species, strains, stocks and individuals can have different nutrient requirements (National Research Council 1995). Regarding the enormous development of many new transgenic strains, it must be taken into consideration that depending on the nature of a transgenic strain, nutrient requirements may vary as well.

c. standardisation
Standard commercial diets usually fulfill nutrient requirements more than sufficiently, at least when transported and stored under the proper environmental conditions. However, there can occur a large variation in composition in natural-ingredient diets (between and within brand variation) due to raw material variation, which will differentially influence experimental results (Beynen et al. 1993, Ritskes-Hoitinga et al. 1991). As between-batch variation can occur, it is advised to buy diets with a batch-analysis certificate so that one is informed about the actual composition of each batch of diet that is being used. For GLP-studies this is a necessity.

Purified diets (Beynen et al. 1993), formulated with a combination of natural ingredients, pure chemicals and ingredients of varying degrees of refinement , have a more standardised composition and give therefore more reproducible results than the use of natural-ingredient diets. However, there is a higher risk of creating shortages of unknown essential nutrients, which are present as "natural contaminants" in natural-ingredient chow diets (e.g. Chromium and Vanadium, National Research Council 1995). Moreover, certain refined ingredients can cause problems (e.g. short-type cellulose fiber can cause intestinal obstruction in rats, Speijers 1987).

For rodents a "cook-book receipe" is available for composing a purified diet, the so-called American Institute of Nutrition diet (AIN-93 diet) (Reeves et al. 1993). The AIN-93 diet fulfills the nutrient requirements for rodents as published in 1995 (National Research Council), except for the vitamin B12 level. The AIN-93 vitamin B12 level must be doubled to live up to the minimum requirements as described by the National Research Council (1995).

Ad libitum food intake is in principle determined by the energy need. This energy need changes according to the stage of life the animal is in (growth, maintenance, pregnancy, lactation). When changing the energy content of the diet (e.g. by adding fat to the test diet), one changes the dietary intake in grams. In order to make sure that only the dietary fat (and carbohydrate) intake will differ between the control and test group, one needs to apply the isocaloric exchange method (Beynen & Meijer 1993).

d. feeding level
Ad libitum feeding is considered normal practice for rodents, however it is considered bad veterinary practice for e.g. pigs, monkeys, rabbits and dogs, as they become obese (Hart et al. 1995). When feeding restrictedly, it must be secured that the restricted feeding level provides enough essential nutrients. Although ad libitum feeding of rodents is considered "normal" practice, this must be questioned intensely. Ad libitum feeding as opposed to restricted feeding has a clear negative impact on rodent health, as it shortens survival time, increases cancer incidence, shortens cancer latency period and increases the incidence of degenerative diseases in kidney and heart (Hart et al. 1995). These effects are very reproducible! Moreover, it increases the number of animals needed if sufficient animals are to survive a 2-year period in long-term toxicological studies.

Keenan et al. (1999) state that ad libitum overfeeding of rodents is at present one of the most poorly controlled variables affecting the current rodent bioassay. Moderate dietary restriction (70-75% of adult ad libitum food intake) is advised as a method that will improve uniformity, increase exposure time and increase statistical sensitivity of chronic bioassays to detect true treatment effects (Keenan et al. 1999). However, moderate dietary restriction will only improve uniformity in individually housed animals, where there is control of individual food intake. A restricted amount of food in group-housed animals is expected to increase variation due to differences in individual food intakes, based on the hierarchy in the group. It will be the challenge to find restricted feeding schedules in group-housed animals, in order to fulfill the animals social needs as well.

e. contaminants

There are several documents stating maximum allowed concentrations of contaminants (GV-Solas 1980, Barqa 1992). One of the guidelines that give maximum limits, to which all toxicologists all over the world are referring to, are issued by the Environmental Protection Agency (1979). As different guidelines state different levels, what to choose as the "correct" maximum tolerated levels? Firstly, one has to decide which guidelines are most appropriate in the experimental setting one is working in. One might even have to develop specific institutional guidelines. Secondly, for each experiment one can do a literature search to figure out whether contaminants, and if yes, which will interfere with the specific purpose of that study. That way concrete maximum levels of specific contaminants can be established. Purified diets have lower contaminant levels than natural-ingredient diets.

II. Diet and well-being

a. welfare and enrichment

From preference testing it is known that rats prefer to work for food instead of obtaining it just like that. For each species there are certain species specific essential needs connected to searching and finding food (e.g. rooting of pigs). If these essential needs are not fulfilled, abnormal behaviour like stereotypies can occur (pigs can develop sham chewing). Enrichment of the environment is possible by letting the animals work and or search for food. Knowledge of the natural feeding time and behaviour are important factors to consider. The time of day at which a restricted amount of food is giving can be an important tool in providing a better welfare (e.g. in rabbits, Krohn et al. 1999). Giving food rewards are important tools to learn and train animals. Which food rewards are chosen and in what amounts need careful consideration: is there interference with the experimental results and or health of the animal?

Certain dietary schedules require individual housing. As individual housing opposes the well-being of social living species, alternative ways of feeding need to be considered. E.g. the animals can be individually fed for a certain period each day and then socially housed for the remaining part of the 24-hour period.

b. transport and acclimatisation

Knowledge of the species is important when transporting animals. Before transport, getting specialist advice for each particular species is needed: e.g. (mini)pigs will vomit when being fed just before transport. Rats and mice will acclimatise faster after transport, when food and water has been provided during the transport (van Ruiven 1996).

III. Diet and animal models

a. Choice of model and experimental conditions

Knowledge and choice of species and experimental (including dietary) conditions will have a major impact on results. The effect of linoleic acid on mammary tumour development in animal models depended on the model system used and type of parameters measured (Ritskes-Hoitinga et al. 1996). Feeding fish oil to rabbits to examine the possible positive influence of fish oil on atherosclerosis, resulted in liver pathology and more atherosclerosis on higher doses of fish oil. This was thought to be the result from the inability of the herbivorous rabbit liver to cope with the long-chained unsaturated fatty acids from fish oil (Ritskes-Hoitinga et al. 1998b).
Feeding by gavage is expected to cause stress, influences metabolism and will therefore lead to other results than voluntary intake (Vachon et al. 1988). Vachon et al. proved that voluntary intake of a certain meal gave results similar to the human, whereas giving the same meal by gavage, did not (Vachon et al. 1988)!

b. Diet and pharmacological studies
The effect/pharmacokinetics of pharmacological substances (e.g. oral antibiotics) are largely dependent on the time of administration in relation to the time of feeding. How long animals need to be fasted before the "bare" effect of pharmacological substances tested can be judged, is an important animal welfare issue (Claassen 1994). A rat will have an empty stomach already after 6 hours (Vermeulen et al. 1997). Fasting for longer periods led to increased locomotory and grooming behaviour (Vermeulen et al. 1997).

IV. The impact of a regular feeding schedule on circadian rhythms[(T)] of physiological and behavioral functions

[[(T)]: Some chronobiological terms are explained at the end of the text]

When individuals of several strains / species of rodents and rabbits are fed a long time ad libitum they tend to become fat, especially so with increasing age and limited space for physical workout (e. g. NZW rabbits kept in cages during longtime maintainance). In order to prevent excessive fattening, the quantity of food, thus, often is restricted: usually a limited amount of food is replenished every day during the working hours. Restricted animals start to eat immediately when food is presented and, in consequence, many biochemical and physiological functions of the gastrointestinal tract and even of the whole organism are phase-shifted in nocturnally active rodents and rabbits. Since the impact of shifted or even inverted circadian rhythms on experiments usually is underestimated this paragraph compiles some basic informations on that. Supplied with food ad libitum, nocturnally active animal species like mouse, rat, hamster and rabbit are consuming almost all of their food during the hours of darkness. Correspondingly many follow-up parameters are on a significantly higher level during the hours of darkness. The differences between the regular minimum and maximum as a rule are so great (can be up to several hundreds of percentages!) that it would be an artefact to ignore them. Few examples would be: mucosal enzymes in the small intestinal tract (Saito et al. 1975), carbohydrate absorption (Hara and Saito 1989), bile flow and composition (Ho and Drummond 1975), serum gastrin and cholecystokinin (Pasley et al. 1987) or serum insulin (Rubin et al. 1988).
When the time of food access is restricted, those functions which are coupled more or less directly to food ingestion are shifted to the time of food access, whether it is during some hours of light or of dark time. This means, that periodic food access can override the light:dark regimen which usually is the main ´zeitgeber´ [(T)] for circadian rhythms of animals and men (Philippens et al. 1977, Rubin et al. 1988, Saito et al 1976 a, b, Saito et al. 1980, Stevenson et al. 1975, Stevenson and Fierstein 1976). However, even many of those functions which are not obviously coupled to food intake, e. g. the 24 h rhythm of locomotor activity (Boulos and Terman 1980,

Boulos et al. 1989, Honma et al. 1983, Jilge et al. 1987, Jilge 1992, Jilge and Staehle 1994, Jilge and Hudson 2001), core body temperature (Jilge et al. 2000), corticosterone (Krieger 1974, Morimoto et al. 1979, Takahashi 1979), heart rate and blood pressure (van den Buuse 1999) are phase-shifted by a shifted feeding regimen.
There are functions, however, which are exclusively synchronized by the light-dark zeitgeber: the enzymes N-acetyltransferase and hydroxyindol-o-methyltransferase and the endproduct catalyzed by them in the pineal organ, melatonin (Reiter 1993, Tamarkin et al. 1985), the disc shedding rhythm of photoreceptors (LaVail 1976) and the mitotic index of the cornea (Burns et al. 1976).

There are two ways how restricted food access affects circadian rhythms:
1. masking and 2. entrainment[(T)] (Aschoff 1986, Aschoff et al. 1982, Aschoff and von Goetz 1986, Mrosovsky 1996, 1999, Pittendrigh and Daan 1976).

1. Masking

Masking means that a periodic environmental factor acts directly upon the overt rhythm without affecting the circadian oscillator[(T)] driving it. As a result the rhythm is synchronized immediately, without transients. When the circadian rhythm of locomotor activity, free-running in constant conditions, is exposed to scheduled food access, the activity rhythm immediately stops to free-run[(T)] and re-assembles around the phase of food access. When - e. g. several weeks later - food is offered ad libitum again, the circadian rhythm continues to free-run at the phase which it had without an interspersed food regimen. That means: periodically restricted food access has an effect on the activity rhythm without affecting the circadian oscillator (Abe et al. 1989; Aschoff and von Goetz 1986).

2. Entrainment

Entrainment means that an external variable like periodic food access has zeitgeber properties (for the definition of zeitgeber see: Aschoff 1958, 1960; Pittendrigh 1960). Scheduled feeding, thus, acts on the oscillator system itself which controls the timing of overt rhythms. The time needed for entrainment – following the instatement of a zeitgeber schedule - depends on the phase relation between the free-running rhythm and the zeitgeber schedule, i. e. the 're-arrangement' of the rhythm around the phase of food access occurs via transitory periods. Their number correlates with the phase relation between zeitgeber and circadian rhythm. As a general rule, the greater the phase difference between function and zeitgeber, the longer the time needed for entrainment. In general the time necessary for entrainment can last up to 50 – 60 days (Pittendrigh and Daan 1976; Jilge et al. 1987, Jilge and Staehle 1993, Jilge 2000). When returning to ad libitum food access again, a free-running rhythm starts out from the phase of the preceding food regimen. In that case, the period length of the free-running circadian rhythm is affected for a couple of cycles by the period length of the preceding zeitgeber. While the honey bee was the first animal in which entrainment of an oscillator with scheduled feeding had been proven (Beling 1929) a 'feeding-entrainable oscillator' (FEO) was shown to exist in some strains of mice, the hamster, rat, rabbit, pigeon, house sparrow and some marsupial species, the parameter recorded most frequently being the activity rhythm (Stephan 1986, Jilge et al. 1987, Jilge and Stähle 1993, Jilge and Hudson 2001, Coleman et al. 1989, Kennedy et al. 1991, Hau and Gwinner 1992, Jilge 1992, Mistlberger 1993, Philipps et al. 1993, Rashotte and Stephan 1996, Marchant and Mistlberger 1997, Challet et al. 1998, Stephan and Davidson 1998, Mistlberger and Marchant 1999, Lax et al. 1999). The FEO is a circadian oscillator in addition to and separate from the 'light entrainable oscillator' (LEO): even when the LEO had been destroyed, hamsters were entrained by periodic food access (reviewed by Mistlberger 1994). While the LEO in mammalian species is known to be located in the suprachiasmatic nuclei of the hypothalamus lying above the chiasma opticum and bilaterally symmetric to the third ventricle, we have no information so far about the location of FEO nor of its afferent and efferent pathways.

In those animals being entrained by periodic food access, in first instance some functions are rearranged immediately after the implementation of scheduled feeding, while simultaneously, but requiring a much longer time, entrainment and restitution of homeostasis of other functions is taking place "unnoticed" (so-called "masking"). The masking of physiological functions appears to be necessary for maintaining vital functions during the time-consuming process of achieving homeostasis for functions implying complete circadian reorganization.

Thus, when food access is restricted to only some hours during the day, one should keep in mind that many digestive and metabolic functions are brought out of phase, especially when nocturnal animals are fed during some hours of the light period. The process of re-entrainment around the phase of food access can require 50 – 60 days and physiological functions like locomotor activity, digestive functions and urine excretion will be affected during this time (Jilge and Stähle 1993)

There are however functions, e. g. the mitotic index of the corneal epithelium and the rhythm of pineal melatonin production which neither are entrained nor masked by periodic feeding but rather remain entrained with the light:dark zeitgeber. Different functions may become permanently internally desynchronized by restricted feeding schedules: the DNA synthesis of the thymocyte for example is coupled to restricted food access whereas the mitotic index in the cornea is not altered by restricted feeding (Pauly et al. 1976). So far we do not know enough about the consequences of the permant temporal displacement of functions e. g. on reproductive, immunologic, intermediary-metabolic or behavioral parameters. It may be that follow-up studies come to the conclusion that (certain) restricted feeding schedules threaten homeostasis.

- circadian rhythm (CR): periodic biological function with a frequency of 1 cycle per 24 $\pm$ 4 h. CR´s are generated endogenously in the suprachiasmatic nuclei (SCN) of the hypothalamus.
- circadian oscillator(system): Neurons generating a CR with a period of about but significantly different from exactly 24 h. The SCN are considered to be the ´masterclock´ of mammals which is entrained by an external zeitgeber. Since the light:dark cycle, entering the SCN via the retinohypothalamic tract (RHT) is the main zeitgeber for mammals, the SCN are referred to as light-entrainable-oscillator (LEO). As delineated above, in some species an additional oscillator has been described so far, which is entrainable by periodic food access. Hence, the name feeding entrainable oscillator (FEO) has been suggested.
- entrainment: synchronization of a CR by an external (or internal) periodic variable within a limit of 24 $\pm$ 4 h.
- free-running rhythm: circadian rhythm (e. g. of locomotor activity) in the absence of any external zeitgeber.
- zeitgeber: external, periodic variable entraining a circadian oscillator.

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#40

February 22, 2006

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Reference: RFI Number NOT-OD-06-011

Dear Dr. Snyder:

The undersigned veterinarians and scientists in the laboratory animal research community are pleased to comment on "Request for Information (RFI): Standards for the Care and Use of Laboratory Animals" (Notice Number NOT-OD-06-011, November 9, 2005), hereafter referred to as the RFI. The RFI specifically requests information related to the need to update the ILAR/NRC publication entitled the *Guide for the Care and Use of Laboratory Animals* (1), hereafter referred to as the 1996 *Guide*.

As individuals actively involved in laboratory research, we represent experience in the areas of laboratory animal medicine, research facility management, neuroscience research, animal welfare, and work in related academic, industrial and commercial settings. Two of the undersigned (Gonder, White) were on the National Research Council Committee that worked on the 1996 revision of the *Guide*. We have jointly examined available recent literature to determine whether new scientific evidence related to the conduct of animal research renders the information contained in the 1996 version of the *Guide for the Care and Use of Laboratory Animals* outdated. We have reviewed literature related to the major topic areas contained in the *Guide* - institutional policies and responsibilities; animal environment, housing, and management; veterinary medical care; and physical plant. We also looked at scientific advances in new topics of laboratory animal care related to state-of-the-art animal research programs. We did not, however, do an exhaustive search of the literature - that task would necessarily fall on the Committee that is charged to write a revision of the *Guide*. We did perform a cursory literature analysis and the results are reported herein. Upon reviewing the literature in this limited search, we found compelling evidence to support a revision of the *Guide* and submit our comments for your review.

Respectfully Submitted,

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DISCUSSION

In some cases recommendations and standards presented in the 1996 *Guide* have been interpreted, clarified and expanded by both the US Department of Agriculture [(USDA), indirectly through enforcement of the Animal Welfare Act Regulations (2)] and publication of the USDA Policy Manual (3), and by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) (www.aaalac.org). Decisions by both USDA and AAALAC have served to identify areas and statements in the 1996 *Guide* that warrant critical review to provide clarification, update or change in the recommendations. Much of the guidance for the care of laboratory animals is based on "best practice" which is more a function of expert opinion and traditional methods than on published data. Each edition of the *Guide* has relied on published data where available. However the science of laboratory animal care as related to research outcomes is not a primary field of study. Even so, when new scientifically-based information appears, it should be evaluated and related to recommendations made in the *Guide* to determine whether those recommendations are still valid.

The following discussion addresses NIH's request for new information and knowledge related to the four chapters of the 1996 *Guide*: 1) institutional policies and responsibilities; 2) animal environment, housing, and management; 3) veterinary medical care; and 4) physical plant. The discussion focuses on science-based information or scientific principles concerning the humane care and use of laboratory animals developed and widely accepted by the research community and not addressed in the 1996 *Guide*. Also presented is newly published science-based information on standard practices for animal environment, housing, management and structural design not cited in the 1996 *Guide*, and other citations for articles published in reputable peer reviewed scientific journals since the development of the 1996 *Guide* (organized in topic areas listed in Appendix A, Selected Bibliography, of the 1996 *Guide*).

Chapter 1 – Institutional Policies and Responsibilities

A. Animal Care and Use Committees

In the decade since publication of the 1996 *Guide*, there has been much discussion regarding the broad responsibilities and oversight of the institutional animal care and use committee (IACUC) with respect to various components of an animal care and use program. In practice these responsibilities have grown beyond just oversight, to direct involvement in implementation. These expectations have been redefined in many settings, and have become the standard of practice that is now in place (4-5), and enforced by the USDA and applied by AAALAC in the accreditation process. Any revision of the *Guide* would need to address these standards and clarify current program expectations.

Expectations for the IACUC in the area of research protocol review have increased. Previous understanding that the IACUC would not be involved in the assessment of scientific merit has changed. In fact, IACUC's, more and more, have been expected to question research approaches and techniques in greater depth. The approach to such review is presented in the NRC report, *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (6), that details the development of animal protocols involving genetically-modified animals. The IACUC is expected to solicit information concerning assessment strategies and endpoints for animals that may spontaneously develop problems that are debilitating or painful. Specifically, in the past decade since the 1996 *Guide,* there is the need for IACUC's to have guidance concerning the determination of estimated numbers of animals to be used in genetically-modified animal experiments (not including experimental manipulations). Several other recent articles provide a basis for developing guidance in this area (including strategies for refinement) (7-10).

Many examples of discussion and validation of humane endpoints have been published (11-22). These expectations have a direct impact on the appropriate provision of veterinary medical care and on protocol review. A critical evaluation of these and other references would serve to provide guidance on these issues that is not currently in the 1996 *Guide.*

Other areas that have come under greater scrutiny, and thus a better understanding, are topics such as antibody production (23-30), introduction of cell lines and other biological materials into animals, population management (see comments under Chapter 2), procurement of surgically-modified animals and oversight of animals kept at other facilities. Expectations of the IACUC in these areas would need to be clarified in any revision of the *Guide*.

B. Occupational Health and Safety of Personnel

The 1996 *Guide* greatly expanded the expectations for providing a sound occupational health and safety program for employees. Since 1996 much has been written on how to appropriately design and implement such a program. Many of the broad recommendations in the 1996 *Guide* have been better defined and put into standard practice. Most notable was the publication, shortly after the 1996 *Guide*, of the ILAR/NRC publication, *Occupational Health and Safety in the Care and Use of Laboratory Animals* (31). This and other more recent publications greatly enhance our understanding of how to fulfill our responsibilities for providing a safe workplace. They include specific information on special considerations for nonhuman primates (32-34), occupational medicine programs (35-36), control and prevention of allergy (37-46), ergonomics (47-49), chemical safety (50) and use of personal protective equipment (51-52).

The events of September 11, 2001, have also had a significant impact on the management of animal research programs. Research involving biohazards has increased, as has the level of regulatory control (53-57), institutional oversight (58-60), security (and biosecurity) (61), personnel safety (62-64), and emergency response preparedness (65). Guidance in preparing for natural disasters is also needed (66-68).

Chapter 2 – Animal Environment, Housing, and Management

A. Cage Space

Cage space requirements for common laboratory animals have been difficult to determine and in previous issues of the *Guide* have been based on limited data coupled with existing practice and professional judgment based on real or perceived adverse findings. In the absence of scientific data to the contrary many have held that providing more space is desirable, has no adverse consequences, and would facilitate adding enrichment devices. With respect to rodents, and in particular rats and mice, few scientific studies have directly addressed cage space needs without the introduction of confounding variables. Comparing studies and building upon previous work is difficult due to the great differences in study design and lack of confirmatory studies. The effects of group size as compared to density have been explored on a limited basis in a few studies (69-71) and the *Guide* should acknowledge the complexities introduced by these when recommending cage space. Still there is a need to bring guidance up to date based upon the new information that exists in the scientific literature.

Several key studies support the view that a range of cage space allocations are equally acceptable for mice and rats and that increased space can have certain adverse outcomes such as increased aggression between cage mates and increased mortality. Recent studies have demonstrated differences in space usage and physiological effects as well as effects based on age, species, strain, genetic background and a variety of other variables including reproductive status and the provision of enrichment materials. The 1996 *Guide* does not address these issues and accompanying complexities. A number of recent references (72-75) provide information in this area.

Careful consideration of the housing environment is crucial because recent evidence suggests that there are substantial benefits to be gained from seemingly slight adjustments in cage sizes (76-77). Clear guidance for both experimenters and regulators is needed because suggestions made in the current version of the *Guide* are often considered to be absolute standards rather than guidance as they were originally intended.

