THE HUMANE SOCIETY OF THE UNITED STATES

OFFICERS
David O. Wiebers, M.D.
Chair of the Board
Anita W. Coupe, Esq.
Vice Chair of the Board
Eugene W. Lorenz
Board Treasurer
Wayne Pacelle
President & CEO
G. Thomas Waite III
Treasurer & CFO
Roger A. Kindler, Esq.
General Counsel & CLO
Janet D. Frake
Secretary

STAFF VICE PRESIDENTS
Andrew N. Rowan, Ph.D.
Executive Vice President Operations
Michael Markarian
Executive Vice President External Affairs
Patricia A. Forkan
Senior Vice President External Affairs International
John W. Grandy, Ph.D.
Senior Vice President Wildlife & Habitat Protection
Heidi Prescott
Senior Vice President Campaigns
Katherine B. Liscomb
Administration & Animal Care Centers
Nicholas Braden
Communications
Richard M. Clugston, Ph.D.
Higher Education
Jonathan R. Lovvorn, Esq.
Animal Protection Litigation
Miyun Park
Farm Animal Welfare
Nancy Perry, Esq.
Government Affairs
Steve Putnam
Business Development & Corporate Relations
Robert G. Roop, Ph.D., SPHR
Human Resources & Education Programs
Melissa Seide Rubin, Esq.
Field & Disaster Services
John M. Snyder
Companion Animals
Martin L. Stephens, Ph.D.
Animal Research Issues
Richard W. Swain Jr.
Investigative Services
Gretchen Wyler
Hollywood Office

DIRECTORS
Leslie Lee Alexander, Esq.
Patricia Mares Asip
Peter A. Bender
Barbara S. Brack
Donald W. Cashen, Ph.D.
Anita W. Coupe, Esq.
Neil B. Fang, Esq., CPA
Judi Friedman
David John Jhirad, Ph.D.
Jennifer Leaning, M.D.
Eugene W. Lorenz
William F. Mancuso
Mary Max
Patrick L. McDonnell
Gil Michaels
Judy Ney
Judy J. Peil
Marian G. Probst
Joshua S. Reichert, Ph.D.
Jeffery O. Rose
James D. Ross, Esq.
Marilyn G. Seyler
Walter J. Stewart, Esq.
John E. Taft
David O. Wiebers, M.D.

March 10, 2006

Dr. William Stokes
Director, NICEATM
National Institute of Environmental Health Sciences
P.O. Box 12233, MD EC-17
Research Triangle Park, NC 27709

Via email to: niceatm@niehs.nih.gov

Dear Dr. Stokes,

On behalf of The Humane Society of the United States (HSUS) and our 9.6 million members and constituents, I am writing in response to NICEATM's *Federal Register* notice of January 27 [FR Doc. E6-1019], which announced a proposed expert workshop on potential alternatives to the LD_{50} assay for assessing the potency of botulinum toxin-based products, to be conducted as a high priority. We appreciate ICCVAM/NICEATM's responsiveness to The HSUS' Test Method Nomination, "Nomination of Alternative Methods to Replace the Mouse LD_{50} Assay for Botulinum Toxin Potency Testing." The HSUS fully supports the decision to hold the expert workshop as a high priority.

The HSUS views the proposed workshop as an important first step in replacing animal use in the manufacture of Botox® Cosmetic and similar products. There are a number of potential alternatives to the LD_{50} assay for this purpose, and these are in various stages of development by different laboratories around the world. What is needed now is a comprehensive assessment of the existing non-animal methods and their associated data, to determine which show the most promise and what needs to be done to complete the development and validation of the most promising test methods. Also needed is an assessment of what interim steps should be taken to reduce and refine animal use for this purpose, while promising non-animal methods are being brought to fruition. The planned workshop is a logical and efficient approach to carrying out these assessments. Of course, ICCVAM/NICEATM's assessment should be coordinated with, and informed by, related efforts in the United Kingdom and the European Union.

The HSUS was pleased with the positive comments made by Food and Drug Administration (FDA) staff at the December 12, 2005 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), in response to ICCVAM/NICEATM's preliminary announcement of its planned expert workshop.