B. Single vs. Group Housing

At the time of publication of the 1996 *Guide,* existing literature supported the concept that single housing of social species likely imposed stress that might affect well-being and impact experimental results. Recent literature also supports that differences exist between individual and social housing but these relationships are complex and can be modified by other variables such as enrichment items. As in the case with cage space, the literature on single vs. group housing is confusing and suffers from a lack of a generally accepted framework for interpretation of results or general agreement on definitions and magnitude of differences that constitute stress. Despite this, recent literature has provided a better understanding of the effects of single versus group housing, which is not reflected in the 1996 *Guide*. A variety of parameters can be affected by social housing, or lack thereof, and hence consistency in housing groups across studies is important. Food consumption appears to be reduced in group-housed males or females, which can impact body weight and potentially longevity (78). Rodents appear to select social contact over environmental enrichment materials (79). Some studies suggest that group housing allows animals to adapt more easily to stressful circumstances compared to singly housed animals although there do not appear to be any significant differences in physiological markers for stress (80-81). The make up of the group and strain/genetic background appear to influence findings (82-84). Singly housed animals appear to be more willing to explore novel environments and to take risks. The 1996 *Guide* does not acknowledge these sources of variation and the parameters affected by it. There are several recent references to consider in this area (85-92).

C. Environmental Enrichment

The 1996 *Guide* does not separately cover the topic of environmental enrichment. Recent proposals for changes in guidance in Europe have put heavy emphasis on this and have proposed substantial changes in other guidance to accommodate enrichment. Substantial amounts of literature have been generated on this topic but the interpretation of the findings remains conflicted and complicated by a lack of consistency in experimental approach and design. It is clear that enrichment imparts variation to experimental findings when not applied consistently or using the same methods (93-100). What constitutes enrichment, how it is to be applied, and what measures of effectiveness will be used to determine success from the perspective of animal well-being remain unclear. Since environmental enrichment is being used in research programs today it is appropriate for the *Guide* to evaluate the current information on enrichment and to provide guidance on its use in animal care and use programs. Current references representative of this body of literature are cited (101-107).

Since 1996, there has been a new appreciation of the beneficial effects of simple environmental enrichment on the behavior and well-being of nonhuman primates (108-115). Moreover, there has been more diligent application of enrichment requirements by regulators and assessment thereof by accrediting agencies. A compilation of guidance is needed to facilitate the appropriate incorporation of the enrichment practices by investigators and caretakers alike.

Novel approaches for enrichment and methods of measuring the effects of these manipulations have developed in the ten years since the last edition of the *Guide* (116-122). In addition, a new appreciation of enrichment as a screening tool for nonhuman primate subjects is just emerging. As stated above, however, all tools that refine the use of nonhuman primates in research, especially in behavioral experiments, may have significant impact on the number of animals used.

D. Temperature and Thermoregulation

The 1996 *Guide* provides temperature recommendations for a number of common laboratory animal species. For some species such as rodents the basis for these recommendations has been unclear. Recent studies have shown that the thermoneutral zones as well as ambient temperatures selected by mice and rats in temperature gradients are considerably higher than those recommended in the *Guide*. This requires anatomic, physiologic, or behavioral adjustment on the part of the animals when housed at temperatures comfortable to humans (e.g. 22° C). These adjustments are measurable and have been described in recent studies as consequences of altered ambient temperature. These adjustments do not appear to be deleterious to the animals and may actually have certain beneficial effects including better reproductive performance. The use of bedding/ nesting materials and the ability to burrow into them or create nests using them has been shown to provide a thermal compensating mechanism to achieve ambient temperatures that approach thermoneutrality. This may provide an alternate explanation to psychological enrichment for the use by rodents of these materials and their preference for them. Similarly, the thermal preferences and effective ambient temperatures differ between single and group housed animals, which may also help to explain their preference for social housing. The *Guide* does suggest that such adaptation is normal but these recent studies provide a clear basis for this. Greater clarification of the section on temperature would enhance an area of the *Guide* that is often misinterpreted. Examples of references that provide expanded information on this topic are cited (123-128).

E. Bedding and Nesting Materials

The present *Guide* discusses bedding materials but does not provide guidance on nesting behavior or considerations for the use of various bedding materials. Recent studies are available that explore these subjects but do not provide a consistent picture as to bedding preferences or experimental effects. They do provide cautionary information with regard to some potential health effects and experimental impact of bedding materials. They also reinforce the intuitive notion that rats (actually nesting behavior in rats is a learned behavior, see ref.129) and mice prefer to build nests which may find additional rational in the issues discussed on thermoregulation. A revision of the *Guide* should include precautionary information regarding the impact of bedding materials on certain health and experimental parameters and its lack of effect on others. Several references (130-141) reflect the recent literature on this subject.

F. <u>Caging and Housing Systems</u>

There has only been limited new information on caging. The issue of the development of foot lesions on wire grid floors has been explored and is much more limited in effect than suggested in the current issue of the *Guide*. Some information is available on the effects of cage color, cage position on racking, and dunging patterns in rodent cages. These may be useful to some investigations and could be considered in a *Guide* revision. Clarification of the issues surrounding wire-bottom versus solid-bottom cages is certainly needed in a new *Guide* revision. References appropriate to this topic are cited (142-148).

Since publication of the 1996 *Guide*, ventilated caging systems for rodents have come into general usage, and have replaced conventional housing in many facilities. The micro-environment in these systems has been a topic of much study, with focus on gas concentrations, air exchange rates, noise, moisture, required sanitation frequency and more. There has been much published on these issues (149-166) that must be critically evaluated in order to provide specific guidance for use of these systems.

The 1996 *Guide* does not present a discussion of housing systems and management issues or refer to such discussions in the literature. An understanding of these systems and how to apply them is often key to providing appropriate housing and disease and allergen control. When the present *Guide* was being constructed microisolation caging was just coming into general use. A few sections of the *Guide* (see for example p. 33 in the *Guide* section on ventilation) did provide some guidance on these housing systems but not in proportion to their current usage. Very specific guidance on topics such as mechanical and environmental parameters for these systems is not yet possible due to the lack of comprehensive controlled studies and industry standards. More general guidance is possible on considerations for bedding types and intervals between bedding changes and cage cleaning frequencies, considerations for handling and disinfection of units in order to meet health goals for animals, and interactions of air exchange rates and noise/ vibration with reproduction and other experimental parameters. Several references explore some of these issues (167-172).

G. Illumination

The 1996 *Guide* provides information of lighting effects on albino animals with respect to light intensity but does not give separate guidance on pigmented animals. Room light intensity recommendations in the 1996 *Guide* do not take into account caging type or other mitigating factors such as nest building on light intensity at animal level. The importance of photoperiod is acknowledged, as is consistency in photoperiod. Recent literature supports these observations but does not appear to extend them. The observation that wavelengths emitted by sodium vapor lighting may be invisible to mice is new and may have some application in certain housing situations. The cited references should be considered as additions to any revision of the *Guide* (173-178).

H. Sanitation

Sanitation practices are an essential component of infection control in animal facilities. The 1996 *Guide* discussed basic processes and provided methods to achieve required sanitation goals. Since that addition, a greater emphasis has been placed on microisolation cage housing and other bioexclusion and biocontainment housing techniques that leave a number of questions unanswered as to the appropriateness and efficacy of husbandry techniques and disinfection methods for these housing systems. The 1996 *Guide* also did not include key references such as the one by Block (179) that provides a comprehensive discussion of disinfectant action and methods for achieving both sterilization and disinfection. Appropriate dosage methods and techniques for physical means of disinfection, including irradiation, also are absent from the *Guide*, as well as references to calibration and validation of such methods for specific load configuration and types. Much of this material is covered in current textbooks, some of which are cited (180-188).

The 1996 *Guide* also does not put sanitation goals into perspective with perceived risks in the typical research environment. It also does not include any discussion of the behavioral and stress consequence associated with frequent disinfection and cleaning of the cage environment, which can have consequences on reproduction and overall animal performance. It also does not discuss extended bedding change or cage washing intervals and it does not discuss the detailed performance criteria for judging the adequacy of these with these specialized housing methods based on the overall husbandry program and infection control goals. A discussion of risk-based performance goals for sanitation programs could provide necessary guidance to institutions.

I. **Population Management and Genetically Modified Animals**

Since publication of the 1996 *Guide* there has literally been an explosion in the use of genetically modified animals in research. With such an increase in animal numbers has come the need for specific guidance in managing the large populations, particularly rodents. Issues facing animal care and use programs include genetics and genetic monitoring, application of assisted reproductive technology, breeding strategies, gnotobiology and record-keeping. Several recent publications review these topics and provide a basis for guidance in this area (189-192).

Chapter 3 – Veterinary Medical Care

A. **Health and Genetic Monitoring**

Health and genetic monitoring have become more complex since the 1996 *Guide* revision as have the techniques for conducting monitoring. The numbers of animals with immunological defects have increased requiring special housing systems that complicate monitoring. The list of organisms with research effects has also increased. Transfer of genetically modified animals between institutions has resulted in the increased prevalence of adventitious infections and disruption of research programs. Methods for detecting organisms and identifying them have become more molecularly oriented especially for confirmatory diagnosis and speciation of both viruses and bacteria. None of these subjects is extensively covered in the 1996 *Guide*, although many of the underlying principles presented in this document still apply. General guidance in these areas could be included in a revision although there is not general agreement on many details or specific approaches. The cited references speak to some of these issues (193-200).

Paradigms for monitoring the health of laboratory rodents are variable, as is the technology available for the purpose (201-207). For serologic testing, antigens that formerly were as crude as cell lysates have been replaced in many instances by purified molecularly expressed proteins. These can be as generic or specific as needed. This is best illustrated by the many antigens used to test for parvovirus seroconversions in mice. A further complication to health monitoring programs is the number of immunodeficient (and animals with immune dyscrasias), genetically modified mice found in contemporary colonies. Their inability (or reduced ability) to mount an immune response leaves the diagnostician in a quandary since methods other than straightforward serologic tests may be required. Although some agents can be detected by use of non-invasive techniques (e.g., polymerase chain reaction on fecal pellets), animals may have to be sacrificed if internal organs are believed to yield the most reliable results. Molecular methods have also largely supplanted the mouse and rat antibody production (MAP, RAP) tests. Diagnostic test methods are evolving rapidly and the *Guide* should include reference to methods that were not available in 1996.

B. Genetically Modified Animals

The present *Guide* provides some limited descriptive information on transgenic animals but was prepared before the widespread use of these animals. Principles in the *Guide* that apply to non-genetically modified animals are still applicable to genetically modified and mutant animals. However, the research community would be better served in any revision with a more extensive section that provides greater guidance with respect to the peculiarities of the care and use of these animals. With the great strain variations that exist with genetically modified animals, such discussions would need to be general in nature. Such discussions would also need to include more in depth information on breeding colony management techniques that are animal and resource conservative to better meet the goals of the three R's. The cited references provide useful information on this topic (208-217).

C. Operant Conditioning

Cooperativity training (aka. operant conditioning) refines experimental procedures and animal management by training an animal to perform a task that would otherwise require capture and/ or stressful restraint including anesthesia. While implied in the *Guide*, alternative methods for eliciting cooperativity when working with nonhuman primates and other species are not documented. Since 1996, several studies have provided evidence that significant strides can be made by using various positive reinforcement methods, not only during experimental procedures, but also during routine animal management (218-224). The benefits of using these techniques are several-fold. First, these increase the ease with which procedures are done. Second, their use is correlated with expression of normal rather than abnormal behaviors. Third, it has been implied that stress hormone levels are lower in animals trained to cooperate.

D. Pain/Anesthesia/Analgesia/Stress

1. Stress

It has long been acknowledged that stress adversely affects the well-being of animals and humans alike. Since the publication of the most recent edition of the *Guide* in 1996, the publication of studies (225-233) in which stress responses were measured has begun to reshape our thinking about common laboratory and housing practices. While it appears that there are no universal truths regarding stress and distress, there are enough new observations that would support guidance regarding stress-inducing situations that could be avoided. Seemingly mundane activities such as cage changing and infrequent sedation for routine procedures can have lasting effects on the biochemistry and the behavior of nonhuman primates. Since one of the goals in experimental control is to reduce variability among subjects, understanding stress-induced variability and the means to control it is crucial. The *Guide* provides few current references that give tangible means for recognizing and controlling stress during experiments and in housing practices. Additional guidance could reduce the variability in experiments and hence reduce the number of animals needed to achieve statistically significant experimental results.

2. Assessment and Recognition of Pain

Since publication of the 1996 *Guide*, much has been written on the assessment and recognition of pain and distress (234-249). Many of the published articles provide information on improved alleviation of pain with the use of newer anesthetics and analgesics. Guidance on utilization of this new information and therapeutic intervention is needed.

3. Abnormal Behavior

Despite careful attention to experimental and housing environments, nonhuman primates, at times, exhibit abnormal behaviors in captivity. Additional methods of recognition, assessment and treatment of these behaviors, and the long-term effects of episodic negative behavior have been documented since the last edition of the *Guide* (250-269). Compilation of this information in an easily accessible place and its updating are crucial for all who treat, use, and manage nonhuman primates. To date, sources containing valuable information are scattered and dissemination of available guidance is less than optimal. A revision of the *Guide* that contains currently available information and that is updated at regular intervals would be beneficial to all concerned with the ethical and appropriate use of animals in science.

E. **Euthanasia**

The present *Guide* utilizes the AVMA panel on euthanasia as the principle authority on appropriate euthanasia techniques to be applied to laboratory animals. Recently there has been controversy as to the use of carbon dioxide for euthanasia particularly for domestic farm animals (especially for use with swine presented for slaughter). These concerns have been extrapolated to rodents where this agent is commonly used. A limited amount of literature is available that has explored its use in rodents. Most of these references suffer from the availability of a standardized framework for interpretation particularly as to what constitutes acceptable limits for stress or imposition of momentary pain (270-273). A variety of studies are in progress at several research institutions that seek to better define its use in rodents and its antemortem effects. A few current references are listed below. Additional peer reviewed studies will be available at the time a revision is undertaken.

F. <u>Minimally Invasive Procedures and Non-Invasive Imaging</u>

Since publication of the 1996 *Guide* there have been many articles written on the use of minimally invasive surgical methods and non-invasive methods of monitoring research animals (274-285). These new technologies require paradigm shifts in how research personnel view the re-use of animals and assess pain and distress.

The widespread use of noninvasive imaging methods in research animals since the publication of the 1996 *Guide* has resulted in the need for development and implementation of specialized animal monitoring programs in facilities that use these new and novel imaging methods. Guidance has been rendered in the ILAR/NRC publication entitled, *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (6) concerning special considerations of animal maintenance in the imaging environment. Several relevant articles are cited (286-289). In addition, the use of imaging facilities, particularly for sequential imaging of rodents, can jeopardize the health of other animals being tested with the same equipment. Guidance on strategies for control of contamination is needed.

Chapter 4: Physical Plant

Since publication of the 1996 *Guide* there have been many articles written on research animal facility design (290-302). Many of these references reflect widely accepted concepts and standard practice, but are not published as scientific peer reviewed journals. Some are published in engineering or trade journals while information on design and management is found in recent texts. Some reflect new technology or new demands on the research facility (e.g., biocontainment) or revised expectations/needs in areas such as surgical facilities. Revision of the *Guide* would necessitate evaluation of these publications in order to provide appropriate guidance.

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#41

HAVERFORD
C O L L E G E

February 20, 2006

Dr. Margaret Snyder
Director, Office of Scientific Affairs
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6705 Rockledge I, Suite 4184 , MSC 7983
Bethesda , MD 20892-7983
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-06-011.html

Re: **Request for Information (RFI): Standards for the Care and Use of Laboratory Animals** (RFI No. NOT-OD-06-011)

Dear Dr. Snyder,

I write to recommend an important expansion of Government Principle V of the *Guide for the Care and Use of Laboratory Animals*[i] : I strongly advocate intraoperative electroencephalographic (EEG) monitoring to ensure adequate anesthesia of laboratory animals during surgery. Specifically, I'm asking that bispectral index (BIS) EEG monitoring be employed whenever an animal undergoes a surgical procedure—and **most particularly in any case where general anesthesia is accompanied by the use of neuromuscular blocking agents.**

Principle V presently states: "Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents." This extremely commendable policy needs serious elaboration; human experience has shown that the efficacy of anesthetic dosage in a paralyzed patient cannot be assumed—it must be gauged objectively and carefully.

Since the last revision of the *Guide* in 1996, there has been tremendous progress in the anesthesiology literature devoted to addressing the problem of "unintentional awareness" (i.e., the persistence, or recovery, of consciousness during surgery). That literature had already documented the disturbing frequency of such events in humans (one in a thousand surgeries, or 30,000 – 40,000 cases per year in the United States),[1,2] despite the obvious intention of anesthesiologists to induce unconsciousness. The problem is most acute when muscle paralysis has been induced. [3,4,5] The testimonials of patients are harrowing to read; they describe a state of paralysis in which every knife-cut and cauterization is felt, and in which a patient who manages to muster any frantic movement at all simply receives an extra dose of the paralytic agent.[6] The research attention given to this problem in the past decade has resulted in a distinct breakthrough: the use of bispectral index (BIS) electroencephalogram (EEG) monitoring as a means of ascertaining and ensuring a patient's depth of anesthesia. This method has been validated as an objective means of assessing the level of sedation in adults and, more recently, in children. [7,8,9,10]

Although animals cannot provide narratives of their ordeals in surgery, they certainly experience unanesthetized vivisection with at least the same statistical frequency as humans—and, logically, with far greater frequency, given the lack of accountability among their anesthetists. I have personally witnessed the vivisection of rats by a well-established medical researcher who used full neuromuscular blockade in combination with subclinical (one might say cosmetic) doses of sedation. He was cheerful and unapologetic about shortchanging the animals of anesthesia; he was satisfied that they lay flaccid as he slit their bellies and extracted organs slowly. I am offering my testimony that such atrocities occur on a daily basis. And they will continue, even in the hands of less cavalier researchers, unless the government drafts strict objective standards for what constitutes anesthesia.

In my view, the use of neuromuscular blocking agents should be eliminated, or at least aggressively circumscribed, so that an animal's sentience can be detected, and remedied with actual anesthetic agents, during surgical procedures. But a further safety net would be the one now eagerly seized upon by humans entering surgery across the country: **BIS monitoring**. I believe that this monitoring (or any better method devised as the technology evolves) should be employed on every animal during surgery to secure, on an objective scale, the deepest level of anesthesia. We cannot simply trust the intuition, let alone the conscience, of the individual researcher to ensure painless surgery.

As you work to turn humane principle into practice, it is imperative that advances in our understanding of anesthesia be incorporated into the treatment of laboratory animals. The NIH's effort to offer these animals a tolerable existence, physically and socially, is violently undone when they are subjected to the all-too-imaginable pain of unanesthetized dissection.

Humans continue to profit from discoveries made in animal-based studies; let us at least share with the animals this agony-preventing innovation developed through human-based research.

Sincerely,

Susan L. Benston MD

Susan L. Benston, M.D.
sbenston@haverford.edu

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[i] Office of Laboratory Animal Welfare (OLAW). Public Health Service Policy on Humane Care and Use of Laboratory Animals: U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training; 1996: 8 http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf

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#42

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February 23, 2006

To: Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
ScientificAffairs@od.nih.gov

From: David Anderson, D.V.M., Acting Director
Stephen Kelley, D.V.M., M.S., DCLAM, Acting Associate Director for Research Resources
Carolyn M. Crockett, Ph.D., Coordinator, Psychological Well-being Program
Keith Vogel, D.V.M.
Maggie Gillen, D.V.M.
Washington National Primate Research Center
Seattle, WA 98195-7330

Re: RFI: Standards for the Care and Use of Laboratory Animals, NOT-OD-06-011

The current Guide for the Care and Use of Laboratory Animals (1996 edition) is a well-written document that was purposely written in general terms describing performance standards and few engineering standards so as to be useful for many years. The current edition has been translated into many languages for use in a variety of countries. Additionally, at least in the area of nonhuman primate husbandry, management, medicine and psychological wellbeing, there is little scientific evidence to justify changing the broad recommendations that are included in the current Guide. For these reasons we question whether the Guide needs to be revised at this time. However, if the preponderance of respondents to the RFI indicates a strong need to revise the Guide at this time, we present the following recommendations:

- Include a summary of how the newly revised Guide differs from the previous 1996 edition. Given that the Guide will also be available on line, this will help owners of the 1996 edition decide whether to purchase the new edition. This will also very helpful to users of Guides in other languages, given that translations likely will lag several years behind the new edition.
- Having chapter references in a combined REFERENCES section would be easier to use, and might shorten the Guide owing to elimination of redundant sources.
- The Selected Bibliography, Appendix A, is likely to become out of date quickly. Rather than a Selected Bibliography (given that the chapters list cited references), we suggest providing on-line sources for citations and lists of useful search criteria. This would be especially useful for new users and users from other countries who might have less experience in on-line literature searches. (This would also shorten the Guide.)
- Appendix B: update to include web addresses of all. Perhaps indicate that the web pages should be consulted for up-to-date information, since this sort of information changes fairly often. Perhaps therefore shorten some of the description.
- Appendices C and D should provide clear information on how to access the regulations on the web.

- For the on-line version of the Guide, provide live-links whenever possible (especially useful for Appendices)

Thank you for your consideration.

David Anderson, D.V.M., Acting Director

Stephen T. Kelley, D.V.M., M.S., DACLAM, Acting Associate Director for Research Resources

Carolyn M. Crockett, Ph.D., Coordinator, Psychological Well-being Program

Keith Vogel, D.V.M.

Maggie Gillen, D.V.M.

#43

Scientific Affairs (NIH/OD)

From: ptinkey@mdanderson.org

Sent: Sunday, February 26, 2006 11:07 AM

To: Scientific Affairs (NIH/OD)

Cc: kgray@mdanderson.org; scraig@mdanderson.org; kanaff@mdanderson.org; ATBorne@mdanderson.org; lcoghlan@mdanderson.org

Subject: RFI No. NOT-OD-06-011 Responses to RFI: Standards for the Care and Use of Laboratory Animals

Attachments: DVMS refence list - FINAL.doc; DVMS references- analgesia, euthanasia, etc.doc

To Whom It May Concern:
Please find attached 2 documents which contains scientific manuscript references relevant to the RFI No: # NOT-OD-06-011 regarding updating of the Guide for the Care and Use of Laboratory Animals. These references were compiled by the laboratory animal veterinarians at The University of Texas M.D. Anderson Cancer Center, Department of Veterinary Medicine and Surgery.
The contact person is:

Peggy T. Tinkey, DVM, DACLAM
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Thank you for the opportunity to offer scientific input into this process.

Peggy T. Tinkey, DVM, DACLAM
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Analgesic Efficacy of Orally Administered Buprenorphine in Rats

Lisa B. E. Martin, DVM,[1] Alexis C. Thompson, PhD,[2] Thomas Martin, BVSC, PhD,[1] and Mark B. Kristal, PhD[2]

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Analgesic Efficacy of Orally Administered Buprenorphine in Rats: Methodologic Considerations

Alexis C. Thompson, PhD,[1] Mark B. Kristal, PhD,[2] Abdullah Sallaj,[2] Ashley Acheson,[2] Lisa B. E. Martin, DVM,[2] and Thomas Martin, BVSc, PhD[2]

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Surgery-induced Immunosuppression and Postoperative Pain Management

Gayle Giboney Page, RN, DNSc, FAAN

Surgery is well known to result in the suppression of some immune functions; however, the role of perioperative pain has only recently been studied. Pain-relieving anesthesia techniques and perioperative analgesia provide some protection against surgery-induced immune suppression and

suppresses immune function; however, the possibility that pain is a mediator of such consequences has been studied only in the past 15 years. Only 20 years ago the first study exploring the impact of nontissue damaging painful stress on the immune system emerged. Studies regarding pain management and immune sequelae of surgery in both humans and animals has been accel-

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ACTA VET. BRNO 2004, 73: 195-199

Endogenous Opioids and Analgesic Effects of Ionizing Radiation in Rats

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Abstract

Kereškényiová E., B. Šmajda: *Endogenous Opioids and Analgesic Effects of Ionizing Radiation in Rats.* Acta Vet. Brno 2004, 73: 195-199.

Some stressors can cause a temporary decrease of sensitivity to pain (stress-induced analgesia, SIA) in mammals. Ionizing radiation belongs to non-specific stressors, too. The aim of this study was to analyze the effects of ionizing radiation on pain sensitivity in laboratory rats and to establish whether the release of endogenous opioids in post-irradiation period is involved in analgesic effect of radiation.

Two-month-old (310-340 g) intact male Sprague-Dowley rats divided in four groups ($n = 10$) housed in groups of five were used in the experiments. Rats were held under an LD 12:12 artificial light regimen at temperature of 22 °C and relative air humidity of 60-70%. Food and water were available *ad libitum*. The rats were tested in a hot plate apparatus with a surface temperature of 55 ± 0.5 °C.

It was found that gamma irradiation with a whole body dose of 6 Gy or with a dose of 10 Gy on the head caused significant prolongation of the hind-paw licking latency in the hot plate test. Intraperitoneal administration of naloxone, a blocker of the endogenous opioid µ-receptors in a dose of 8 mg.kg^{-1} b.m. 30 min before testing on the hot plate significantly ($p < 0.05$) reversed the post-irradiation analgesia in both irradiation models, while a dose of 4 mg.kg^{-1} was ineffective

Lab Anim. 2005 Apr;39(2):137-61.

Related Articles, Links

Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats.

Conlee KM, Stephens ML, Rowan AN, King LA.

ATLA 33, 119-127, 2005 119

Anaesthesia and Post-operative Analgesia Following Experimental Surgery in Laboratory Rodents: Are We Making Progress?

Claire A. Richardson and Paul A. Flecknell

REVIEW ARTICLE Laboratory Animals 2005 (39):137-161

Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats

K M Conlee[1], M L Stephens[1], A N Rowan[1] and L A King[2]

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uction of germfree mice

ransfer and facility de-
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paritis virus. Lab Anim.

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Euthanasia of Mouse Fetuses and Neonates

Contemporary Topics 2004 43(5):29-34

BRENDA A. KLAUNBERG, MS, VMD,[1] JAMES O'MALLEY, DVM, MPH,[2] TERRI CLARK, DVM,[3] AND JUDITH A. DAVIS, DVM, MS[2]

We sought to determine whether any of the common methods of euthanasia for adult rodents would lead to an acceptable death fetuses or neonates. We wanted to identify a method that was rapid, free of signs of pain or distress, reliable, and minimally distressfı the person performing the procedure and that minimized the amount of handling required to perform the procedure. We evaluatec methods of euthanasia, with and without anesthesia, in three age groups of mice: gravid mice (E14-20) and neonatal pups (P1-P7 and P14). Euthanasia methods included: halothane inhalation, carbon dioxide inhalation, intraperitoneal sodium pentobarbital, intraven potassium chloride, and cervical dislocation with and without anesthesia. Noninvasive echocardiography was used to assess heartl during euthanasia. With cardiac arrest as the definition of death, no method of euthanasia killed fetal mice. Halothane inhalation (5% vaporizer) was not an acceptable method of euthanasia for mice of the age groups tested. Intraperitoneal administration of sodi pentobarbital for euthanasia required a higher dose than the previously established dose, and there is a risk of reduced efficacy in pregn

Histopathologic changes in laboratory animals resulting from various methods of euthanasia. Fledman DB, Gupta BN. Lab Animal Science.1976 Apr 26(2pt1):218-221

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February 24, 2006

RFI No. NOT-OD-06-011

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

Dear Dr. Snyder:

Thank you for the opportunity to make recommendations as the PHS considers updating its *Guide for the Care and Use of Laboratory Animals* (hereafter: the *Guide*). We wish to draw your attention to the following two literature reviews we recently completed, a copy of each of which is enclosed with this letter:

1. Balcombe JP, Barnard N, Sandusky C. 2004. Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science*. 43(6): 42-51.

   summary: Significant changes in stress indicators (e.g., concentrations of corticosterone, glucose, growth hormone or prolactin; heart rate, blood pressure, and/or behavior) are associated with handling, blood collection, and gavage in rats, mice, monkeys, dogs, rabbits, hamsters, and birds. These changes are rapid, pronounced, non-transient, and animals do not readily habituate to them. Thus, significant fear and stress appear to be predictable consequences of routine laboratory procedures.

2. Balcombe JP. in press. Laboratory environments and rodents' behavioural needs: A review. *Laboratory Animals*.

   summary: Published studies indicate that rats and mice value opportunities to take cover, build nests, explore, gain social contact, and exercise some control over their social milieu, and that thwarting these needs is physically and psychologically detrimental, leading to impaired brain development and behavioural anomalies (e.g., stereotypies). Adding environmental enrichments to small cages does not eliminate these problems; substantial changes in housing and husbandry conditions are needed.

Based on these data and reviews, several aspects of the current *Guide* need addressing. It is not so much that the *Guide* fails to make decent recommendations, but rather that laboratory conditions are not responsive to them. The following comments are focused primarily on the most used and least welfare-considered of the animal species in labs: rats and mice, though for the most part they apply also to other species used in research and testing.

The following statements from the *Guide* refer to the need to provide animals with suitable surroundings which allow them to perform natural behaviors:

"Animals should be housed with the goal of maximizing species-specific behaviors and minimizing stress-induced behaviors." p 22

"The environment in which animals are maintained should be appropriate to the species, its life history... ." p 22

"Acceptable primary enclosures .. allow for the normal .. behavioral needs of the animals." p 23

In that the PHS *Guide* includes rodents in its purview, these recommendations reflect an awareness that—as we outline in the *Laboratory Animals* review mentioned above—rodents are no different from other mammals in being highly motivated to perform behaviors natural to them. Preference studies and other observations show that they value opportunities to hide, explore, forage, exercise, burrow, choose social partners, and otherwise to escape the close confines of their cage. Given the chance, they also prefer to forage for food than to merely gnaw at dried pellets through the cage roof.

The problem is that minimum housing standards prescribed by the Animal Welfare Act—and which define the vast majority of commercially available caging systems currently in use—are totally inadequate for meeting the *Guide's* recommendations. With a barren, cramped environment over which they have little control, they are resigned to a monotonous existence that stunts brain development and, for an estimated 50 percent of all mice in labs, leads to behavioral stereotypies (Mason & Latham 2004).

A recent survey of animal facilities at the US National Institutes of Health indicates that a slight majority of rats and mice at these facilities are now being provided with nesting and structural (shelter) enrichment (Hutchinson *et al.* 2005). Other indicators that rodent housing conditions are improving include the availability of commercially-produced resources for nesting, shelter, gnawing, and play (Key 2004), and a sharp rise since the late 1980s in the number of citations using keywords "environmental enrichment" and "rodent" (Hutchinson *et al.* 2005). Considering that two decades ago environmental rodent enrichment was scarcely being discussed, these are laudable trends. But practically all laboratory-housed rodents continue to live in small "shoe-box" cages. Both scientific and ethical arguments support an approach that provides these species with living environments more akin to their natural existence.

Preference studies show that mice in laboratories favor a variety of environmental features still commonly absent in laboratory housing conditions. A review of 40 studies published between 1987 and 2000 concluded that mice prefer more complex cages, and will work for nesting material, shelter, raised platforms, a running wheel, and larger cages (Olsson & Dahlborn 2002). While merely adding structure to a standard cage had limited effects on behavior, providing a considerably larger and more complex cage had significant effects, including increased activity or reduced signs of anxiety in open field trials, exploration tests and elevated plus maze trials, or a reduced latency to emerge in emergence tests (ibid).

Similar patterns were obtained for rats (reviewed in Balcombe, in press). And despite hundreds of generations of captivity, rats and mice retain most or all of their ancestral behavior patterns (Berdoy 2002, Patterson-Kane 2002, Sluyter & van Ootmerssen 2000 Olsson & Dahlborn 2002).

The feeding regime for mice and rats in laboratory settings is deleterious in two ways. First, it is monotonous, providing little of the variety that wild mice and rats would normally eat. Wild rodents draw from comparatively diverse and seasonally changing food sources, compared with the formulated dry pellets provided freely in the laboratory. Expanded diets that include seeds, fresh vegetables, fruit, and bread, are more palatable than pelleted diets, offering a variety of textures and flavors (Jennings et al. 1998). Second, because their food is provided *ad libitum*, the animals are given no challenge to obtain food. In the wild, foraging takes up probably the largest proportion of the animals' waking time, and is an important part of these animals' psychomotor experience. As such, the unchallenging feeding environment in the laboratory is highly unnatural, and this may contribute to serious psychological deficits stemming from caged confinement.

If the *Guide* is to be effective in getting research facilities to meet its laudable recommendations, then rats and mice (and other species) must be provided with housing responsive to the issues summarized above; that is, housing that affords them, at the very least, opportunities to hide, explore, exercise and forage. Current housing fails to do this, and constitutes a profound violation of the *Guide*'s aims. It is noteworthy, for instance, that almost half of laboratory housed rodents are currently not given nesting materials or a shelter.

A revision to the *Guide* should articulate this current gap between intentions and practice, as well as address the latest understanding of how current conditions in labs cause stress and thwart natural behaviors. We urge readers to make substantive reforms in housing conditions that allow rodents specifically to perform natural behaviors. Such reforms should ensure that all animals be provided with:

- enough shelter space for each animal to hide
- enough space and environmental complexity that animals can bound and climb
- fresh, natural and varied food, and opportunities to forage for and manipulate it
- materials with which to make nests

Sincerely,

Jonathan Balcombe, Ph.D., Research Scientist
Chad Sandusky, Ph.D., Director of Toxicology & Research

APPENDIX I: Literature Cited

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APPENDIX II: Supporting papers (attached)

Balcombe JP, Barnard N, Sandusky C. 2004. Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science*. 43(6): 42-51.

Balcombe JP. in press. Laboratory environments and rodents' behavioural needs: A review. *Laboratory Animals*

NAME:	Johnathan Balcombe/Physical Committee for Responsible Medicine
ARTICLE/CONTENT:	Laboratory Routines Cause Animal Stress
SOURCE:	Contemporary Topics, 2004 (AALAS)

 #415

From: Anita Conte [conte@mail.csi.cuny.edu]
Sent: Tuesday, February 28, 2006 9:35 PM
To: Scientific Affairs (NIH/OD)
Subject: NOT-OD-06-011

NOT-OD-06-011
In reply to RFI: Standards for the Care and Use of Laboratory Animals with regard to housing of birds;specifically pigeons and chickens. Traditionally avian housing is single wire caging which allows no contact with conspecifics and no opportunity to engage in species-specific behaviors ie. foraging, flying and bathing. In the past two years I have conducted some pilot studies using flight cage as an enriched environment for pigeons and chickens. Preliminary results show corticosterone levels of birds in the enriched environment reveal lower stress levels then birds in home cages and in a crowded (2 per cage) condition (fecal samples are assayed using an (EIA) enzyme immunoassay). Further, behavioral observations reveal that birds form relationships with conspecifics when they are given the opportunity to do so and engage in species-typical behaviors such as nest building, defending space and foraging. Since my findin! gs are unpublished I would like to suggest that an excellent reference regarding lab birds, their housing, welfare and husbandry is; Laboratory birds: refinements in husbandry and procedures. Fifth report of BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Laboratory Animals 2001 October 35 Suppl 1:1-163 Thank you for the opportunity to share information and if you have any questions please contact me.

Sincerely,
Anita Conte, MA
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Scientific Affairs (NIH/OD)

46

From: Dr.Bennett [btb@uic.edu]
Sent: Tuesday, February 28, 2006 2:36 PM
To: Scientific Affairs (NIH/OD)
Subject: NOT-OD-06-011

February 28, 2006

Dr. Margaret Synder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge 1, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

Dear Dr. Snyder:

This correspondence is in response to NOT-OD-06-011 in which you request information on the need to update the laboratory animal welfare standards of the Guide for the Care and Use of Laboratory Animals (Guide). You specifically ask for new knowledge related to the four chapters in the Guide with documentation of that knowledge. In making the decision to submit the comments that follow I reviewed the current edition of the Guide. My focus was on what new information is in the literature that would markedly change the impact that the Guide would have on the existing review process of animal facilities and programs as required by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (PHS Policy). I came to the conclusion that there was not a significant new body of information that would warrant a complete revision of the Guide. There may be areas where a more detailed review of the literature would find articles that could be added to the Selected Bibliography contained in Appendix A, but I believe this could be done without a complete re-write of the document.

The primary purpose of this correspondence is to provide comment on why I believe that there is not more new scientific information in the laboratory animal science literature that would necessitate a rewrite of the Guide. As a laboratory veterinarian with 36 years of experience and the former director of a postdoctoral training program in laboratory animal medicine, I find the paucity of new scientific information on the care and management of laboratory animals to be a problem. I believe that this situation is largely related to the change in the nature of the training programs funded by the National Center for Research Resources. Now that the emphasis of these programs is completely on research training and not a mixture of research and clinical/management training the number of articles being published that relate to the day-to-day care of laboratory animals has declined. Spending time to do the type of applied research that led to many of the articles that are referenced in the current edition of the Guide is no longer an integral part of the current NIH funded training programs. For those training programs that are currently supported on institutional funds, the ability to support such research is compromised by the cost of supporting salaries and benefits for the trainees. Not only have the number of pertinent articles being published declined, so have the number of scientific presentations. For example the national meeting of the American Association of Laboratory Animal Science (AALAS) has evolved into largely a seminar oriented meeting with only a limited number of platform sessions.

A secondary purpose of this correspondence is to request that your office not support a revision of the Guide that would involve expanding the current scope of the document as it relates to compliance with the PHS Policy. I am aware that there have been suggestions concerning a revision of the Guide that would add considerable information to the existing format as a means of making the document more international in scope and as means of dealing with management issues that are only tangentially related to the implementation of the PHS Policy. The Guide has served us well over the years in implementing and improving quality animal care programs, and it can continue to do so without major revisions. Revisions that would change the original intent of the document should not be supported with NIH funds.

Thank you for the opportunity to comment. If you have any questions concerning my comments or need additional information, do not hesitate to contact me.

B. Taylor Bennett DVM, PhD
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Scientific Affairs (NIH/OD) #47

From:	Brown, Patricia (NIH/OD) [E]
Sent:	Wednesday, February 22, 2006 5:12 PM
To:	Scientific Affairs (NIH/OD)
Cc:	Taylor, James (MAC) (NIH/OD); Person, Brenda (NIH/OD) [E]
Subject:	Response to RFI No. NOT-OD-06-011
Attachments:	EnrichOSA RFIMemo.pdf

EnrichOSA
IMemo.pdf (268 KB)

Enlosed please find a response from Dr. James Taylor to the RFI No. NOT-OD-06-011 submitted on behalf of the NIH Animal Research Advisory Committee.

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CAPT, VC, USPHS
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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

February 22, 2006

TO: Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH

FROM: Director, Office of Animal Care and Use, OIR, OD

SUBJECT: RFI No. NOT-OD-06-011

This correspondence is in response to the Request for Information No. NOT-OD-06-011(RFI): Standards for the Care and Use of Laboratory Animals. As you are aware, the Animal Research Advisory Committee (ARAC) develops policies and guidelines for use by the 21 Animal Care and Use Committees with oversight of animal activities in the NIH intramural research program. In addition, ARAC has appointed advisory panels of scientists, veterinarians and others to develop white papers on issues involving animal care and use to assist the animal community in developing best practices that benefit research animals.

In 2004, an ARAC advisory panel created a paper entitled "Enrichment Strategies for Rodents in the Laboratory" which provides an overview of the current literature with respect to the provision and impact of environmental enrichment on laboratory rats and mice. This paper makes recommendations on social housing, nesting and cage structures for rats and mice and was endorsed by ARAC in September 2004. A copy is attached and can be found at the web site: http://oacu.od.nih.gov/wellbeing/RodentEE.pdf.

The RFI specifically solicited information related to "structural and social environment of animals" which seems a certain match to the topics that the "Enrichment Strategies for Rodents in the Laboratory" covers.

Please contact me if additional information is required.

James F. Taylor

James F. Taylor, D.V.M., M.S.

Attachment

cc: Dr. Wyatt

NAME: James Taylor/NIH-OACU

ARTICLE/CONTENT: Enrichment Strategies for Rodents in the Laboratory

SOURCE: Endorsed by ARAC, 9/2004

Enrichment Strategies for Rodents in the Laboratory

Endorsed by ARAC - 9/8/04

This document provides a brief overview of the current literature with respect to the provision and impact of environmental enrichment on laboratory rats and mice. The development of enrichment programs for non-regulated species, e.g., rats, mice, birds, and other laboratory animals, continues to receive attention from the scientific community. A proactive, systematic, and consistent approach addressing enrichment programs for all laboratory animals is encouraged throughout NIH.

One goal of the Guide for the Care and Use of Laboratory Animals (NRC, 1996) is to "promote the humane care of animals… and provide information that will enhance animal well-being". Environmental Enrichment (EE) is the provision of stimuli that encourage species appropriate behavior and provides for an individual animal's physical and psychological needs. EE is achieved by modifying a captive animal's environment with the goal of providing the animal with a wider range of behavioral opportunities (Mellen and Ellis, 1996; Shepherdson, 1992). Thus, successful EE programs take into account all aspects of a species' natural behavior, including social organization, foraging behavior, and daily activity of the animal (DVR Environmental Enrichment Plan, 2004; Poole and Dawkins, 1999; Steward and Raje, 2001). Non-species specific factors, including the impact of economic and ergonomic considerations, as well as the possible implications to on-going research, must also be weighed when designing an enrichment program (Olsson and Dahlborn, 2002; van Loo et al., 2002).

The Guide (NRC, 1996) describes three elements that should be addressed when managing animal behavior. They are the structural environment, the social environment, and activity. The structural environment refers to components of the primary enclosure such as "cage furniture, equipment for environmental enrichment, objects for manipulation by the animals, and cage complexities" (NRC, 1996). Shelves, perches, nesting material, tunnels, and other objects that promote the expression of species typical behavior are examples of structural enrichment. The social environment addresses attempts to meet an animal's social needs. This can be accomplished by allowing members of the same species to have physical and/or visual, auditory, or olfactory contact with one another. Activity can mean providing an animal the opportunity for exercise, but can also allow an animal to engage in cognitive learning and social contact.

Several recent publications have summarized and reviewed the effectiveness and usefulness of providing environmental enrichment to rodents (Bayne et al., 2002; Jennings et al., 1998; Mortell, 2001; Olsson and Dahlborn, 2002). The following describes some of the major research findings upon which recommendations in each of these categories are based.

In their review of enriched environments for mice, Bayne et al., (2002) noted that performance in open field tests, animal docility, corticosterone levels, and adrenal gland weights did not appear to be affected by the application of long term enrichment. Some evidence does exist which suggests that singly housed mice may have a compromised immune system when compared to socially housed mice (Schwartz et al., 1974). Moreover, mice developed tumors faster when individually housed than when kept in groups (Riley, 1981). In rats, the provision of increased structural complexity has the potential to promote modifications in brain structure, physiology (Park et al. 1992), and function (Goldman et al., 1987; Renner and Rosenzweig, 1987). These changes are mediated via increased cortical thickness (Diamond et al., 1987), increased dendritic spine density and increased concentrations of oligodendrocytes (Katz and Davies, 1984). In situations in which these changes may impact the outcome of the research, Animal Care and Use Committees and investigators should work together to balance animal welfare concerns with study objectives.

Social enrichment: Whenever possible, rodents should be housed socially in compatible groups. Mice can be successfully group housed if the social structure is limited to one male with several females and if the dominance hierarchy has been well established. Female mice can be kept in groups consisting of familiar animals (Wolfensohn and Lloyd, 1998). Escalating aggression in male mice of some strains may preclude social housing, but male rats seem to adapt well to group housing situations (Barnett, 1975; Brain 1992; Mortell, 2001).

Nesting: Mice build nests in the wild. The type and kind of nest built varies by species (Brain and Rajendram, 1986). Providing nesting material to laboratory rodents is relatively simple. Previous studies have shown that mice readily use several different types of nesting materials including shredded paper, paper towels, paper strips, commercial nesting fiber, wood shavings and wood wool (Blom et al., 1996; Sherwin, 1997; van de Weerd et al., 1996, 1997, 1998a, Table 1 & 4, Olsson and Dahlborn, 2002). The addition of nesting material to a mouse cage addresses both activity and structural enrichment.

Cage structures: As with nesting materials, several different types of structural enrichment have been tested with mice. Tunnels, nest boxes, Perspex boxes, opaque and cardboard tubes are just a few of the structural items available. The resultant effects of providing structural enrichment items to mice are not as conclusive as they are for nesting materials. The Canadian Council on Animal Care (1993) reported that results obtained from experiments examining appliance usage, i.e., cage structures, by mice were equivocal. However, positive effects of providing structural enrichment for mice are noted elsewhere. For example, cages with nest boxes are preferred to cages without a nest box (van de Weerd et al., 1998b; several different types of nest boxes tested). Additionally, structural enrichment of cages housing male mice resulted in increased exploratory behavior, less bar gnawing, and less drinking behavior (Leach et al., 2000, provided plastic inserts with raised platforms and shelters). Another study reported that some male mice explored more and slept less initially following the addition of nest boxes, but this effect disappeared over time (van Loo et al., 1996). Haemisch and Gärtner (1994) noted increased aggression between male mice following changes to their structural environment. The Rodent Refinement Working Party (Jennings et al., 1998) recommends structural enrichment with the proviso that it may be contraindicated if increased aggression among male mice is observed. In preference tests, laboratory rats consistently choose environments with nest boxes and shredded paper over unenriched environments (Manser et al., 1998). When given a choice between a nest box and shredded paper, the rats choose the environment with the nest box (Patterson-Kane, 2000). Rats demonstrated a preference for cages with wooden platforms, wood chips and paper towels over a barren or empty cage (Bradshaw and Poling, 1991).