The FDA has an important role to play as ICCVAM/NICEATM moves forward with the workshop and subsequent activity, given that the FDA has regulatory authority over botulinum toxin-based products used for cosmetic and therapeutic purposes. The FDA's active involvement in this process would be consistent with the agency's stated commitment to alternative methods in general, and to alternatives to the LD_{50} assay in particular.

The HSUS notes that other federal agencies also have an interest in testing methods for botulinum toxin, including the Department of Defense, the Centers for Disease Control and Prevention, the Department of Agriculture, the Department of Homeland Security, and the Environmental Protection Agency. The HSUS also encourages these entities to play an active role in ICCVAM/NICEATM's planned workshop and subsequent activities.

Apart from government agencies, The HSUS believes that manufactures of botulinum toxin-based products, including Botox manufacturer Allergan, Inc., can and should make an enormous contribution to the expert workshop and follow-up activities. Allergan should have considerable data on the unspecified alternatives that the company claims to be working on, as well as years of data on LD_{50} testing of Botox products. ICCVAM/NICEATM should encourage Allergan and other manufacturers to submit their data promptly, to allow for prompt scheduling and preparation of the workshop.

The HSUS would also like to caution against an over-reliance on LD_{50} data for assessing and validating the proposed alternative methods. Mouse LD_{50} data will undoubtedly correlate poorly with biological events in humans, especially in this case where the animal test is not even assessing the clinically-relevant endpoint of muscle paralysis. With respect to the botulinum toxin, unlike in so many areas previously investigated by ICCVAM/NICEATM, the human mechanism is well characterized and serves as the basis for some of the non-animal methods, such as the SNAP-25 assay. The panel should be able to determine approaches for the validation of the proposed alternative methods that will not require additional animal studies be carried out, or that—in the short-term—can be coordinated with routine testing currently required for regulatory approvals.

We encourage NICEATM to look for experts for the workshop that will bring the essential expertise in neurotoxin mechanisms and neural cell culture to the panel. Based on our review of the technical literature, The HSUS would like to nominate the following people as experts for the workshop: Dorothea Sesardic, Elaine Neale, Edwin Chapman, and Lance Simpson. Attached, you will find further details on their expertise and contact information. We have not contacted them to ask whether they would be willing to participate in the workshop, but we recommend that they be invited to do so. We would also like to nominate Dr. Sherry Ward, an in vitro toxicologist. Dr. Ward has a comprehensive perspective on potential 3R alternatives for assessing the potency of botulinum toxin-based products, having taken the lead in drafting The HSUS' Test Method Nomination mentioned above. As a consultant to The HSUS, Dr. Ward's participation in the expert workshop would give representation to the animal protection community. I would be happy to forward her CV and contact information.

The HSUS believes that ICCVAM/NICEATM can play a crucial role in eliminating the animal suffering associated with the testing of Botox-like products, and in doing so contribute further to the worldwide effort to move beyond the heavily criticized LD_{50} assay. The consensus of the December 2005 SACATM meeting was that the use of the LD_{50} assay for assessing the potency of Botox-like products is an excellent example of the crude, outdated, and inhumane types of animal tests that ICCVAM/NICEATM should be targeting. We urge ICCVAM/NICEATM to fast track this endeavor.

Sincerely,

Martin L. Stephens, Ph.D.
Vice President, Animal Research Issues

Promoting the protection of all animals
2100 L Street, NW, Washington, DC 20037 ▪ 202-452-1100 ▪ Fax: 202-778-6132 ▪ www.hsus.org

HSUS recommendations for alternatives to mouse LD_{50} expert panel:

Name: Dorothea Sesardic
Address: Division of Bacteriology, National Institute for Biological Standards and Control, Hertfordshire, UK [2006]
Expertise: SNAP-25 assay; mouse LD50 assay

Name: Elaine A. Neale
Address: Chief of the Section on Cell Biology, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland
Expertise: neural cell-based assays

Name: Edwin R. Chapman
Address: Departments of Physiology and Food Microbiology and Toxicology, and the Neuroscience Training Program, University of Wisconsin, Madison, WI 53706
Expertise: mechanisms, sensors, neural cell-based assays

Name: **Lance L. Simpson**
Address: Department of Medicine, Jefferson Medical College, Philadelphia, PA
Expertise: Cellular mechanisms research and isolated phrenic nerve-hemidiaphragm preparations used in some expts.