The ILAR Rodent Guide (NRC 1996) suggests that individually housed rodents prefer sheltered areas within their home cage. This may offer rodents opportunities to control the amount of light and/or to seek out higher areas within their cage. Shelters may be an effective way to enrich the environment (NRC, 1996).

Conclusions

Based on this review, the following guidelines are suggested for housing rats and mice in the laboratory.

- Rats and mice benefit from being socially housed whenever possible.
- Mice benefit from being housed on nestable bedding or being provided with a suitable substrate with which to build a nest.
- Rats benefit from being provided with increased structural complexity, i.e., nest box, platforms or paper towels.

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#48

Response to:

Request for Information (RFI): Standards for the Care and Use of Laboratory Animals

RFI No. NOT-OD-06-011

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On behalf of:

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Over the past 10 years, research at The Jackson Laboratory (TJL) into the conditions that best suit the housing of mice has produced results that suggest a revision of the *Guide* should be considered. Some of the most relevant of these are summarized below:

1. Cage changing frequency and the cage microenvironment. Monitoring of cage ammonia (NH_3) and carbon dioxide (CO_2) concentrations, as well as relative humidity (RH) and temperature, demonstrated that safe levels could be maintained for many mouse strains housed on pine shavings, with a two-week cage-change regimen provided the air-changes per hour (ACH) were kept around 60 (Reeb et al., 1997; Reeb et al., 1998; Reeb-Whitaker et al., 2001). The welfare of the animals was either maintained or improved under this two-week cage change protocol. No differences were observed in weanling weight, growth rate, plasma corticosterone, immune function, breeder mortality or productivity among animals maintained under different cage change regimes (Reeb-Whitaker et al., 2001). Moreover, pup mortality decreased when cage changing was reduced from every 7 days to either 14 or 21 days. Similar results were also observed for many of the currently available bedding materials (Smith et al., 2004b).

2. Frequency of changing water bottles and cage lids. In unpublished studies, we examined the effect of decreased frequency of changing either water bottles or cage lids. For water bottles containing acidified water (pH: 2.8 – 3.1), changing every 2 weeks rather than every week resulted in no growth of pathogenic organisms and no significant increase in non-pathogenic bacteria or change in water pH. General animal health and breeding performance were unaffected over a 6-month period. In another study, cage lids were changed either every 2 weeks or at the end of a breeding rotation, i.e. 32 weeks and tested for bacterial growth on the lids as well as in the food hoppers every 2 weeks at the time of cage changing. Whilst statistically significantly more organisms were recovered at three time points only (14, 18 and 26 weeks) animal health was unaffected as indicated by weaning weight of pups, parental reproductive performance and general health.

3. Impact of increased housing density. We have completed initial studies on the impact of increased animal density on the cage microenvironment as well as on the animals. Although only carried out for a maximum of eight weeks (from weaning until 11 or 12 weeks of age), these studies demonstrated no untoward effects on single-sex group housed animals at densities approximately twice those recommended in the current *Guide*. These studies recorded no environmental parameters at levels considered to be of danger to mice and in nearly all instances these were well within *Guide* recommendations (Smith et al., 2004a; Smith et al., 2005). Apart from male FVB/NJ, there was no evidence of aggressive behavior at any of the densities studied for 8 weeks. There was no difference in growth rates, food consumption or general health and well-being of the mice in these studies. Since these studies were of limited duration with a

small number of strains, TJL has started a program of long-term studies to examine more closely the biological impact of increased housing density. Mice will be single-sex housed in groups of 4, 6 and 8 with 12.9, 8.6 and 6.5 in^2/mouse of floor space, respectively. As well as monitoring their general state of well-being mice will have blood samples taken for hematology, biochemistry, hormone assays as well as fecal collection for corticosterone metabolite measurement. A sample of mice will also have telemetry devices implanted to enable remote monitoring of heart rate, body temperature and activity. Furthermore, the interactive behavior of the mice within the cages will be recorded using infra-red illuminators and video cameras, after which it will be assessed for differences between groups. The first studies will last 16 weeks, with plans to follow these with studies of up to 9 months.

4. Environmental enrichment. As part of an investigation into barbering in C57BL/6J mouse colonies at TJL, we conducted several short, preliminary investigations into the impact of environmental enrichment (EE) on this behavior. The premise for this study arose from the findings of Garner and colleagues (Garner et al., 2004a; Garner et al., 2004b) in which aberrant behavior appears to be an inciting cause of barbering. The first stage of our investigation was a test to evaluate and compare the interactions of mice with various readily available enrichment devices. Of those tested, mice interacted most with large metal rings suspended from the wire cage lid, nesting materials (Nestlet™ or standard tissue), Plexiglas Igloos® or metal cage tag-holders. Following these initial observations, 4-week old female mice were housed for 12 weeks either under standard (pine shavings as bedding) or enriched conditions (pine shavings plus 2 1-3/4" steel spilt rings suspended from the cage lid and one new Nestlet™ each week at cage changing). Over this period there was no difference in growth rates; however, the enriched group had a delayed onset of barbering, fewer affected mice and fewer cages with affected mice. This effect was most pronounced by 10 weeks of age, after which the groups were reduced in size according to standard husbandry practice, which altered the cage dynamics and made further data interpretation difficult. We plan to repeat this study at a time when there again is significant barbering within our colonies; currently the incidence is very low which would require very large numbers of animals to detect any significant differences.

5. Analgesia. We conducted several preliminary studies to examine the efficacy of analgesia in association with surgery and the effective dose of different analgesics in a test setting. In one study, mice underwent either splenectomy or sham thymectomy and received either no analgesia or a single dose of buprenorphine or morphine. Mice were monitored for metabolic function and ambulatory activity using the Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH). Due to logistical and practical issues at the time of this study, no conclusive results were forthcoming. However, it did indicate the validity and usefulness of this approach to the study of analgesic efficacy. Another study involved the Formalin test and three analgesics administered at three dose rates. Low dose buprenorphine (0.05 and 0.01 mg/kg)

and morphine (2.5 and 5 mg/kg) were effective in reducing/eliminating both the acute and tonic pain responses to injected formalin. On the other hand, carprofen at 5, 10 and 15 mg/kg appeared to have anti-analgesic effects in this assay. The analgesics were only administered 15 minutes prior to the formalin injection and the animals only observed for 60 minutes after this. Therefore, the time of onset of carprofen may have been delayed relative to the onset of the painful stimulus. Earlier administration of carprofen in this test system may have resulted in a better analgesic response.

6. Use of CO_2 for euthanasia. Pritchett et al. (2005) documented the conditions required for euthanasia of neonatal mice of various strains and ages by CO_2. Not surprisingly, they found that mice up to 6 days of age required the longest exposure to CO_2 to ensure they were dead. Therefore mice this young should be euthanized either by exposure to 100% CO_2 for 60 minutes or by decapitation. Further studies have been undertaken to eliminate the addition of live mice into euthanasia/cull jars containing previously euthanized mice. Our solution, presently being used in several TJL Production areas as a pilot study is to hold mice to be euthanized in standard TJL weaning cages (approx. 113 in^2) and deliver the CO_2 through a specially constructed lid that fits these cages. In this way, up to 40 mice can be euthanized simultaneously with minimal stress to the animals. Based on the initial success of the pilot study, we are implementing this approach throughout TJL animal rooms.

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#49

Scientific Affairs (NIH/OD)

From:	Shalin Gala [ShalinG@peta.org]
Sent:	Thursday, March 16, 2006 4:50 PM
To:	Scientific Affairs (NIH/OD)
Subject:	Comments concerning RFI No. NOT-OD-06-011
Attachments:	Comments concerning RFI No. NOT-OD-06-011.pdf; Brief on primate fear.pdf; Appendix A.pdf

March 16, 2006

Dr. Margaret Snyder, Director

Office of Scientific Affairs

Office of Extramural Research, OD, NIH

6705 Rockledge I, Ste. 4184, MSC 7983

Bethesda, MD 20892-7983

Re: Comments concerning RFI No. NOT-OD-06-011

Dear Dr. Snyder:

In response to your "Request for Information (RFI): Standards for the Care and Use of Laboratory Animals" (Notice Number: NOT-OD-06-011), we respectfully submit comments on behalf of the more than one million members and supporters of People for the Ethical Treatment of Animals (PETA). We appreciate the March 31, 2006, deadline extension described in RFI No. NOT-OD-06-040. Attached to this e-mail you will find PETA's comments along with two supporting documents. We are grateful for the opportunity to provide our input and hope to see our recommendations incorporated in the revised *Guide*. If you should have any questions or concerns, please contact me directly at ShalinG@peta.org or 757-962-8325. Thank you.

Sincerely yours,

Shalin Gala

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Please include all previous correspondence when responding. Many thanks!

<<Comments concerning RFI No. NOT-OD-06-011.pdf>> <<Brief on primate fear.pdf>> <<Appendix A.pdf>>

March 16, 2006

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Re: Comments concerning RFI No. NOT-OD-06-011

12 pages via mail and e-mail: ScientificAffairs@od.nih.gov

Dear Dr. Snyder:

In response to your "Request for Information (RFI): Standards for the Care and Use of Laboratory Animals" (Notice Number: NOT-OD-06-011), we respectfully submit comments on behalf of the more than one million members and supporters of People for the Ethical Treatment of Animals (PETA).

Standardized Institutional Animal Care and Use Committee (IACUC) Evaluations Are Needed

The *Guide for the Care and Use of Laboratory Animals* ("*Guide*") details the IACUC's role in reviewing protocols that involve the use of animals in research, testing, or education. The reliability of these protocol reviews is poor, as evidenced by a comprehensive 2001 study published in the journal *Science* titled, "Reliability of Protocol Reviews for Animal Research." Researchers in the departments of psychology at Wesleyan University and Western Carolina University asked various IACUCs to review the same protocols and found that "IACUC protocol recommendations exhibit low interrater agreement."[1] They note:

> [T]he rating dimensions we used represent key aspects of the protocol review process (e.g., justification for the number and type of animals in the study). Thus, to the extent that unreliability arose from a failure to consider these dimensions during the original protocol review, these results become even more serious. Only 2% of the animal research protocols submitted to us had been disapproved by the original IACUC; in the context of low interrater agreement, this base rate implies that IACUCs will rarely disapprove of protocols that other committees feel should be rejected.

This problem of unreliable reviews is most noticeable when IACUCs consider the use of non-animal methods in place of methods using animals. According to Policy #12 in the federal *Animal Care Policy Manual*, "A fundamental goal of the AWA [Animal Welfare Act] and the accompanying regulations is the minimization of animal pain and distress via the consideration of alternatives and alternative methods. . . . [A] written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives or alternative methods."[2] This directive is consistently disregarded even though scientifically validated non-animal testing methods are often available.

In its September 2005 audit, the U.S. Department of Agriculture's (USDA) Office of the Inspector General (OIG) stated that "33 of the top 50 (66 percent) research facility violators in the nation were educational institutions, suggesting that IACUCs at universities are less effective . . . . [Some] facilities were resistant to change, showing a general disregard for APHIS regulations. VMOs informed [the OIG] that some institutional officials were not supportive of IACUC activities and APHIS regulations, resulting in significant issues with animal care at the facilities."[3]

To comply with the spirit of federal regulations governing animal welfare, if an approved non-animal method exists, then it should be used (and not merely "considered" and disregarded) in place of methods using animals. A brief review of various animal welfare policies shows that such a stance is now mainstream around the world:

- The Medical Research Council (MRC) states that "[t]he use of animals must be essential to the research. *Where an appropriate alternative exists, it must be used*" [*emphasis added*].[4]
- Belgian law states that "*experiments on animals are forbidden if any valid alternative method not using animals is available*" [*emphasis added*].[5]
- GlaxoSmithKline (GSK) states: "*If a non-animal method is developed to replace animals, then it must be used*" [*emphasis added*].[6]
- The U.S. Department of Defense (DOD) states: "Alternative methods to the use of animals *must be considered and used* if such alternatives produce scientifically valid or equivalent results to attain the research, education, training, and testing objectives" [*emphasis added*].[7]

NIH can easily solve the troubling IACUC reliability problem by incorporating the following measures into the *Guide*:

1. **Standardize the evaluative criteria by which IACUCs determine whether non-animal methods are acceptable for use in place of animals, such that all IACUCs—following the same procedures—should reach the same conclusions.**
2. **Weigh multiple IACUC judgments when analyzing the feasibility of using non-animal methods in place of animals. If the majority of those judgments call for the use of a non-animal method in a particular instance, then each IACUC should follow suit and replace its use of animals with the non-animal method.**
3. **Catalogue alternatives and non-animal methods that are deemed by IACUCs to be acceptable replacements for animals in a national registry that is searchable and available to the public.**

4. **Adopt and enforce the MRC, Belgian, GSK, and/or DOD regulations that mandate the use of approved non-animal methods in place of animals.**

<u>Enforcement of Non-Animal Method Use Is Needed</u>
A variety of non-animal test methods are currently approved and validated; however, they are not being fully utilized. Examples of validated and/or internationally accepted non-animal methods include dermal absorption (OECD Test Guideline No. 428), skin corrosion (OECD Test Guideline No. 431), phototoxicity (OECD Test Guideline No. 432), the ECVAM-validated embryonic stem cell test for embryotoxicity, and *in vitro* bioreactors for monoclonal antibody production. OECD Test Guidelines Nos. 420, 423, and 425 for acute oral toxicity should always be used in place of the traditional LD-50 (OECD Test Guideline No. 401), which has been replaced, and preferably, a basal cytotoxicity test should be used as a dose-setting measure beforehand. In the case of pyrogenicity testing, experimenters must use human-blood-based methods such as Endosafe-IPT®.

We urge the NIH to amend the *Guide* to explicitly state that experimenters will be subject to penalties (e.g., grant suspensions) and/or enforcement proceedings whenever and wherever the agency finds that experimenters are using animals when accepted non-animal methods exist.

<u>Cost-Saving Considerations Are Not Acceptable Reasons to Continue Animal Experimentation</u>
Jodie A. Kulpa-Eddy, a staff veterinarian at USDA-APHIS-Animal Care, states that "While the cost of utilizing an alternative may be a factor presented to the IACUC, cost savings alone has never been considered an adequate explanation for requiring animals to endure pain or distress."[8]

We ask the NIH to amend the *Guide* to explicitly state that animal use is not to be decided on the basis of cost-savings when non-animal methods are available. Rather, the merit of non-animal methods should rest solely on their successful passage through appropriate scientific validation trials. If acceptable non-animal methods are available, then the NIH should *require* that they be used in place of animal tests.

<u>The Scientific Integrity of Primate Experimentation Is Compromised by the Stress Factor</u>
The *Guide* notes that the criteria for timely intervention and removal of animals from a study should be considered in the preparation and review of animal care and use protocols. One often overlooked, yet highly important, criterion is the confounding factor of stress that nonhuman primates (NHP) regularly face in laboratory experiments. These experiments are inherently compromised by the pervasive biochemical, physiological, epidemiological, behavioral, social, psychological, and cognitive contamination caused by stress and the impossibility of accurately defining and controlling the myriad causes and effects of stress. (Please refer to the enclosed brief.)

On February 17, 2005, distinguished cardiologist John J. Pippin, M.D., F.A.C.C., presented a 23-page brief before the U.S. Food and Drug Administration's (FDA) Arthritis Advisory Committee and Drug Safety and Risk Management Advisory

Committee detailing how animal experiments misled scientists in the development of Vioxx and the other COX-2 inhibitors. He stated:

> Such basics of laboratory animal studies as manual handling, blood drawing, intravascular or intracavitary blood drawing, intravascular or intracavitary injections, orogastric gavage, vascular or other instrumentations, and anesthesia produce profound and lingering physiological alterations .... Even such routine measures as entering an animal's room, moving its cage, using different types of bedding, lighting, noise, water availability, and dietary changes may alter animal behavior and physiology. Typical alterations include behavioral changes (anxiety, fear, hyperactivity), increases in biochemical stress markers (corticosterone, epinephrine and norepinephrine, glucose, thyroid hormones, growth hormone, prolactin), and increases in physiological stress markers (blood pressure and heart rate) .... *The introduction of physical and mental stress, with the attendant physiological disruption, is inseparable from manipulation of the animals for evaluation. Such changes likely compromise or invalidate data obtained from the animals* [*emphasis added*].[9]

On scientific grounds, we urge the NIH to amend the *Guide* such that IACUCs shall no longer approve protocols involving the use of NHPs (or at the very least, great apes) in laboratories.

Such a move has widespread support and precedent. The use of great apes in experiments has been banned in Great Britain, New Zealand, Sweden, the Netherlands, and Austria, and Japan has halted invasive experimentation on great apes. The Honorable Elisabeth Gehrer, Austria's education, science, and culture minister, praised the country's progressive stance: "Great apes are the animals that are most closely related to humans. It is of particular concern for me that there is this explicit prohibition. This will ensure that no such animal experiments will be carried out in the future either."[10]

Furthermore, on February 28, 2006, the Honorable David Drew—Labour and Cooperative Party MP for Stroud in England—introduced Early Day Motion 1704, which called for an end to the use of primates in laboratory experimentation. Specifically, Drew stated that "[T]his House ... notes that [NHPs'] level of sentience and highly developed social instincts make it extremely difficult to meet their behavioural needs in a laboratory setting; further notes that physical differences between human beings and other primates may make it impossible to predict reliably human outcomes from primate procedures; further notes public opposition to the use of primates; calls upon the Government to extend the current ban on the use of great apes to all primates as a matter of urgency; and further calls on the Government to press for an EU-wide ban on primate experiments as part of the impending review of European Union Directive 86/609/EEC."[11]

The NIH Can Improve the Welfare of NHPs Used in Experimentation

Notwithstanding a complete ban on the use of NHPs or great apes in laboratory experiments, there are ways in which experimenters can mitigate some of the confounding factors of stress, including those discussed below.

Environmental Enhancement

In 1997, APHIS interviewed its animal care inspectors, many of whom felt that too many primates were unnecessarily single-housed, especially at research laboratories.[12] According to Kulpa-Eddy *et al.*, "Prolonged single caging does not promote well-being, especially when it is started at an early age (Lutz *et al.* 2003; Turner and Grantham 2002). In one modified preference test, the value level of social companionship was so high that primates chose it in lieu of food (Dettmer and Fragaszy 2000)."[13, 14, 15, 16] When social group housing is not feasible, Kulpa-Eddy *et al.* suggest that "[f]acilities should consider partial forms of social grouping (e.g., adjacent grooming compartments, connector tunnels, and social rotations) …."[17]

We urge the NIH to revise the *Guide* by requiring that experimenters utilize pair or social group housing and to make exemptions to such housing only when the rationale has been thoroughly scrutinized by a review committee; when exemptions are given, the *Guide* should specify that Kulpa-Eddy *et al.*'s environmental enhancement protocols must be implemented.

Environmental Enrichment

Kulpa-Eddy *et al.* also state that "[w]hen home cages are of minimum legal size, enlargements or exercise areas can be an aid to enrichment, as long as meaningful complexities are arranged within them (Jensvold *et al.* 2001; Prescott and Buchanan-Smith 2004; Buchanan-Smith *et al.* 2004). Examples of such space displacing items are shelves, hammocks, perches, swings, nest boxes, large toys, or another animal."[18, 19, 20, 21]

We encourage you to amend the *Guide* such that experimenters are required to incorporate Kulpa-Eddy *et al.*'s environmental enrichment recommendations into their experiments. In addition, we recommend the inclusion of other types of enrichment, such as feeder probes, puzzles, boards, and other items that can be manipulated manually or orally (e.g., gnawing sticks), and a mirror in good condition. Also, foods that require processing (corn, peanuts, etc.) should be provided in conjunction with a variety of other food items to supplement chow and water or the protocol diet.

Husbandry and Handling

Kulpa-Eddy *et al.* state that "some primates develop increasingly fearful reactions to caretaker cues that signal the onset of involuntary restraint. It is possible to reduce or eliminate the potential confounding effects of handling stress on research through patience and the use of rewards (Reinhardt and Reinhardt 2000)."[22, 23]

Specifically, we ask that the NIH revise the *Guide* such that the manual capture and restraint currently used for routine husbandry and data collection should be replaced with positive-reinforcement training (PRT) for voluntary participation by nonhuman primates. It should be required that procedures with documented

success (see Appendix A) be implemented immediately, and there should be a written plan and timeline for expansion to other aspects of husbandry and to novel experimental protocols as they are developed. Furthermore, introduction of monkeys to new procedures, equipment, and housing must include a reasonable habituation period supported by PRT. Additionally, all personnel performing husbandry or data collection must complete training and testing in appropriate PRT methods.

Kulpa-Eddy *et al.* also commend various new initiatives in the research community, such as "[r]esearchers realizing the benefits of using normally developed primates and requesting that suppliers leave infants with their natal groups longer."[24]

We ask that the *Guide* be amended to require that, per International Primatological Society guidelines for the acquisition, care, and breeding of nonhuman primates, infants not be weaned artificially or removed from their mothers prior to 18 months of age.[25]

In a paper published in a 2000 issue of *The Journal of Neuroscience*, Darlene D. Francis *et al.* state: "In rodents or nonhuman primates, prolonged periods of maternal separation (MS) in early life increase the magnitude of neuroendocrine and fear responses to stress and thus vulnerability for stress-related illness (Higley *et al.*, 1991; Plotsky and Meaney, 1993; Suomi, 1997; Hall *et al.*, 1999; Caldji *et al.*, 2000; Ladd *et al.*, 2000; Liu *et al.*, 2000; Meaney, 2001). A synopsis of the paper states: "Postnatal maternal separation increases hypothalamic corticotropin-releasing factor (CRF) gene expression and hypothalamic-pituitary-adrenal (HPA) and behavioral responses to stress. We report here that environmental enrichment during the peripubertal period completely reverses the effects of maternal separation on both HPA and behavioral responses to stress, with no effect on CRF mRNA expression. We conclude that environmental enrichment leads to a functional reversal of the effects of maternal separation through compensation for, rather than reversal of, the neural effects of early life adversity."[26, 27, 28, 29, 30, 31, 32, 33, 34]

For those animals who currently suffer from the deleterious effects of maternal separation, we urge you to amend the *Guide* to require that all the aforementioned environmental enrichment methods be implemented immediately.

Kulpa-Eddy *et al.* also offer praise for other new initiatives that are being used in the research community, including the following:

- Socialization, habituation, and training programs for dogs established by laboratory animal suppliers and utilized by research facilities (Adams *et al.* 2004; Hubrecht 1995).
- Improved design of dog runs to increase cage complexity and human interaction (Hubrecht 1993; Loveridge 1998).
- Providing treats and toys to dogs (where appropriate) to encourage human interaction (Wells 2004).
- Increasing use of training of primates as an enrichment strategy to reduce handling and procedural stress and to facilitate other

enrichments such as resocialization or release into exercise cages (Laule *et al.* 2003).

- Group caging of primates in large indoor built-in runs.
- Use of exercise areas (Storey *et al.* 2000), connector tunnels, very large windows, skylights, swimming tubs, and outdoor access. Large windows between rooms and service corridors give primates an opportunity to observe and habituate to humans under nonthreatening circumstances.
- Requests to primate suppliers to randomize and pair animals in advance of shipment. Socializing and training continue through quarantine.
- Personality profiling that allows faster re-pairing with new candidates for primates that have been separated during a study.
- Use of psychoactive drugs from human medicine to treat primates for self-injurious behavior, stereotypy, or depression (Hugo *et al.* 2003; Troisi 2002).
- Voluntary enrichment of species other than primates and dogs, especially swine, cats, and rabbits.[35, 36, 37, 38, 39, 40, 41, 42, 43, 44]

In its revision of the *Guide*, we encourage the NIH to endorse and include a requirement that experimenters implement each of Kulpa-Eddy *et al.*'s "new initiatives," listed above, with the following stipulations:

- **Regarding the increased use of training of primates as an enrichment strategy, we urge the NIH to require that all training follow a PRT protocol and be wholly voluntary for the animals, such that the animal can move away from the part of the enclosure where the training is taking place. Also, the training should be an activity that falls outside the skills that the trainers are trying to teach (e.g., learning to play a game or solve a puzzle that was not part of a test, with a wide range of responses rewarded).**

- **Regarding Kulpa-Eddy *et al.*'s suggestion about the use of psychoactive drugs, medicating symptoms cannot take precedence over addressing the cause of the problems. In the case of self-biting, for example, animals are effectively put into a chemical restraint; the frequency and intensity of self-biting are often reduced, but so are all behaviors. Mother-infant separation and the proportion of time spent in single caging are two of the strongest correlates of self-injuring. For animals who are adults now, drugs may be a necessary part of intervention. But, for a prospective psychological well-being plan, leaving babies with their mothers and implementing social housing should be the highest priorities.**

<u>Adoption of Humane Bleeding Methods for Mice Is Needed</u>

In a paper published in the October 2005 issue of *Lab Animal*, Dr. William T. Golde—a microbiologist at the USDA's Plum Island Animal Disease Center—and his colleagues rightly argue that "[a]lthough Institutional Animal Care and Use Committees will approve protocols including several blood collection methods, *none are particularly simple or humane*" [*emphasis added*].[45]

For scientific and ethical reasons, we ask the NIH to amend the *Guide* such that IACUCs shall no longer approve protocols involving the use of retro-orbital, cardiac puncture, tail clip, tail laceration, and saphenous vein puncture bleeding methods for mice.

Retro-Orbital Blood Collection

Golde *et al.* state:

> In the United States, the most common rodent bleeding method is retro-orbital, puncturing the orbital sinus behind the eye. … Nevertheless, poor technique can blind the animal, and several countries have banned this method because officials consider it to be inhumane.[46]

Cardiac Puncture Blood Collection

Golde *et al.* state:

> This procedure requires anesthesia, which may alter parameters of the experiment. … This is not a simple method and is only humane when the procedure goes very well, leaving minimal damage to cardiac and pericardial tissues along the needle track. Missing the heart or passing the needle completely through the heart could lead to undetected internal bleeding or other complications. Because the chance of losing animals is so great, investigators choosing this method often supplement the number of animals requested for the research so as to accommodate loss during an experiment.[47]

Tail Clip Blood Collection

Golde *et al.* state:

> A major disadvantage is that to leave enough tail for several future bleeds, the portion of tail excised must be small, thus yielding a small blood sample of a few drops (<0.1 ml). Another problem with this method is that it could lead to cannibalism among cagemates and is not at all humane, especially for several blood draws.[48]

Tail Laceration Blood Collection

Golde *et al.* state:

> This technique yields as much as 0.5 ml of blood; however, it usually requires anesthesia, and an incision made too deeply can complicate repair.[49]

Saphenous Vein Puncture Blood Collection
Golde *et al.* state:

> [T]his procedure is slow, requiring extensive time working with each animal, and is not compatible with large trials of pharmaceuticals or biologicals. The time required to do a large trial (*e.g.*, 50–100 animals) would cause researchers to design smaller experiments using fewer animals. The investigators describing this method limit the amount of blood collected to 0.3 ml, and in practice, the blood volumes collected are even less. This commonly would yield ~0.1 ml of serum and limit analysis to a few very small-volume assays.[50]

We urge the NIH to require all experimenters who perform blood collection procedures on mice to use only a submandibular bleeding lancet (such as the GoldenRod animal lancet developed by Golde *et al.*, which is available through MEDIpoint, Inc.).

Golde *et al.* note:

> The new mouse lancet for submandibular bleeding is a humane, efficient, and economical method for bleeding laboratory mice. Similar styles of blood lancet have been in use for decades to draw blood from many mammalian species, especially humans, resulting in very little pain, discomfort, and tissue damage. We believe that this method will not only improve scientific design and results in studies using laboratory mice, but may also have application to other laboratory animals, including rats, hamsters, and gerbils.[51]

We appreciate the opportunity to provide our input and hope to see our recommendations incorporated in the revised *Guide*. If you should have any questions or concerns, please contact me at ShalinG@peta.org or 757-962-8325. Thank you.

Respectfully submitted,

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enclosures: Brief on primate fear
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Fear, Anxiety, and Stress in the Laboratory: Why Nonhuman Primates Make Poor Research Subjects

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We have compiled the following executive brief for the convenience of IACUC personnel to help negotiate and summarize the recent literature on this subject. It indexes and appraises the recent studies on the causes and effects of stress on primates in laboratories, including the reasons these factors can never be eliminated or controlled. The brief is organized as follows:

1. Specific Laboratory Stressors of Primates

1.1 Housing and Social Stressors

Laboratory cages are physically confining and socially restrictive living spaces for primates, and these conditions impose unreasonable stresses upon them. Recent studies have confirmed the causes and effects of housing and social stressors on primates, including primates who are subjected to solitary lives in cages or those who are housed in cramped, crowded conditions. Other studies have shown the harmful consequences of separating primates from their cage mates and placing them together arbitrarily into new groups, altering power dynamics and systems of social support. In all these cases, imposing unnatural physical and social configurations on primates resulted in profound disruptions of species-specific behavior and physiological issues.[1,2,3,4,5,6,7,8,9,10,11]

- Cross, Pines, and Rogers (2004) and Soltis, Wegner, and Newman (2003), for example, demonstrated that both the presence of conspecifics or separation from conspecifics can be causes of acute stress. [12,13]

- Shapiro *et al.* (2000) and Reinhardt and Rossel (2001) documented how individual caging constitutes such a potent stressor as to produce immunosuppression.[14,15]

- Chase *et al.* (2000) and Bellanca and Crockett (2001) demonstrated that singly housed, socially restricted primates paced more, locomoted significantly less, were more aggressive, and manifested significantly more abnormal behaviors.[16,17]

- Boyce *et al.* (1998) noted that when confinement space is reduced, the crowded conditions result in a five-fold increase over six months in the incidence of violent injuries.[18]

- Cross, Pines, and Rogers (2004) documented how separating animals with social bonds stimulates a response consisting of behavioral agitation and adrenal activity, and Pines, Kaplan, and Rogers (2004) demonstrated how marmosets are negatively affected by any events adversely affecting a roommate. [19,20]

- Crockett *et al.* (2000) and Reinhardt (2000) demonstrated that even subtle changes in conditions of captivity such as different cage sizes and cage levels can be extremely stressful to primates.[21,22]

1.2 Environmental Stressors

Laboratory environments differ enormously from natural habitats, and recent studies have demonstrated that several of a laboratory's environmental conditions contribute to unacceptable levels of stress in primates, including ambient temperature, lighting conditions, loud noises, cage locations, and even the mere presence of humans in primate rooms. Although some laboratories have been able to make some small modifications in the environmental conditions of their laboratories, it is not possible for primates to live in laboratories and participate in experiments without suffering from environmental stress.[23,24,25,26,27,28,29,30,31,32,33,34,35]

- Reinhardt and Reinhardt (2000a) demonstrated that poor lighting in laboratories frequently provides a cave-like housing environment for primates, particularly for those who are forced to live ground-dwelling lifestyles in bottom-tier cages. Reinhardt concludes that these conditions impair well-being and invalidate research data.[36]

- Cross, Pines, and Rogers (2004) documented how noise adversely affects primates in laboratories. Their mean levels of salivary cortisol during periods of disturbance were four times higher than normal.[37]

- Reinhardt and Reinhardt (2000b) recorded that primates exhibit apprehension and fear when an investigator or technician even enters the room.[38]

1.3 Husbandry Stressors

Primates in laboratories are subjected to a variety of routine animal husbandry procedures, all of which are experienced as stressful even when a laboratory follows best practices. The most sensitively conducted non-invasive and non-experimental procedures can create stressful conditions in captive primates. A study by Balcombe (2004) on the effects of routine husbandry on rats concluded that non-invasive manipulation occurring as part of routine husbandry, including lifting an animal, cleaning or moving an animal's cage, etc., resulted in "significant changes in physiologic parameters correlated with stress (e.g., serum or plasma concentrations of corticosterone, glucose, growth hormone or prolactin, heart rate, blood pressure, and behavior."[39] The effects on primates are that much more complex and profound. For example:

- Carstens and Moberg (2000) cautioned, "What might be viewed as innocuous manipulation of the animal may confound experimental results," and Wolfe (2000) confirmed that stress results from "both experimental and non-experimental sources."[40,41]

- Suzuki (2002) documented how plasma cortisol levels increased when a large adult male researcher entered the room, as macaques instinctively assumed the researcher to be a predator or rival.[42]

- Line *et al.* (1989) demonstrated that primates become significantly stressed when their room or cages are cleaned or they are tested for tuberculosis. Heart rates can remain elevated for hours after these events, and primates do not habituate to them.[43]

Capture is especially stressful for primates, and they frequently reveal their distress in obvious ways such as crouching, assuming defensive postures, diarrhea, fear grinning, attempting to flee, grimacing, suffering from rectal prolapse, screaming, struggling, or making aggressive displays. Primates are frequently restrained and captured in laboratories, and they always experience restraint as stressful regardless of the method used. Common methods of restraint and studies that have demonstrated their stressful effects include anesthetics such as ketamine, board restraints, chair restraints, chute restraints, guillotine panels, manual restraint, squeeze cages, table restraints, tethering, and transfer boxes. In addition to capture and restraint, recent studies have demonstrated that primates are also significantly stressed by other routine husbandry procedures such as feeding, medical procedures, palpation, pregnancy examinations, and weighing.[44,45,46,47,48,49,50,51,52,53,54,55,56,57]

1.4 Protocol Stressors

All research protocols are stressful to primates, even those that are not specifically designed to produce stress. Most of these involve at least some of the following standard components which multiple studies have proved produce stress and skew data: behavioral testing, blood sampling, novel situations and environmental manipulation, stool sampling, reproduction techniques such as penile vibratory stimulation or electroejaculation, venipuncture, and saliva or urine sampling.[58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75]

- McAllister (2004) and Reinhardt and Reinhardt (2000) documented how using cortisol levels as a measure of stress are complicated by the use of invasive techniques that may increase hypothalamic-pituitary-adrenal HPA axis activity during sample collection.[76,77]

- Yeoman (1998) and Cui (1996) demonstrated the detrimental effects of stress on sperm yield and quality on samples collected through the highly stressful and painful method of electroejaculation.[78,79]

1.5 Pre-laboratory Stressors (When Applicable)

The effects of stress are persistent and may have begun before a primate enters a laboratory. These unknown variables, which may have already altered physiology and behavior as well as receptivity to new procedures, further complicate attempts at establishing reliable controls.

a) Prenatal and Early Rearing Sources of Stress

- Gorman and Coplan (2002) and Clarke *et al.* (2004) demonstrated that prenatal stress can produce profound alterations in biological factors such as regulation of hypothalamic-pituitary-adrenal (HPA) axis, biogenic amines, and immune function. Coe (2003) confirmed that the prenatal environment can alter behavior, dysregulate neuroendocrine systems, and affect the hippocampal structures in primates in a persistent manner.[80, 81, 82]

- Barr *et al.* (2003) and Lutz *et al.* (2003) documented that macaques with histories of early-life stress have also have exhibited impulsive aggression, incompetent social behavior, and increased behavioral and endocrine responsivity to stress. Tiefenbacher (2005) demonstrated that chances of primates developing self-injurious behavior is heightened by adverse early experiences and subsequent stress exposure.[83,84,85]

b) Capture and Transportation/Relocation Sources of Stress

- Laudenslager *et al.* (1999) described the magnitude of stress associated with original capture, noting that during the period of captivity, plasma cortiosol rose, plasma prolactin and growth hormone fell, and there was a significant rise in insulin.[86]

- Honess, Johnson, and Wolfensohn (2004) documented the stress caused by air transport and re-housing and reported that the behavioral changes which occurred never returned to levels at the original breeding facility within the first month, an experience that "may result in the compromising of the welfare of the study animals."[87]

2. Specific Effects of Laboratory Stressors in Primates

2.1 Biochemical, Physiological, and Epidemiological Effects

There is a wealth of information detailing the extent to which stress disrupts the major physical functions of primates and leads to the development of disease and other pathologies.

- Carstens and Moberg (2000), for example, report that the cumulative effects of several stressors on primates leads to diversion of resources that results in their suffering from immune incompetence and other pathologies such as loss of reproductive abilities.[88]

Laboratory stress in primates affects the biochemistry of their endocrine, immune, and reproductive systems. The endocrine system is the adrenal gland, including the cortex and the medulla, adrenal hormones, including adrenal androgens, cortisol, adrenal corticoids, corticosteroids, and glucocorticoids. It also includes the pituitary gland and its hormones, including trophic hormones, the pituitary-adrenocortical-hypothalamic system, thyroid gland hormones, catecholamines, luteinizing hormones, lymphoids, prolactin, and opiate hormones.[89,90,91,92,93,94,95,96,97,98,99,100]

Stress affects the immune system of primates in laboratories by altering general antibody responses, the character of lymphocytes—including B cells, CD4+ cells, CD8+ cells, and T cells—cytokine, interferon, hematocrit, hemoglobin, monocytes, natural killer cell (NK) activity, prostaglandins, and white blood cells.[101,102,103,104,105,106,107,108,109,110]

The reproductive system undergoes general changes as well. The organs affected are the pituitary-gonadal hormones, ovaries, placenta, the follicular phase and luteal phase of menstruation, testosterone, dihydrotestosterone, progesterone, pregnenolone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, 20a-dihydroprogesterone, estrone, estradiol, DHA and DHAS, semen volume, and motility.[111,112,113,114,115,116,117,118,119,120]

The known physiological effects of stress in primates in laboratories include arteriosclerosis, osteoporosis, diabetes, changes in blood pressure, body temperature, circadian rhythms, ECG patterns, enzymatic shifts, heart rate, leukocytosis, metabolism, respiratory rates, sleep patterns, and weight gain or loss.[121,122,123,124,125,126,127,128,129,130,131,132,133,134,135]

- Gilmer and McKinney (2003) reported that the physiological effects of stress in primates included an altered hypothalamic-pituitary-adrenal response to stress, changes in diurnal temperature regulation, and alteration in immune function; Schapiro (2000) documented how diminished immune response is the most frequently observed consequence of prolonged or intense stress exposure.[136,137]

- Fuchs and Flugge (2004) documented how one month of stress reduced cell proliferation in the dentate gyrus and decreased the total hippocampal volume. . . . Stress also induced a constant hyperactivity of the hypothalamic-pituitary-adrenal axis and suppressed both motor and marking behaviors.[138]

These biochemical effects also make primates more susceptible to diseases, including bacterial infections, neutrophilia, parasitic infestations, and viral infections as well as doubling the possibility of endometrial cancer. Shivley (2004) and Boere *et al.* (2003) documented additional stress-induced pathologies such as higher incidences of diabetes, consumptive disorders, osteoporosis, arteriosclerosis, and gastric-duodenal ulcers. Bailey (2004) recorded how even prenatal stress altered bacterial colonization.[139,140,141,142,143,144]

- Shively (1999) concluded from studies of monkeys that social stress caused by low social status may be the underlying mechanism affecting pathophysiology and disease.[145]

2.2 Behavioral and Social Effects

The myriad behavioral abnormalities that characterize primates in laboratories have been well known for decades and include bizarre postures such as floating limbs, self-biting, self-clasping, self-grasping, and saluting; stereotyped motor acts such as pacing, head-tossing, head-weaving, bouncing in place, somersaulting, and rocking; appetite disorders such as uncontrollable eating, insufficient eating, frequent drinking, feces-eating, and paint-eating; sexual disorders such as inappropriate orientation, homosexual behavior, sexual dysfunction, and autoerotic stimulation; disturbed activity patterns such as inactivity, hyperactivity, and temporally inappropriate behavior; and agonistic disorders such as hyper-aggressiveness, fear-grinning, screaming, acute diarrhea, struggling and refusing to enter the squeeze cage; and self-abusive behavior such as self-biting, hair pulling, and self-scratching leading to physical harm.[146,147,148,149]

- Gilmer and McKinney (2003) demonstrated that early adverse experiences in primates can lead to behaviors including repetitive idiosyncratic behavior, increased self-directed behaviors, inappropriate expressions of aggressive behavior, nonmodulated patterns of consumption, and inappropriate sexual and maternal behavior.[150]

- Reinhardt and Rossel (2001) and The National Research Council (1998) documented how self-biting typically occurs in individually caged primates.[151,152]

2.3 Psychological and Cognitive Effects

Many of the social and behavioral effects of stress in captive primates have already been discussed in previous sections of this brief, and additional studies also illustrate its ill effects on primate psychology and cognitive functioning. These effects include degradations in their ability to engage in species-typical activities such as exercising, mating, raising children, maintaining mental well-being, engaging in normal forms of social companionship, performing routine tasks, and the ability to recognize predators.[153,154,155,156,157,158,159,160,161,162,163,164]

- Shivley (2005) documented how female cynomolgus monkeys suffered from signs of depression when they were isolated and exhibited lethargy, hormone disruptions, and higher heart rates—all of which are indicative of depression.[165]

- Gilmer and McKinney (2003) documented how early adverse experiences affected primates cognitively, resulting in such animals' requiring longer habituation time for any task. Arnsten and Goldman-Rakic (1998) and Moghaddam and Jackson (2004) demonstrated that noise stress impairs prefrontal cortical cognitive function in monkeys.[166,167,168]

3. General Characteristics of Stress for Primates in Laboratories

3.1 Primates Do Not Habituate to Laboratory Stressors

Experimenters frequently claim that primates in laboratories habituate to stress after a period of acclimatization, but this is untrue. Several recent studies have demonstrated that primates do not habituate to many stressors, even after years of exposure.[169,170,171,172,173,174,175,176,177]

Consider the following:

- Schnell *et al.* (1997) argued that it is impossible to completely inhibit the defensive reactions of primates to experimental procedures—even after long-term training. He demonstrated that primates in laboratories respond to restraint and venipuncture with marked, acute, and chronic increases in their heart rate and blood pressure even after years of experience as research subjects. Moreover, experienced primate research subjects have learned to anticipate restraint and venipuncture events by developing sustained patterns of cardiovascular stress.[178]

- Line *et al.* (1989) demonstrated that primates do not habituate to the stressors of room cleaning, cage cleaning, or tuberculosis testing. Line *et al.* documented how they became significantly stressed when their rooms or cages were cleaned or when they were tested for tuberculosis. Heart rates remained elevated for hours after these events, and primates did not habituate to them.[179]

- Gordon *et al.* (1992) demonstrated that experimentally naïve primates do not habituate to blood sampling procedures even after six weeks of exposure.[180]

- Honess, Johnson, and Wolfensohn (2004) reported that levels of stress a month after relocation from a breeding facility never returned to normal.[181]

- Lilly *et al.* (1999) demonstrated that primates did not acclimate to new housing situations even after 23 weeks in a new situation.[182]

- Golub and Anderson (1986) found that primates never adapted physiologically to the stresses of weekly blood sampling and manual palpation, even though they may have adapted behaviorally. Heart rate, blood pressure, respiration rate, and cortisol levels always rose during these procedures, even in primates who have experienced these procedures for 23 weeks.[183]

- Laudenslager *et al.* (1985) discussed how primates who are forced to endure separation experiences from their mothers or troop members frequently suffer from abnormal heart rates, body temperatures, circadian rhythms, EEG patterns, cellular immune function, and behavioral and neurological pathologies more than three years after the separation event. These changes persist for several years after the separation experience and may be permanent for some primates.[184]

3.2 Laboratories Cannot Eliminate Stressors

Sometimes experimenters and laboratory staff believe that they can improve or modify their laboratory environments and procedures to reduce or eliminate unwanted stress in the lives of the primates under their care. But this is almost always an impossible goal, even in the best of primate sanctuaries. Primates are simply too sensitive to stress, and laboratory environments are inherently too stressful for primates to live in them without suffering the unnatural and data-contaminating condition of ceaseless stress.

- Barros and Tomaz (2002) and Tatoyan and Cherkovich (1972) demonstrated that the mere presence of a human observer is capable of eliciting defensive attack and anxiety-related behavior. In many cases, the presence of human beings is even more stressful to primates than being restrained.[185,186]

- Schapiro *et al.* (2000) demonstrated that every type of laboratory housing for primates degrades the effectiveness of at least some components of their immune systems.[187]

3.3 Primates Hide Symptoms of Stress, and Many Symptoms of Stress Are Difficult to Diagnose and Detect

It is widely documented that primates not only hide symptoms of stress as defensive measures, but that symptoms of stress may be indiscernible or invisible to the investigator. Many primates in laboratories may look fine, but inwardly they are suffering from the damaging effects of stress in their biochemistry, physiology, psychology, and sociability. Usually only the most extreme forms of fear, pain, or suffering will cause primates to show the visible effects of their distress.[188,189,190]

- Coe *et al.* (1987) demonstrated that primates who are separated from their troops suffer from diminished immune system response, even though they do not appear debilitated or depressed. Coe concluded that it is not possible to visually identify the effects of diminished immune system response in primates that are suffering from separation experiences.[191]

Making diagnoses of stress more problematic is that the primate subject may also not be conscious of the physical effects of stress:

- For example, Carstens and Moberg (2000) discussed "stress-induced analgesia" and how psychological distress in primates can increase or decrease pain perception.[192]

Carstens and Moberg discussed as well how a tumor, for example, may elicit stress responses in an animal not conscious of the cancer. In a laboratory setting, such induced physiological pathologies are often an integral component, and many symptoms may not even be recognized as stress or be attributed to stress, as they may be the product of complex, interacting, and ambiguous physiological origins.

3.4 The Effects of Stress in Primates Are Complex and Interact

Stress is a complicated phenomenon, affecting multiple, interconnected systems, so that it is difficult to isolate as a single variable or effect. Primates react to stress in highly individualized and complex ways, especially at the biochemical level where the sympathetic nervous system, the hormonal systems, and the immune systems all interact with each other in response to stressful conditions. The complexity of these responses means that experimenters are frequently unable to know if the data that they collect reflect the results of the experimental procedures or the stressed condition of the primate in the laboratory. The results, therefore, are ambiguous because experimenters cannot reliably identify the causes of the effects they measure. Included in this brief are indexed dozens of studies that demonstrate this fact. But a few studies deserve special mention because they have examined the complex reality of stress in primates directly:

- Norcross and Newman (1999) identified that stress "can differentially affect the hormonal response without differentially affecting the behavioral [response]."[193]

- Carstens and Moberg (2000) stated that the most reasonable strategy for measuring stress would be to monitor the responses of the four major defense systems (behavior, autonomic nervous system, neuroendocrine system, and immune system) since they are responsible for the biological changes that occur during stress; however, they argued that none of the monitoring has proved to be a reliable measure of stress or *distress* since no single system responds to all stressors.[194]

- Shively (2005) described depression in primates as a "whole-body disorder."[195]

- Schapiro *et al.* (2000) demonstrated that even though stress indexes in primates are usually measured singly for purposes of experimental clarity, the actual biochemical realities of stress in primates are extremely complicated. Every single measurable stress effect interacts with all of the others, making it impossible to limit the biochemical and physiological effects of stress to only a few biological systems.[196]

- Goncharov *et al.* (1979) demonstrated that stressors evoked not just a few, initial hormone responses, but generally elicited a broad range of multiple, concurrent responses involving much of the neurological and endocrine systems.[197]

- Coe *et al.* (1987) demonstrated that the endocrine and immune systems of primates in laboratories do not change in simple ways in response to stress and concluded that we must not underestimate the true complexity of the total effects that stress has on them.[198]

3.5 Stress Affects Individual Primates Uniquely

Stress is a highly variable phenomenon affecting individual primates in unique ways and making statistically reliable data problematic.

- Carstens and Moberg (2000), for example, stated that because there is currently no litmus test for distress, trying to recognize distress must be done on almost a case-by-case basis. They added the caveat that the same stressor can be manifested in a variety of responses in the same animal.[199]

Further complicating stress measurements are the intra-animal differences in how the four general defense systems respond in attempting to cope with the stressor. Early experience, genetics, age, and physiological state are examples of a multitude of moderators that influence the nature of a stress response. With traditional laboratory animals such as rodents, many of these variables can be more easily controlled and accounted for in the experimental design, but for some laboratory animals (e.g. nonhuman primates or random-source animals), it is extremely difficult to account for these modulators of the stress response because simple measures of hormones, autonomic nervous system activity, or immune response may be unreliable measures of stress outside the experimental paradigm.

- Gust *et al.* (1994) demonstrated that the biochemical reactions of individual primates to social stressors vary widely. Gust concluded that because social stressors are one of the most common and upsetting forms of stress among primates housed in laboratories, the large effects of social stress and the wide variability in responsiveness among individuals make it difficult to interpret experimental data derived from them.[200]

- Sapolsky (2001, 1993) demonstrated how stress affects primates uniquely and how primates respond to stress in highly individualized ways.[201, 202]

3.6 Stress Variables Cannot Reliably Be Controlled, Factored, or Generalized

The scientific integrity of studies involving laboratory-confined primates is inherently compromised because of the pervasive contamination of stress and the impossibility of accurately defining and controlling the spectrum of causes and effects of stress. (Bentson *et al.* 2003).[203]

- Moberg (1999) argued that not only can pain and stress cause distress, the biologic effects can also compromise experimental results. Carstens and Moberg (2000) further cautioned that there are neither "agreed-upon definitions" for terms such as pain and stress nor are there absolute, objective measures because animals cannot verbalize what they are experiencing.[204,205]

- Hawkins (2003) reported that indicators of pain, suffering, and distress in primates are largely subjective.[206]

- Reinhardt (2004) concluded that there is no control over the time during which an environmental disturbance is occurring, a factor that must be mentioned to explain possible incongruities of data.[207]

- Schnell *et al.* (1997) demonstrated that the acute effects of stress in primates have broad implications for the evaluation of pharmacological profiles of drugs used in biomedical research.[208]

3.7 Cross-Species Misconceptions

Despite overwhelming evidence, there are still researchers who do not recognize the significance of stress factors in research on primates.

According to Haller (DD 2001), "There is an important discrepancy between animal models of anxiety and human anxiety patients: While experimental animals are usually unstressed, patients usually have a long history of stress."[209]

However, an equivalent mistake is the assumption that stress research on primate models can be meaningfully extrapolated to humans. Just as pharmacological efficacy has great variation between nonhuman and human primates, the experimental data obtained from nonhuman primates have little generalizability beyond the simple, tautological recognition that induced stressors cause symptoms of stress.

4. Recommendations

Laboratories are stressful environments, and the primates who are held within them endure lives of ceaseless anxiety, pain, and fear. Some laboratories are more stressful than others, but no laboratory can reduce the stresses that primates experience significantly enough to raise animal-welfare conditions to an acceptable level, and no laboratory can reduce the stressors sufficiently to produce meaningful and reliable scientific data. Clearly disturbing experiments such as those conducted at Columbia University have little scientific import and egregious ethical consequences. In these studies, monkeys had metal pipes surgically implanted into their skulls for the sole purpose of inducing stress in order to study the connection between stress and women's menstrual cycles. We urge all IACUCs and affiliated institutions not to accept or approve further protocols involving primates in laboratories.[210]

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Appendix A

Procedures and Protocols That Should Be Immediately Replaced With Positive Reinforcement Training (PRT)

Voluntary Presentation

- Venipuncture
- Oral swab or saliva collection
- Semen collection
- Vaginal or rectal swabs
- Urine collection
- Subcutaneous injections
- Intra-muscular injections
- Presentation of any parts of the body for limited veterinary examination

Translocation

- Change cages for husbandry, experimental protocol, veterinary care, and welfare management
- Return to cage after escape, in-room protocol, or transfer between locations

Social Relationships

- Facilitate positive social interactions between monkeys (using positive reinforcement for affiliation or neutral interactions)
- Minimize negative interactions between monkeys (using positive reinforcement for behavior that replaces excessive agonism between animals or that soothes agitated animals, e.g., assuming a neutral position in the cage, touching a specific feature in the cage, etc.)

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Snyder, Margaret (NIH/OD) [E] #50

From: Frank E. Barber [frankeb@olemiss.edu]
Sent: Friday, November 11, 2005 1:17 PM
To: Scientific Affairs (NIH/OD)
Subject: Animal Use

Probably the greatest fault in the system for limiting the use animals for research is the continuation of animal use when there are no valid or useful results from the animal experiments. This occurs when a case for use is made and approved, but:

A. In the course of the experiments the desired results are not obtained (the experiment doesn't work); or,
B. It's bad research, obfuscated inadvertently; a more expert peer reviewer would have denied approval or required modifications.

AND:
1. the investigator fails to acknowledge or report the fact when it becomes known to him/her, and
2. the investigator continues the experiments anyway.

Why this occurs is not clear but there are several possible reasons:

A. "beating a dead horse" (pardon the pun); continuing experiments in a vain hope that they might work in the future
B. concern that getting future animal use approvals may be difficult if the current experiments don't go well
C. belief that it is necessary to obtain a complete set of data to meet some contractual need
D. pride and/or inertia - to meet some personal commitment to oneself or others.
E. failure to "fix" a bad experiment because that would mean going back through a painful approval process

AND:
1. belief/knowledge that no one will know, or care, or dare to rat (pardon the pun) on a fellow scientist
2. knowledge that once a protocol is approved there is no penalty for continuing it to the
end regardless of the outcome (within limits, of course). (Masking bad research as a negative outcome.)

Possible solution is self policing, ie requires the investigator(s) to evaluate each experiment and sign off on a simple multiple choice form.
There would be no action required by anyone not participating in the experiment unless there is a violation.

Required certification by the investigators (persons doing the experiments), to the department head, on an animal by animal basis for large animals, or some other period when multiple animals are used simultaneously. Report would simply certify in writing, by checking a box and signing the form.

Box A. the experimental protocol is working and the experiments will progress as planned, or
Box B. the experimental protocol is not working but the investigator certifies that the problem has been fixed and subsequent experiments will continue on schedule, or
Box C. the experimental protocol is not working and the experiments will be suspended temporarily until the problem is fixed. In this case, the investigator must sign off on B certification before the experiments can start up again.
Box D. the experimental protocol is flawed (or other conditions) and the experiments are being terminated.

Designated senior investigators present at the experiment would be require to concur and sign. If there is disagreement, it must be worked out before continuing in a manner to be determined at the department level.

checking a box and signing the form.

Box A. the experimental protocol is working and the experiments will progress as planned, or

Box B. the experimental protocol is not working but the investigator certifies that the problem has been fixed and subsequent experiments will continue on schedule, or

Box C. the experimental protocol is not working and the experiments will be suspended temporarily until the problem is fixed. In this case, the investigator must sign off on B certification before the experiments can start up again.

Box D. the experimental protocol is flawed (or other conditions) and the experiments are being terminated.

Designated senior investigators present at the experiment would be require to concur and sign. If there is disagreement, it must be worked out before continuing in a manner to be determined at the department level.

Except for keeping the records, there is no work involved by anyone except that which is assumed to be done anyway; that is self-evaluation of experimental results on an animal by animal basis (and checking and signing a form.)

#51

Snyder, Margaret (NIH/OD) [E]

From: Selzer, Michael [Michael.Selzer@uphs.upenn.edu]

Sent: Wednesday, November 09, 2005 3:00 PM

To: Scientific Affairs (NIH/OD)

Subject: care of laboratory animals

I work on sea lampreys, a jawless primitive vertebrate without a boney spine. I think this species, and perhaps fishes should be exempt from the need to file animal welfare plans. Is there a possibility that this will be considered?

Michael E. Selzer, M.D., Ph.D.
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Scientific Affairs (NIH/OD) #52

From: Tull, Whitney [wtull@asmusa.org]
Sent: Thursday, March 30, 2006 3:26 PM
To: Scientific Affairs (NIH/OD)
Subject: RFI No. NOT-OD-06-011
Attachments: ASM Comments on RFI No. NOT-OD-06-011.doc

Dr. Margaret Snyder
Director, Office of Science Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

Dear Dr. Snyder,

Attached, please find comments from the American Society for Microbiology (ASM) regarding the identification of new scientific information that might warrant the NIH issuing a contract for a new or updated edition of the *Guide for the Care and Use of Laboratory Animals (The Guide)* (RFI No. NOT-OD-06-011).

Thank you,
Whitney

Whitney Tull
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Office of Public Affairs
American Society for Microbiology
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AMERICAN SOCIETY FOR MICROBIOLOGY

Public and Scientific Affairs Board

[RFI No. NOT-OD-06-011]

March 31, 2006

Dr. Margaret Snyder
Director, Office of Science Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

Dear Dr. Snyder:

The American Society for Microbiology (ASM) is responding to the National Institutes of Health (NIH) request for information in seeking to identify new scientific information that might warrant the NIH issuing a contract for a new or updated edition of the *Guide for the Care and Use of Laboratory Animals (The Guide)* (RFI No. NOT-OD-06-011). The following comments were developed by the ASM Committee on Agriculture and Food Microbiology (Committee), of the Public and Scientific Affairs Board.

The ASM is the largest single life science society with more than 42,000 members, including scientists in academic, industrial, clinical, and government institutions, working in areas related to basic and applied research, the prevention and treatment of infectious diseases, laboratory and diagnostic medicine, the environment, animal health, and water and food safety. The ASM applauds the NIH's efforts to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate.

Comments on the Guide for the Care and Use of Laboratory Animals

After carefully reviewing the publications in this area since the last revision of *The Guide* in 1996, the Committee detected two separate issues:

1- The number of publications in all the four areas addressed by *The Guide* has not increased as would have been expected based on the expansion and increase in the use of laboratory animals over the past 15 years. This is most likely attributed to the cuts in funding for the National Center for Research Resources (NCRR) during the early 1990s (3,4). Progress in the area of Laboratory Animal Medicine and Science will only occur if there are appropriate sources of funding.

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2- The scope of *The Guide* is very broad and its recommendations are used as benchmarks by many scientists. Changes in some of the base recommendations such as cage size, frequency of bedding and cage washing, and environmental enrichment may be required due to recent studies published that show certain modifications can improve the lives of laboratory animals (1,2,5,6,7,8,9,10,11,12,13).

The Committee notes that there have been publications that increase the body of knowledge in many areas regarding the four main chapters of *The Guide*, making it imperative to update the literature cited and the appendixes. Additionally, *The Guide* should be accessible electronically, which would facilitate the ability of scientists to be well informed and up to date. This would enable NIH to constantly update the reference list and make all of the reference documents electronically accessible in their entirety. *The Guide* should also be available for download onto PDAs and other types of mobile electronic apparatus.

Sincerely,

Ruth Berkelman, M.D.
Chair, Public and Scientific Affairs Board

Michael Doyle, Ph.D.
Chair, Committee on Agriculture and Food Microbiology

Susan Sanchez, Ph.D.
Member, ASM

Reference List

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Scientific Affairs (NIH/OD)

#53

From: Ellen Paul [ellen.paul@verizon.net]
Sent: Friday, March 31, 2006 9:07 AM
To: Scientific Affairs (NIH/OD)
Subject: RFI No. NOT-OD-06-011 (Attention: Dr. Margaret Snyder) - REVISED

Attachments: NIH-revision of Guide.doc

NIH-revision of Guide.doc (42 ...

Please substitute this document for the letter submitted yesterday. Minor errors have been corrected. Hard copy on letterhead is in the mail.

Thanks.

Ellen Paul

--
Ellen Paul
Executive Director
The Ornithological Council
Mailto:ellen.paul@verizon.net
Phone (301) 986 8568
Ornithological Council Website: http://www.nmnh.si.edu/BIRDNET "Providing Scientific Information about Birds"

The Ornithological Council

PROVIDING SCIENTIFIC INFORMATION ABOUT BIRDS

American Ornithologists' Union

Association of Field Ornithologists

CIPAMEX (Sección Mexicana del Consejo Internacional para la Preservación de las Aves)

Cooper Ornithological Society

Neotropical Ornithological Society

Pacific Seabird Group

Raptor Research Foundation

Society for the Conservation and Study of Caribbean Birds

Society of Canadian Ornithologists/ Société des Ornithologistes du Canada

Waterbird Society

Wilson Ornithological Society

27 March 2006

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184 , MSC 7983
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RE: RFI No. NOT-OD-06-011

Dear Dr. Snyder,

The Ornithological Council appreciates the opportunity to comment on the possible revision of the Guide for the Care and Use of Laboratory Animals (*Guide*). As a consortium of eleven scientific societies of ornithologists in the Western Hemisphere – seven of them in the United States – we are keenly concerned with the highly influential guidance published by the National Research Council's Institute for Laboratory Animal Welfare, the National Institutes of Health's Office of Laboratory Animal Welfare, APHIS, and their various private partners. The research conducted by the scientists we represent is judged by the Institutional Animal Care and Use Committees who use this guidance document in reviewing research protocols.

The Request for Information seeks "new scientific information that might warrant NIH issuing a contract for a new or updated edition of the *Guide* ." We are surprised that the question was asked in this manner, as it presupposes that the *Guide* establishes specific handling and care standards that would change if and when new research evaluating each standard becomes available. In fact, this is not the case. Perhaps the more appropriate question would have been, "Should the Guide be revised, and if so, why and how?"

Earlier this week, I attended the ARENA meeting in Boston, where a panel discussion was held on the potential revision of the Guide. Panelists confirmed that, as the preface states, the Guide was meant to provide principles and was outcome-oriented. It was not intended to be prescriptive or to provide "engineering standards." In our view, this was and still is an appropriate and useful purpose for the Guide. As principles of animal welfare have not changed, and as the desired outcomes have not changed, we suggest that there is no compelling reason to revise the Guide.

David E. Blockstein, Ph.D.
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It has always seemed odd that the *Guide* includes some very specific engineering standards, including recommended space allocations for group-housed animals and dry-bulb temperatures. If a revision is undertaken, perhaps these anomalous specifications could be removed to an appendix or even a separate publication. We also suggest that it would be appropriate to establish taxon-specific groups to study husbandry conditions (analogous to the might be appropriate before a revision is undertaken. We wonder if it is appropriate for the single most authoritative document – whose use is mandated for federal agencies – to establish standards that have not been tested through experimentation and subjected to peer review. However, while the standards should be based on peer-reviewed studies, those standards do not belong in the text.

It would be impractical, if not impossible, to revise the guide to incorporate "new scientific information" pertaining to the many different research methods and species studied in biomedical research and wildlife biology. Each subdiscipline of wildlife biology, for instance, has its own guidance for ethical and humane treatment of animals in research. The Ornithological Society publishes Guidelines to the Use of Wild Birds in Research. We are about to commence on our second major revision since the document was first published in 1988. We do not attempt to describe specific methods for every avian species. Like the *Guide*, our guidance sets out general principles and desired outcomes. The American Society of Mammalogists is just completing a revision to the 1998 edition of its Guidelines for the Capture, Handling and Care of Mammals.

Thus, as the *Guide* does not purport to be a compendium of specific methods and standards, new scientific information pertaining to existing or new methods does not mean that the Guide should be revised.

As to new scientific information pertaining to methods, we suggest that NIH could and should establish an online database of methods papers (full text to be contributed voluntarily). Making proper use of metadata, the users could search by taxon and method. In this way, researchers and IACUC members will have easy access to a wide range of literature to aid in designing suitable methods and in assessing those methods. The database will also serve to supplement the *Guide* in a way that will increase the utility of the *Guide* and that will avert need for periodic revision to incorporate "new scientific information."

We recognize that minor revisions are needed. For instance, the *Guide* does not refer to the 2000 Report of the AVMA Panel on Euthanasia, or to our updated *Guidelines to the Use of Wild Birds in Research.* A possible means to achieve this minor revision include the publication of an addendum or the conversion to an online publication (e.g., abandon the print edition entirely) that can be updated on a regular basis.

Should the Guide be revised, we hope that the revision will be undertaken in a manner that is appropriate to the scope of the research assessed by those who use the *Guide* in making those assessments. We have long been concerned, and have expressed our concern, that the 1996 edition of the Guide is inadequate with regard to wildlife biology. The definition of "field studies" under the Animal Welfare that purports to exempt most field studies instead comprises three very broad, undefined exceptions that collectively function to bring most field studies under IACUC review. And, in fact, the Public Health Service makes no distinction between field and laboratory studies, and universities make no such distinction. Therefore, a statement that "biomedical and behavioral investigations occasionally involve observation or use of vertebrate animals under field conditions" could only have been written and reviewed by scientists who have no contact with, or knowledge of, the very substantial field of wildlife biology. In the United States alone, seven peer-reviewed ornithological journals are published, and many other papers reporting ornithological research are published in other journals. Some ornithological research is conducted in a laboratory environment (often with wild birds that have been brought into captivity) but most is conducted in the field. There are many other wildlife biology subdisciplines.

To simply state, as does the *Guide*, that "some of the recommendations listed in this volume are not applicable to field conditions…" leads to a malfunction in the system of animal welfare oversight, which is, by design, a peer review system. In fact, most of the recommendations in the *Guide* are unsuited for wildlife biology. As a result, the *Guide* is of questionable relevance for IACUCs assessing protocols submitted by wildlife biologists. We therefore urge the NIH, ILAR, and other federal agencies that might undertake a revision to be sure to include among the writers, editors, and reviewers wildlife biologists who are knowledgeable about animal welfare principles and who can assure that any statements pertaining to wildlife biology are accurate, complete, and useful. We know that the National Academy is now sensitive to the issues of balanced representation, as required by the 1997 amendments to the Federal Advisory Committee Act and, of course, the National Institutes of Health is bound by the original FACA requirements. Legalities aside, it is inappropriate, and perhaps unethical, to provide animal welfare guidance without having the appropriate expertise. In addition to including wildlife biologists in every stage of the revision, we also urge that a revision provide that IACUCs must strive to attain appropriate knowledge – through consultation or otherwise – before assessing protocols for wildlife biology.

Should the NIH or other federal agency choose to revise the *Guide*, the Ornithological Council would like to have the opportunity to recommend ornithologists to serve on the as authors, editors and reviewers for the appropriate sections, and to serve on relevant panels and committees. We would also be glad to serve as a conduit to other wildlife societies.

Thank you for considering our comments. We hope they prove useful.

Sincerely,

Ellen Paul
Executive Director

#54

Scientific Affairs (NIH/OD)

From: Gross, Lauren [lgross@aai.org]
Sent: Friday, March 31, 2006 11:42 AM
To: Scientific Affairs (NIH/OD)
Cc: Ellen Kraig
Subject: RFI No. NOT-OD-06-011
Attachments: AAIcomments.AnimalWelfareGuideUpdate.033106.pdf

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184 , MSC 7983
Bethesda , MD 20892-7983

Dear Dr. Snyder:

Please find attached a letter from Ellen Kraig, Ph.D., Chair of the Committee on Public Affairs of The American Association of Immunologists (AAI), responding on behalf of AAI to NIH's *Request for Information (RFI): Standards for the Care and Use of Laboratory Animals: Notice Number: NOT-OD-06-011*.

Please let me know if you have any questions or if you have any difficulty accessing the attached document.

Thank you.

Sincerely,

Lauren G. Gross
Director of Public Policy and Government Affairs
The American Association of Immunologists

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THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

March 31, 2006

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge I, Suite 4184, MSC 7983
Bethesda, MD 20892-7983

by email to: ScientificAffairs@od.nih.gov

Re: RFI No. NOT-OD-06-011

Dear Dr. Snyder:

The American Association of Immunologists (AAI) appreciates having this opportunity to comment on the question of whether there is a need to update the laboratory animal welfare standards of the Guide for the Care and Use of Laboratory Animals ("Guide").

AAI has carefully reviewed the Guide and assessed whether it needs updating in view of changes in science and technology since 1996. In our view, the Guide is thorough, balanced, and flexible enough to accommodate changes and emerging needs, while continuing to ensure adequate protection of laboratory animals. Therefore, AAI does not see a need for the Guide to be updated at this time.

Please feel free to contact us if you have any questions.

Sincerely,

Ellen Kraig

Ellen Kraig, Ph.D.
Chair, AAI Committee on Public Affairs

9650 ROCKVILLE PIKE, BETHESDA, MARYLAND 20814-3994
PHONE: 301-634-7178 • FAX: 301-634-7887
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 #55

From: Joe Erwin [jerwin@agingapes.org]
Sent: Friday, March 31, 2006 3:09 PM
To: Scientific Affairs (NIH/OD)
Cc: jerwin@agingapes.org
Subject: Erwin re: NOT-OD-06-011

TO: Dr. Margaret Snyder, Director

FROM: Joseph M. Erwin, PhD
Semi-retired Biomedical & Behavioral Consultant,
Senior Scientist, Innovative Biosafety Systems, Inc., and
Executive Director, Foundation for Comparative and Conservation Biology (FCCB)

SUBJECT: Comments Regarding Revision of the GUIDE

Some advances in fundamental knowledge (especially in comparative genomics); research priorities (especially emphases on biodefense and infectious disease research, as well as the risk of a bird flu pandemic); and increased experience with biocontainment housing for research requiring elevated levels of biosafety, all warrant re-examination and revision of laboratory animal care and use.

My interest and expertise is principally with regard to research involving nonhuman primates (NHPs), and my comments here are mainly directed toward issues affecting NHPs.

Other areas of concern include the following: (1) research on aging that involves maintaining individual primates throughout the lifespan should be recognized as having some special considerations and priorities for care; (2) increased capacities for data mining using bioinformatics databases provide special opportunties to study a variety of spontaneously occurring diseases and disorders, such as obesity, diabetes, metabolic disorders, arthritis, osteoporosis, and neurodegenerative disorders, as well as the natural processes associated with aging (e.g., menopause) and healthy aging; (3) improved imaging technologies offer increased opportunities to study normal and abnormal processes across the lifespan; (4) the NRC primate nutrition guide has been updated and contains information that could be relevant for the guide, and it should at least be cited; (5) phenotypic characterization of primates (and probably other animals) is now more important than ever, due to increased availability of genetic and genomic information, and efforts to integrate data from various sources can be more profitable than ever before (thus making it even more important to identify source populations and maintain detailed records on individual primates); and (6) advances in robotics and telemetric monitoring should be recognized, along with the need to design housing systems that make use of these techniques to learn more from each individual animal with less exposure and greater safety for animal care personnel.

I would welcome an opportunity to supply specific information on any or all the above listed topics. Please contact me directly for additional information.

Joseph M. Erwin, Ph.D.
Adjunct Professor of Biomedical Sciences & Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine
at Virginia Tech
4139 Gem Bridge Road
Needmore, PA 17238
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jerwin@agingapes.org

Scientific Affairs (NIH/OD)

#56

From: Megha Even [meven@pcrm.org]
Sent: Thursday, March 30, 2006 1:54 PM
To: Megha Even
Subject: Serum-free hybridoma culture
Attachments: Serum-free hybridomas.pdf

Thank you for your interest in hybridoma technology for the production of monoclonal antibodies. Attached please find the *Trends in Biotechnology* opinion article, **Serum-free hybridoma culture: ethical, scientific and safety considerations**. I hope you find it useful for your work.

Please feel free to contact me with any questions.

Megha Shah Even, M.S.
Research Analyst
Physicians Committee for Responsible Medicine
5100 Wisconsin Ave NW, Suite 400
Washington DC 20016
T: 202.686.2210 ext. 327
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meven@pcrm.org
www.pcrm.org

NAME:	Megha Even/Physicians Committee for Responsible Medicine
ARTICLE/CONTENT:	Serum Free hybridoma culture: ethical, scientific ans safety considerations,
SOURCE:	Opinion - Trends in Biotech Vol. 24 No. 3, 3/2006

#57

The Jackson Laboratory

Beverly Paigen, Ph.D.
Senior Staff Scientist
207-288-6388 (Voice)
207-288-6078 (Fax)
[illegible] (E-mail)

March 31, 2006

Dr. Margaret Snyder
Director, Office of Scientific Affairs
Office of Extramural Research, OD, NIH
6705 Rockledge 1, Suite 4184, MSC 7983
Rockville, MD 20817

RE: request for information NOT-OD-06-011

Dear Dr. Snyder,

I am sending you this letter by email to make the deadline and am also sending you by FedEx the reprints referred to in this letter. Most of these studies have been published but the most recent are manuscripts in preparation and I wanted to alert you to the major conclusions.

This is a summary of studies carried out at The Jackson Laboratory over the last decade on mouse husbandry including:

the microenvironment of animal cages, particularly ammonia levels
ventilation of animal cages and rooms
frequency of cage changing
density of animals in cages
culling of pups
transmission of disease among animals
reduction of mouse allergen in animal facilities to protect workers.

These studies were started with the motivation of understanding the cause of the high prevalence of laboratory animal allergy and reducing its incidence. As a result of these studies, we found that housing animals in individually ventilated cages (PIV) and changing the cages under ventilation reduced airborne allergen 10-fold and reduced the number of animal caretakers reporting allergic symptoms from 50% to 10%. However, the cost of buying PIV cages and ventilated changing stations for animal rooms was high, so we began exploring means of reducing the costs of managing the animal facility but still maintaining animal health. This led to studies on the frequency of cage changing and housing density. We found that cages could be changed every two weeks and that animals could be housed at approximately twice the density recommended by the Guide.

Both changes greatly reduce cost and result in a slight improvement in animal health without any negative impact.

May I also suggest that a new Guide would be very timely and that moreover, with the development of technology, the Guide could be released in a loose-leaf form in a binder so that updated sections could be released over the web at more frequent intervals.

Below I have briefly summarized the major findings with the references. I will be sending the reprints that I have available under separate cover.

Sincerely yours,

Beverly Paigen

Beverly Paigen, Ph.D
Senior Staff Scientist, The Jackson Laboratory
Bar Harbor Maine, 04609
207-288-6388

Brief summary of findings

The allergen: The mouse allergen, Mus m1, is carried on particulates. Low humidity increases particulates and levels of allergens (1). Allergen is the only air contaminant present at high enough concentrations to cause symptoms in humans ; ammonia and volatile organics are too low to be significant (2). Particulates and allergen exposure to workers can be significantly reduced by changing cages on a table that has ventilation (2).

Room ventilation rates: Room ventilation more than 5 air changes/hour does not improve ventilation within animal cages (3). The air changes within cages is driven by the thermal heat load of the mice. Increased room ventilation may be important for human comfort but it is not important for animal health.

Individually ventilated cages- cage changing frequency: Based on several measures of health and cage microenvironment, the optimal frequency of changing cages was once every two weeks for breeding pairs or breeding trios (4, 5). Pup mortality increased with weekly changes; corticosterone levels tended to decrease (but not significantly) with decreased changing. Detailed histology of nasal passages of pups exposed to the highest levels of ammonia showed no abnormal changes (5).

Reducing allergens: We tried several strategies to reduce allergens including increased cleaning of room (no effect), tops for animal cages, using positive or negative pressure for the PIV cages, using or not using ventilated changing tables (6). We found that the best reduction was achieved with PIV cages under negative pressure and changed with ventilated changing tables (6, 7). This caused a 10-fold reduction in allergen levels in the air and the percentage of caretakers reporting allergic symptoms daily fell from 50% to 10% (7).

Negative pressure and the transmission of animal disease: We found that negative pressure did not increase the transmission of disease. In fact it was difficult to transmit disease except by direct exposure to a sick animal (cohabitation) or its bedding. Even transmission by a caretaker handling a sick animal and then a healthy one was not very efficient (8)

Housing density: Using a variety of measures of health and well-being, it was found that C57BL/6 mice could be housed at approximately twice the density recommended in the Guide (9). This study was followed by replication using three commonly used strains of mice with a reputation for being aggressive (BALB/c, FVB) or heavy soilers (NOD) (10). All except FVB males could be housed at twice the density; FVB males were aggressive at each housing density. In these previous studies, the number of parameters measured for health were limited to weight, health, hormones, aggression and stress. We next housed C57BL/6 mice at normal density and twice the recommended density and put them through the phenotyping protocol described at pga.jax.org, measuring complete blood counts, hematology parameters, blood pressure, lung function, blood chemistries, electrocardiograms, weight gain, hormones, obesity, and bone density over a 9-month period. Both the 5-mice/pen and 9-mice/pen groups were equal except that the mice at higher density had a significantly reduced reticulocyte count, probably explained by a nonsignificant decrease in heart rate. This is probably due to their greater calmness, reduced heart rate, thus reducing the need for new red blood cells. Everything else was similar. The manuscript describing this latter study is in preparation (11).

Culling of pups: Although the Guide does not state that pups should be culled, the recommendations for density based on weight/ space are widely interpreted to mean that no more than 12 pups should be in a cage before weaning. We carried out a study to test the effect of culling on survival and weight gain. We used a hybrid strain and trio matings so that it was common to have more than 12 pups in a pen. We compared three groups: not culled, culled to 12 pups, culled to 8 pups. There was no difference in survival or weight at weaning of the pups among all groups. The manuscript describing these results is being prepared (12).

Publications

1. Jones RB, Kacergis JB, MacDonald MR, McKnight FT, Turner WA, Ohman JL, Paigen B. 1995. The effect of relative humidity on mouse allergen levels in an environmentally-controlled mouse room. Am J Ind Med 56:398-401.

2. Kacergis JB, Jones RB, Reeb CK, Turner WA, Ohman JL, Ardman MR, Paigen B. 1996. Air quality in an animal facility: particulates, ammonia, and volatile organic compounds. Am Ind Hyg Assoc J 57:634-640.

3. Reeb CK, Jones RB, Bearg DW, Bedigian H, Paigen B. 1997. The impact of room ventilation rates on mouse cage ventilation and microenvironment. Contemp Top Lab Anim Sci 36:74-79.

4. Reeb CK, Jones RB, Bearg DW, Bedigian H, Myers DD, Paigen B. 1998. Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. Contemp Top Lab Anim Sci 37:43-49.

5. Reeb-Whitaker CK, Paigen B, Beamer WG, Bronson RT, Churchill GA, Schweitzer IB, Myers DD. 2001. The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. Lab Anim 35(1):58-73.

6. Reeb-Whitaker CK, Harrison DJ, Jones RB, Kacergis JB, Myers DD, Paigen B. 1999. Control strategies for aeroallergens in an animal facitity. J Allergy Clin Immunol 103(1 Pt 1):139-146.

7. Schweitzer IB, Smith E, Harrison DJ, Myers DD, Eggleston PA, Stockwell JD, Paigen B, Smith AL. 2003. Reducing exposure to laboratory animal allergens. Comp Med 53(5):487-492.

8. Myers DD, Smith E, Schweitzer I, Stockwell JD, Paigen BJ, Bates R, Palmer J, Smith AL. 2003. Assessing the risk of transmission of three infectious agents among mice housed in a negative caging system. Contemp Top Lab Anim Sci 42(6):16-21.

9. Smith AL, Mabus SL, Stockwell JD, Muir C. 2004. Effects of housing density and cage floor space on C57BL/6J mice. Comp. Med 54:66-663. (reprint not available)

10. Smith AL, Mabus SL, Muir C, Woo Y. 2005. Comp. Med. 55:368-376. (reprint not available)

11. Paigen B, Svenson KC, Peters L, Smith AL. in preparation.

12. Smith AL, Mabus SL. Effect of culling on survival and growth of pups. In preparation.

NAME: Beverly Paigen/The Jackson Laboratory

1. ARTICLE/CONTENT: The Effect of Relative Humidity on Mouse Allergen Levels

SOURCE: Am. Ind. Hygiene Assoc., 1995

2. ARTICLE/CONTENT: Air Quality in an Animal Facility

SOURCE: Am. Ind. Hygiene Assoc., 1995

3. ARTICLE/CONTENT: Impact of Room Ventilation

SOURCE: Contemporary Topics, Jan. 1997

4. ARTICLE/CONTENT: Microenvironment in Ventilated Animal Cages

SOURCE: Contemporary Topics, March 1998

5. ARTICLE/CONTENT: Control Strategies for aeroallergens in an animal facility

SOURCE: J Allergy Clin Immunol 139-146

6. ARTICLE/CONTENT: Reducing exposure to Laboratory Animal Allergens

SOURCE: Comp. Med 487-492

7. ARTICLE/CONTENT: Assessing the Risk of Transmission of Three Infectious Agents Among Mice

SOURCE: Contemporary Topics November 2003

Implications for Animal Health of Changing Water Bottles on Mouse Cages Once Every Two Weeks

Many facilities prefer to use water bottles rather than automatic watering for mice because of the potential for problems with automatic watering in this species. At The Jackson Laboratory (TJL), all mice are watered from bottles filled with filtered, acidified (pH 2.8-3.1) town water. Bottles are typically changed weekly. Changing bottles at 2-wk intervals was investigated as a means of reducing costs and labor. Studies were undertaken to determine: 1) the stability of the water pH over 2 wk; 2) the growth of bacteria in the water over time; 3) the ability of a single 450-ml bottle to sustain a cage of mice over 2 wk; and 4) possible effects on performance of the mice. Test bottles were placed on breeding cages (pairs/trios ± offspring) of mice of various strains. Fifty-five bottles were drilled polycarbonate with plastic caps, 70 were drilled polysulfone with metal caps, and 50 were glass with rubber stoppers and metal sipper tubes. pH at the end of 2 wk was within the target range (2.8-3.1) in 105 bottles, but was higher (3.15-3.62) in 21 bottles and lower (2.39-2.76) in 4 bottles. Filtered samples from 115 bottles were aseptically transferred to blood agar to assess bacterial contamination after 1 and 2 wk on the mouse cages. Spore-forming bacteria were cultured from 44% of the 1-wk samples and 64% of the 2-wk samples. There was no growth of non-spore formers in any of the bottles. Contamination with spore formers was more common in bottles with sipper tubes (1 wk = 68%; 2 wk = 92%) than in drilled bottles (1 wk = 26%; 2 wk = 43%). There was no bacterial growth from bottles used on cages with pathogen-free, defined-flora mice given sterilized feed. For most mouse strains tested, a single 450-ml bottle provided more than enough water to maintain a breeding cage for 2 wk (mean volume remaining after 2 wk = 239 ml). However, with some strains that wean large litters (e.g., C57BL/6J), up to 20% of bottles did not last 2 wk. To date (6 mo), there have been no changes in breeding performance among mice maintained on the 2-wk regimen. The results show that, under the conditions at TJL, 450-ml bottles are adequate to sustain most breeding units for 2 wk. Although there was some increase in the number of spore-forming bacteria in bottles kept on cages for 2 wk vs. 1 wk, water quality after 2 wk was adequate to protect animal health. Breeding performance appears to be unaffected by 2-wk vs. 1-wk bottle changing.

Effect of cage lid sanitization frequency on bacterial contamination of the lids and breeding performance of C57BL/6J mice.

The ILAR *Guide* states that cage accessories should be sanitized at least every 2 weeks. A study was undertaken to determine whether a significant decrease in the sanitization frequency of lids on mouse cages would adversely affect breeding performance or bacterial contamination of the lids. Three groups of C57BL/6J breeding trios, 80 females and 40 males per group, were maintained in ventilated caging for 32 wk. Cages in all groups were changed every 2 wk, but cage lids were treated differently according to group. In Groups A and B, wire bar lids were changed every 2 wk at the time of cage changing. Fresh feed was given to Group A at each change, whereas in Group B, feed was transferred from the old lid to the new lid. In Group C, the wire bar lid containing the old feed was transferred to the new cage at each cage change, remaining with the mice for the entire 32 wk. Records were kept of the number of litters born, number of pups per litter, number weaned, and weight at weaning; pups were weaned at 4 wk. Bacterial contamination was determined using RODAC plates; samples were collected from the bottom surface of the lid's food hopper. Plates were incubated for 48 hr and bacterial colonies identified and counted by a registered medical technologist. Baseline samples were taken from the clean lids prior to placing them on the cages. Subsequent samples were taken just before cage change in Groups A and B, and monthly in Group C. Bacterial counts from Group A and B lids were comparable throughout the study and were similar to those from Group C lids at most time points. However, counts from Group C lids were higher (Wald Chi-square) than those from Group A lids at 14 wk ($p<0.02$) and 18 wk ($p<0.01$) and higher than Group B at 26 wk ($p<0.001$). The predominant organisms identified from all lids were coagulase negative Staphylococci and spore formers. There were no pairwise significant differences among the 3 groups in breeding performance. Mean litters per female ranged from 4.75 to 5.04, the mean number of pups weaned per litter ranged from 5.0 to 5.4, and the born to wean ratio ranged from 75.9% to 78.8%. The mean weight of pups at weaning ranged from 12.9 to 13.3gm; weanlings from Groups B and C were heavier (two-sample t-test) than those from Group A ($p<0.004$). These data indicate that sanitization of cage lids as infrequently as every 32 wk – vs. every 2 wk as recommended in the *Guide* has little effect on bacterial contamination of the lids and no effect on breeding performance of C57BL/6J mice. However, less frequent sanitization of cage lids at The Jackson Laboratory has resulted in estimated minimum cost savings of $280,000 per year.

#58

Scientific Affairs (NIH/OD)

From: Göran Hellekant [hellekant@svm.vetmed.wisc.edu]
Sent: Wednesday, April 12, 2006 10:46 PM
To: Scientific Affairs (NIH/OD)
Attachments: The Scientist - How regu#383357

The scientific community working with animals would benefit greatly if some of the suggestions below would be taken into consideration. It is an article from the The Scientist. Unfortunately the deadline for suggestions had escaped me, but I mail this anyway, because these suggestions are important for medical science.

I propose the following:
1. Level the differences in legislation between animals used in research - the AWA - and agriculture.

2. Unify the animal protocol forms across all research institutions.

3. Have the AWA demand expertise of all members of the local Animal Care Units.

4. Make the ACUCs a normal part of the committee system at the institution and elect its members from active researchers.

5. Mandate use of the institutional legal system and state laws to deal with AWA allegations.

The whole article is attached.
Sincerely,
G. Hellekant
professor DVM, PhD

--

Goran Hellekant Office: (608) 262-1056
Dept. of Animal Health and Biomedical Sciences Home: (608) 271-7715
University of Wisconsin - Madison Email: hellekant@ahabs.wisc.edu
1656 Linden Drive, Madison, WI 53706 Office: rm. 113, AHABS bldg

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NAME: G. Hellekant/University of Wisconsin

ARTICLE/CONTENT: Comparison of Carbon Dioxide Argon & Nitrogen inducing unconsciousness

SOURCE: Lab Animal Science/2006

THE HUMANE SOCIETY OF THE UNITED STATES

Comments of The Humane Society of the United States
In Response to Request for Information (RFI): Standards for the Care and Use of Laboratory Animals
RFI No. NOT-OD-06-011
March 30, 2006

The Humane Society of the United States, on behalf of our 9.5 million members and constituents, is writing in response to the Request for Information regarding standards for the care and use of laboratory animals. The HSUS believes that there is a need to update the *Guide for the Care and Use of Laboratory Animals* (which will be referred to as the *Guide*), which is used by thousands of PHS-assured institutions in the United States, as well as numerous AAALAC-accredited institutions worldwide; we, therefore, urge the National Institutes of Health to move forward on this effort. Numerous developments in the field of animal research have occurred since the last revision of the *Guide* in 1996, particularly in regards to issues of great importance to animal welfare, such as pain, distress, animal housing, and environmental enrichment, among others. The stated purpose of the *Guide* is "to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate;" the 1996 edition is no longer meeting this purpose to the extent that it can and should.

The HSUS has chosen to provide references published since 1996 that correspond to the categories that are found in Appendix A of the 1996 edition of the *Guide*. In addition to Appendix A, many of these references can be incorporated into the text of the chapters. We must express our concern, however, that Appendix A does not include a category for distress—this issue is of enormous importance (both legally and ethically) and we urge inclusion of this category in the next and subsequent revisions of the *Guide* (as an individual category and not combined with another category, such as "anesthesia, pain and surgery").

Appendix I of these submitted comments lists our recommended references; hard copies of many of these references are enclosed (one copy only due to the number of articles provided). Appendix I indicates not only which references are enclosed as hard copies (each are assigned a number and can be found in the enclosed binder), but those that can be accessed electronically as well (web links are provided).

Aside from published information, it would certainly be useful for the *Guide* to include websites of information. While some websites may become outdated, there are many websites that continually update information in regards to animal research; this would be valuable to your audience because any hard copy revision of the *Guide* will quickly become outdated due to the nature of the field. As a result, we have included a list of websites that we believe are valuable and relevant to the issues addressed in the *Guide*. We have provided websites that correspond to some categories found in Appendix A--see Appendix II of these submitted comments.

Finally, we strongly urge NIH to include animal welfare scientists/ethologists on the committee that will be tasked with revising the *Guid*e. Animal welfare science is a burgeoning field in regards to animals used in research and the contributions of these experts would be valuable. We, of course, also urge inclusion of animal protection representatives on the committee as well.

The HSUS appreciates the opportunity to provide comments and information in regards to the care and use of laboratory animals. We do hope that NIH will move forward with revising the *Guide* in order to reflect current and valuable information that could improve the welfare of tens of millions of animals used in research in the United States and worldwide.

Kathleen M. Conlee
Director of Program Management
Animal Research Issues
On behalf of The Humane Society of the United States

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
Administration and Management	Canadian Council on Animal Care. February 1997. CCAC Guidelines on Animal Use Protocol Review.Ottawa, Ontario: Canadian Council on Animal Care. (Online at: http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/PROTOCOL/PROTGDE.HTM)	1	√
	Hampshire, V., and DeRenzo, E. 2002. Moving Research from the cage to the bedside: The need for IACUC/IRB cooperation. Lab Animal. 31(4): 27-31.	2	
	Silverman, J. , Suckow, M. A. , and Murthy, S., eds. 2000. The IACUC Handbook. CRC Press, New York.		
	Wolfensohn, S., and Lloyd, M. 1998. Handbook of Laboratory Animal Management and Welfare, Second Edition. Black Science Ltd., Oxford, UK.		
Alternatives	Carlsson, H. E., J. Hagelin, and J. Hau. 2004. Implementation of the 'Three Rs' in Biomedical Research. Vet Rec. 154(14): 467-70.	3	
	Goldberg, A. M. , and Locke, P. A. July/August 2004. To 3R is humane. The Environmental Forum: 18-26.	4	
	Impact of Noninvasive Technology on Animal Research. ILAR Journal. 2001. 42(3): entire issue; http://dels.nas.edu/ilar_n/ilarjournal/42_3/		√
	Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods. March 2005. Expert panel report: Evaluation of the current validation status of in vitro methods for identifying ocular corrosives and severe irritants. Research Triangle Park, North Carolina. http://iccvam.niehs.nih.gov/methods/ocudocs/EPreport/ocuEPrpt.pdf		√
	Kramer, K. et al. January 2001. The Use of Radiotelemetry in small animals: recent advances. Contemporary Topics in Laboratory Animal Science. 40(1): 8-16.	5	
	Kreger, M. April 2000. The Search for Refinement Alternatives: When You've Just Got to Use Animals. Lab Animal. 29: 22-29.	6	

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
	Lab Animal. July/August 2002. 31(7): entire issue. (dedicated to the three R's)	7	
	McArdle, J. 1997-1998. Alternatives to Ascites Production of Monoclonal Antibodies. AWIC Newsletter. 8: 1-18.	8	
	Reinhardt, V. 2003. Compassion for animals in the laboratory: Impairment or refinement of research methodology? Journal of Applied Animal Welfare Science. 6(2): 123-130.	9	
	Stephens, M. L., et al. 2002. Possibilities for Refinement and Reduction: Future Improvements Within Regulatory Testing. ILAR Journal. 43(Supplement) : S74-S79.	10	
	The Working Group on Refinement. January 2001. Refining Procedures for the Administration of Substances. Laboratory Animals. 35(1): 1-41.	11	
	Turner, et al. 2003. Refinements in the Care and Use of Animals in Toxicology Studies-- Regulation, Validation, and Progress. Contemporary Topics in Laboratory Animal Science. 42(6): 8-15.	12	
Alternatives And Sample Size and Experimental Design	Humane endpoints for animals used in biomedical research and testing. ILAR Journal. 2000. 41(2): entire issue; http://dels.nas.edu/ilar_n/ilarjournal/41_2/		√
	Morton, D. B., Scharmann, W. and H. R. P. et al. Jones. Humane Endpoints in Animal Experiments for Biomedical Research: C. F. M Hendriksen and D. B. Morton. UK: Royal Society of Medicine Press, Ltd., 1999.		
	Morton, David. 1998. The Importance of Non-Statistical Design in Refining Animal Experiments. ANZCCART News. 11(2): 1-12 (Insert).	13	
Amphibians, Reptiles, and Fishes	Canadian Council on Animal Care. 2005. Guidelines on the Care and Use of Fish in Research, Teaching and Testing; http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/PDFs/Fish%20Guidelines%20English.pdf		√
	O'Rourke, D.P. 2002. Reptiles and amphibians as laboratory animals. Lab Animal. 31(6): 43-47.	14	

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
	Reed, B.T. May 2005. Guidance on the Housing and Care of the African Clawed Frog, Xenopus laevis. West Sussex, England: Royal Society for the Prevention of Cruelty to Animals. www.rspca.org.uk/xenopus		√
Anesthesia, Pain, and Sugery And Distress*	Carstens, E. and Moberg, G. 2000. Recognizing pain and distress in laboratory animals. Institute for Laboratory Animal Research. 41(2): 62-71.	15	
	Stephens, M. , Mendoza, P. , Weaver, A. , and Hamilton, T. 1998.Unrelieved pain and distress in animals: An analysis of USDA data on experimental procedures. JAAWS. 1(1): 15-26.	16	
Anesthesia, Pain, and Surgery	(2003) A collection of articles on pain management that originally appeared in *Veterinary Technician*	17	
	American Veterinary Medical Association. 2002. Animal welfare forum: Pain management. JAVMA. 221(2): 201-237.	18	
	Heavner, J.E. 2001. Anesthesia update: Agents, definitions, and strategies. Comparative Medicine. 51(6): 500-503.	19	
	Hellebrekers, L. J. , ed. 2000. Animal Pain: A Practice Oriented Approach to an Effective Pain Control in Animals. Van Der Wees, Utrecht, The Netherlands.		
	Paul-Murphy et al. 2004. The need for a cross-species approach to the study of pain in animals. Journal of the American Veterinary Medical Association. 224(5): 692-697.	20	
	Roughan, J. V. and Flecknell, P. A. 2003. Pain Assessment and Control in Laboratory Animals. Laboratory Animals. 37(2): 172.	21	
	Rutherford. 2001. Asessing Pain in Animals. Animal Welfare. 11: 31-53.	22	
	Schofield, J., and Williams, V. 2004. Recent advances in anesthesia in guinea pigs. ANZCCART News. 17(3): 7-8.	23	

* Distress is a proposed category.

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
	Soulsby, L. and Morton, D. (Eds.) 2001. Pain: its nature and management in man and animals. London, UK: Royal Society of Medicine Press Ltd.	24	
	Stasiak et al. 2003. Species-Specific Assessment of Pain in Laboratory Animals. Contemporary Topics in Laboratory Animal Science. 42(4): 13-20.	25	
Anesthesia, Pain, and Surgery And Alternatives	Not Included	26	
Anesthesia, Pain, and Surgery And Amphibians, Reptiles, and Fishes	Jackson. 2003. Laboratory Fish: Impacts of Pain and Stress on Well-Being. Contemporary Topics in Laboratory Animal Science. 42(3): 62-70.	27	
Anesthesia, Pain, and Surgery And Cats and Dogs	Taylor, P. M. and Robertson, S. A. 2004. Pain Management in Cats--past, present and future. Part 2. Treatment of Pain--clinical pharmacology. Journal of Feline Medicine and Surgery. 6(5): 313-320 and 321-333.	28	
Anesthesia, Pain, and Surgery And Farm Animals	Anderson, D. E. and Muir, W. W. 2005. Pain management in ruminants. The Veterinary clinics of North America: Food animal practice. 21: 19-31.	29	
Anesthesia, Pain, and Surgery And Rodents and Rabbits	Karas, A. 2002. Postoperative Analgesia in the Laboratory Mouse. Lab Animal. 31(7): 49-52.	30	
	Leach, M. C., et al. 2002. Aversion to Gaseous Euthanasia Agents in Rats and Mice. Comp Med. 52(3): 249-57.	31	
	Sharp et al. 2003. Recovery of Male Rats from Major Abdominal Surgery After Treatment with Various Analgesics. Contemporary Topics in Laboratory Animal Science. 42(6): 22-27.	32	

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
Anesthesia, Pain, and Surgery And Welfare	Hawkins, P. 2002.Recognising and Assessing Pain, Suffering and Distress in Laboratory Animals. A Survey of Current Practice in the UK With Recommendations.	33	
	Pain and Distress Recommended Resources List (HSUS).http://www.hsus.org/animals_in_research/pain_distress/pain_and_distress_recommended_resources/	34	√
Design and Construction of Animal Facilities	Reinhardt, A. and Reinhardt, V. 2006. Variables, Refinement and Environmental Enrichment for Rodents and Rabbits kept in Research Institutions. Washington, DC: Animal Welfare Institute. awionline.org/pubs/rabrodent/rodrab.html		√
	Baumans, V. , Schlingmann, F. , Vonck, M. , and Van Lith, H. A. 2002. Individually ventilated cages: Beneficial for mice and men? Contemporary Topics in Laboratory Animal Science. 41(1), 13-19.	35	
	Hockly et al. 2002. Environmental Enrichment Slows Disease Progression in R6/2 Huntington's Disease Mice. Annals of Neurology. 51: 235-242.	36	
	Holley, D. C. , Said, B. , Howard, A. , and Ward- Dolkas, P. 2003. Monitoring lab animal feeding by using subcutaneous microchip transponders: Validation of use with group-housed rats. Contemporary Topics in Laboratory Animal Science. 42(3): 26-28.	37	
	Lutz, C. , Well, A., and Novak, M. 2003. Stereotypic and Self-Injurious Behavior in Rhesus Macaques: A Survey and Retrospective Analysis of Environment and Early Experience. American Journal of Primatology. 60: 1-15.	38	

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
Enrichment	ILAR Journal. March 2005. Enrichment Strategies for Laboratory Animals. 46(2): http://dels.nas.edu/ilar_n/ilarjournal/46_2/html/		√
	Olsson, A. S. and Dahlborn, K. 2002. Improving housing conditions for laboratory mice: a review of 'environmental enrichment'. Laboratory Animals. 36(3): 243-270.	39	
Ethics And Administration and Management	Smith, J. A. , and Jennings, M. 2003. A resource book for lay members of local ethical review process. West Sussex, England: Royal Society for the Prevention of Cruelty to Animals.		
Ethics And Laboratory Animal Care	Sherwin, C. M. , et al. Guidelines for the Ethical Use of Animals in Applied Ethology Studies. Applied Animal Behaviour Science. 81(3): 291-305.	40	
Ethics And Welfare	King, L. A. 2003. Behavioral Evaluation of the Psychological Welfare and Environmental Requirements of Agricultural Research Animals: Theory, Measurement, Ethics, and Practical Implications. ILAR J. 44(3): 211-21.	41	
Euthanasia	Close, B. , Banister, K. , Baumans, V. , Bernoth, E. M. , Bromage, N. , Bunyan, J. , Erhardt, W. , Flecknell, P. , Gregory, N. , Hackbarth, H. , Morton, D. , and Warwick, C. 1996. Recommendations for euthanasia of experimental animals: Part 1. DGXI of the European Commission. Lab Animal. 30(4): 293-316.	42	
	Close, B. , Banister, K. , Baumans, V. , Bernoth, E. M. , Bromage, N. , Bunyan, J. , Erhardt, W. , Flecknell, P. , Gregory, N. , Hackbarth, H. , Morton, D. , and Warwick, C. 1997. Recommendations for euthanasia of experimental animals: Part 2. DGXI of the European Commission. Lab Animal. 31(1):1-32.	43	
	Conlee, K. M. , Stephens, M. L. , Rowan, A. N. and King. L. A. 2005. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. Laboratory Animals. 39: 137-161.	44	

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
Exotic, Wild, and Zoo Animals	The Canadian Council on Animal Care has produced new guidelines regarding the care and use of wildlife for research purposes, including general considerations, restraint, surgical procedures, marking, and euthanasia. For more information, please visit http://www.ccac.ca/english/gdlines/wildlife/Wildlife.pdf. These guidelines are accompanied by new species-specific guidelines on bats; please visit http://www.ccac.ca/english/gui_pol/GUFRAME.HTM .		
General References And Distress	Grandin, T. and Deesing, M. 2002. Distress in animals: is it fear, pain or physical stress? American Board of Veterinary Practitioners. 2002.	45	
Laboratory Animal Care	Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. 2003. Guidelines for the care and use of mammals in neuroscience and behavioral research. Washington, DC: National Academies Press. http://darwin.nap.edu/books/0309089034/html		√
	Mench, J. 1998. Why it is important to understand animal behavior. ILAR Journal. 39(1): 20-26.Online at: http://dels.nas.edu/ilar_n/ilarjournal/39_1/39_1Why.shtml	46	√
	Poole, T., ed. 1999. The UFAW Handbook on the Care and Management of Laboratory Animals, Seventh Edition. Blackwell Science, Oxford, UK.		
	Reinhart, V. 2004. Common Husbandry-Related Variables in Biomedical Research With Animals. Laboratory Animals. 38(3): 213-235.	47	
Laboratory Animal Care And Distress	The Humane Society of the United States. 2005. Pain and distress associated with polyclonal antibody production: discussion and recommendations. The Humane Society of the United States, Washington, DC. http://www.hsus.org/web-files/PDF/ARI/pain_and_distress_associated_with_polyclonal_antibody_production.pdf	48	√
	Moberg, G. 1999. When does stress become distress? Lab Animal. 28(4): 22-26.	49	

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
Rodents and Rabbits And Laboratory Animal Care	Jennings, M., et al. 1998. Refining Rodent Husbandry: the Mouse. Report of the Rodent Refinement Working Party. Lab Animal. 32(3): 233-59.	50	
Rodents and Rabbits And Welfare	Harkin et al. 2002. Physiological and Behavioral Responses to Stress: What does a Rat Find Stressful? Lab Animal. 31(4): 42-50.	51	
	Jegstrup et al. 2003. Characterization of Transgenic Mice: A Comparison of protocols for welfare evaluation and phenotype characterization of mice with a suggestion on a future certificate of instruction. Laboratory Animals. 37(1): 1-9.	52	
	Penny Hawkins, David Anderson, Ken Applebee, David Key, Jim Wallace, Gianpaolo Milite, Judy Macarthur, Clark Robert Hubrecht, Maggy Jennings. 2003. Individually Ventilated Cages and Rodent Welfare: Report of the 2002 RSPCA/UFAW Rodent Welfare Group Meeting. Animal Technology and Welfare: 23-34.	53	
	Wurbel, H. 2001. Ideal Homes? Housing Effects on Rodent Brain and Behaviour. Trends Neurosci. 24(4): 207-11.	54	
Sample Size and Experimental Design	Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. 2000. Organization for Economic Cooperation and Development (OECD).	55	
Sample Size and Experimental Design And Alternatives	Festing, M. F. W. , Overend, P. , Das, R. S. , Borja, M. C. , Berdoy, M. 2002. The design of animal experiments: Reducing the use of animals in research through better experimental design. London: Royal Society of Medicine Press.		
Serial Publications	Pain & Distress Report, The Humane Society of the United States, Washington, D.C. http://www.hsus.org/animals_in_research/pain_distress/	56	√
Technical and Professional Education	Festing, M. F. W. , Overend, P. , Das, R. S. , Borja, M. C. , Berdoy, M. 2002. The design of animal experiments: Reducing the use of animals in research through better experimental design. London: Royal Society of Medicine Press.		

Appendix I. Recommended References

Appendix A Categories	Reference	Hard Copy Provided	Electronic Access
Welfare	Balcombe et al. 2004. Evidence that Laboratory Routines Cause Animal Stress. Contemporary Topics in Laboratory Animal Science. 43(6): 42-51.	57	
	Poole, T. 1997. Happy Animals Make Good Science. Lab Anim. 31(2): 116-24.	58	
	Reinhardt, V. (Ed.). 2002. Comfortable quarters for laboratory animals. Washington, D.C.: Animal Welfare Institute. http://www.awionline.org/pubs/cq02/cqindex.html		√
	The Institute for Laboratory Animal Research. Guidelines for the Humane Transport of Research Animals. http://darwin.nap.edu/books/0309101107/html/		√
Welfare And Amphibians, Reptiles, and Fishes	Sherwin, C. M. 2001. Can Invertebrates Suffer? Animal Welfare. 10: S103-118.	59	
Welfare And Anesthesia, Pain, and Surgery And Rodents and Rabbits	The Rodent Welfare Group Report, issued by the Royal Society for the Prevention of Cruelty to Animals(RSPCA) and the Universities Federation of Animal Welfare (UFAW). 2002. Animal Technology and Welfare. 1(1): 3-12.		
Welfare And Distress	Moberg, G. P. , and Mench, J. A ., eds. 2000. The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. CABI Publishing, New York, New York.		
	Pekow, C. 2005. Defining, Measuring and Interpreting Stress in Laboratory Animals. Contemporary Topics in Laboratory Animal Science. 44(2): 41-45.	60	

Appendix II. Recommended Websites

Appendix A Categories	Website
Administration and Management	Animal Welfare Information Center (AWIC) Workshop Online: "Meeting the Information Requirements of the Animal Welfare Act": http://www.nal.usda.gov/awic/awicworkshops/awicworkshops.htm
	IACUC.org: www.iacuc.org . This website is produced by AALAS and is an online resources for IACUCs.
	The Laboratory Animal Management Association (LAMA), supported by the Office of Laboratory Animal Welfare, has created a web-based resource for disaster planning and management in laboratory animal research facilities. The website can be viewed at: http://www.lama-online.org/OLAW-1.html.
Alternatives	Sharing and Collaboration Across Borders: Information on Alternatives Databases: http://oslovet.veths.no/databasesintro.html .This website links to 26 databases and organizations that have websites and sorts them by reduction, refinement and replacement.
	Altweb focuses on replacement, reduction and refinement alternatives and assists scientists with alternatives searches, promotes information sharing, and provides news, information and resources regarding alternatives. A project team of regulatory agencies, animal protection organizations, universities and industry organizations provide vision and direction for the site. For more information, go to http://altweb.jhsph.edu/
	The website of the Netherlands Centre for Alternatives and Animal Use contains 15 databases on alternatives, alternatives to testing organizations, animal care and animal welfare and a wealth of useful information. Visit http://prex.las.vet.uu.nl/nca/
	The National Library of Medicine has created a special database for alternatives to animal testing, which contains over 7,500 citations from TOXLINE and MEDLINE regarding methods, tests and procedures that refine, reduce and replace animal testing. To search this database, go to http://toxnet.nlm.nih.gov/altbib.html.
	The 8th edition of the Merck Veterinary Resource Manual is now available online at http://www.merckvetmanual.com/mvm/index.jsp. The manual, a service of Merck & Co., Inc. and Merial Limited, includes over 12,000 indexed topics and over 1200 illustrations. By using the advanced search option, information can be searched by topic, species, disease, organ system and keyword.
	The HSUS produced a manuscript regarding refinements in toxicological testing from a workshop of international experts, please visit http://www.hsus.org/animals_in_research/animal_testing/workshop_on_refinements_in_toxicology_testing/index.html .

Appendix II. Recommended Websites

Appendix A Categories	Website
	A new website allows scientists to share data and computer models of cells, organs, and whole organisms that can be stored, improved, updated, or used by other scientists in their experiments, such as, for example, testing the effect of therapeutic drugs or toxins on cells. The Ark website, hosted by the University of Bath (Bath, England), can be found online at: www.bath.ac.uk/mech-eng/ark/.
	The University of California, Davis' Center for Animal Alternatives website lists a number of sources for finding reduction, refinement and replacement alternatives regarding the use of animals in research, testing, and education. The website can be found at: www.vetmed.ucdavis.edu/Animal_Alternatives/weblinks.htm .
	NORINA (Norwegian Inventory of Audiovisuals) has updated its website--http://oslovet.veths.no --which contains a database of over 3500 alternatives with links to suppliers, a database of laboratory animal science textbooks with links to bookstores, as well as information on legislation, education, and ethics.
	The Report Alternative (Non-Animal) Methods for Cosmetics Testing: Current Status and Future Prospects, A Report Prepared in the Context of the 7th Amendment to the Cosmetics Directive for Establishing the Timetable for Phasing Out Animal Testing (Edited by Chantra Eskes and Valerie Zuang (2005), ATLA 33,Supplement 1), can be downloaded at the European Centre for the Validation of Alternative Methods (ECVAM) website at http://ecvam.jrc.it/index.htm .
Anesthesia, Pain, and Surgery	AWIC's bibliography pertaining specifically to analgesia and analgesics in animals: http://www.nal.usda.gov/awic/pubs/awic200002.htm
Anesthesia, Pain, and Surgery And Welfare	www.uchsc.edu/animal: To view the slides from a presentation by Dr. Ron Banks on "Pain/Distress Assessment and Obviation" at a recent ARENA conference, click on "Animal Use Planning" and then "ARENA meeting slides and references."
	HSUS' Pain and Distress Report: http://www.hsus.org/animals_in_research/pain_distress/
	References for animal pain, stress and capture myopathy can be found at: http://www.npwrc.usgs.gov/resource/tools/telemtry/refanim.htm. This website is from the US Geological Survey's Northern Prairie Wildlife Research Center.

Appendix II. Recommended Websites

Appendix A Categories	Website
Anesthesia, Pain, and Surgery And Welfare And Laboratory Animal Care	The Animal Welfare Information Center (AWIC) has compiled an online bibliography on the recognition and alleviation of pain and distress in research animals, which can be found at http://www.nal.usda.gov/awic/pubs/awic200003.htm. The bibliography is divided according to species (e.g., mice, rats, and dogs) and also has a section devoted to general pain and distress references. According to AWIC, this publication provides "a starting point for those concerned about the welfare and humane care of animals used in research."
Cats and Dogs	The Department of Veterans Affairs has created a video entitled "Working with the Laboratory Dog" for those who work with laboratory dogs in a research setting. To view this video, visit http://grants.nih.gov/grants/olaw/TrainingVideos.htm#dog
Enrichment	www.enrichmentonline.org is a database of environmental enrichment created by the Fort Worth Zoo.
Enrichment And Alternatives	Part II of the Annotated Database on "Refinement of housing and handling conditions and environmental enrichment for laboratory animals" www.awionline.org/lab_animals/biblio/lbfarm.htm
Enrichment And Nonhuman Primates	The Animal Welfare Institute's Annotated bibliography on Refinement and Environmental Enrichment for Primates Kept in Laboratories. http://www.awionline.org/lab_animals/biblio/index.html
Enrichment And Welfare And Laboratory Animal Care	AWI has created a new database provides information on refinement of housing and handling conditions, including environmental enrichment, for all species used in research, testing, and teaching institutions. The online database, a service of the Animal Welfare Institute, has over 2500 entries of which over 500 are full text documents . To access the Refinement and Environmental Enrichment for all Laboratory Animals database, please go to http://www.awionline.org/lab_animals/biblio/laball.htm
Laboratory Animal Care	Using Animals in Science online, a website that provides information about the use of animals in research, teaching and testing and is intended for a wide range of readers. http://anzccart.rsnz.govt.nz
	The Animal Welfare Institute (AWI) announced a new website for reporting specific concerns related to the well-being of animals used for research, testing, and education. www.labanimalissues.org is a secure and confidential site that individuals can use to report their concerns anonymously. AWI will follow-up by taking actions that may include, but are not limited to, the following: inspecting the animal(s) involved, filing a complaint(s) with the appropriate oversight agency, and informing the media and/or Congress.

Appendix II. Recommended Websites

Appendix A Categories	Website
Laws, Regulations, Policies	The Animal Legal & Historical Web Center at www.animallaw.info, a project of the Michigan State University-Detroit College of Law, contains information on US federal and state statutes, foreign national law and international materials that govern the care of captive animals, collection of specimens, and protection of endangered species.
	Australia's Animal Research Review Panel and Animal Welfare Unit have created a website of international legislation and policies, information regarding care and use of animals for research, education and training information, please visit http://www.animalethics.org.au/
Welfare And Laboratory Animal Care	An independent website for University of Edinburgh students provides information on animal welfare and husbandry and can be found at http://www.vet.ed.ac.uk/animalwelfare/index.htm. A range of topics are addressed, including animal pain, legislation, animal behavior, husbandry and health of laboratory animals.