NICEATM — *National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods*

ICCVAM — *Interagency Coordinating Committee on the Validation of Alternative Methods*

Alternatives in Laboratory Animal Medicine

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C.L.Davis Workshop in Laboratory Animal Medicine
North Carolina State University
May 7-8, 2009

Outline

- Introduction to Alternatives
- Alternatives: Laws, Regulation, and Policies
- Government Alternatives Organizations
- Examples of Accepted Alternative Methods
- Refinement as an Alternative

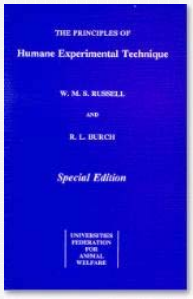
Alternatives in Laboratory Animal Medicine: Introduction

- How are animal use alternatives defined?
- What two scientists published a book describing the concept of the 3Rs?
  - What was the name of the book, and when was it published?

"The Principles of Humane Experimental Technique"

- Published in 1959
  - William M.S. Russell
  - Rex L. Burch
- The 3Rs approach:
  - Reduction
  - Refinement
  - Replacement

If we are to use a criterion for choosing experiments to perform, the criteria of humanity is the best we could possibly invent.



Russell, W.M.S.& Burch,R.L.(original1959;reprinted 1992). The Principles of Humane Experimental Technique, 238pp.Potters Bar, Herts, UK: UFAW

Workshop on the 3RS: The Way Forward

May 30-June 3, 1995
Shearingham, Norfolk, UK

Humane science is good science.

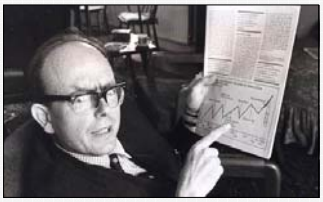
This is best achieved by vigorous application of the Three Rs: reduction, refinement, and replacement alternatives.

Balls M, Goldberg AM, Fentem CL, et al (1995).The Three Rs: The Way Forward (ECVAM Workshop Report 11). *ATLA* 23: 838-866.

Professor William Russell 1925-2006

"The greatest scientific experiments have always been the most humane and the most aesthetically attractive, conveying that sense of beauty and elegance which is the essence at its most successful."

Russell and Burch, 1959



Alternatives; Laws, Regulations, and Policies

- What Federal law and implementing regulation requires consideration of alternatives whenever a procedure will involve more than slight or momentary pain or distress?
- What Federal Policy requires consideration of alternatives before any animal use is approved?

Alternatives: U.S. Legal and Statutory Mandates

- U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals
- USDA Animal Welfare Act Regulations (9CFR)
- Effectively require consideration and use of 3Rs alternative methods, including humane endpoints

Pain and Distress: U.S. Regulations and Policies

- More that momentary or slight pain or distress:
  - Must be limited to that which is unavoidable for the conduct of scientifically valuable research
  - Must be conducted with appropriate sedatives, analgesics, or anesthetics, unless withholding such agents is justified for scientific reasons in writing by the PI
  - Will continue for only the necessary period of time
- *Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure, or if appropriate, during the procedure*

U.S. Regulations and Policies: Consideration of 3Rs Alternatives

- **Principal Investigators (PI)**
  - Must consider alternatives to procedures that may cause more than momentary or slight pain or distress
  - Must provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including refinements, reductions, and replacements
- **Institutional Animal Care and Use Committees (IACUCs)**
  - Must determine that investigators have appropriately considered alternatives
  - Not just non-animal Replacement Alternatives, but also refinement and reduction!

Relevant US Laws: Development and Validation of 3Rs Alternatives

- NIH Revitalization Act of 1993: Public Law 103-43
  - NIH directed to conduct research to reduce, refine, and replace animal use
  - NIEHS directed to develop and validate alternative methods for acute and chronic safety testing
  - NIEHS directed to develop a process to achieve the regulatory acceptance of scientifically valid alternative methods
- ICCVAM Authorization Act of 2000: P. L. 106-545
  - Established Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) as a permanent committee
    - Directed to review scientific validity of new, revised, and alternative test methods
    - Agencies must consider ICCVAM recommendations in 180 days

Legal/Statutory Mandates: Europe

- 7th Amendment to the Cosmetics Directive
  - Animal testing and marketing bans:
    - 2004: ALL finished cosmetic products
    - March, 2009: All cosmetic ingredients
      - exceptions for repeat dose testing
    - 2013: Complete ban for all ingredients
- REACH: Chemical testing data required for >30,000 substances
  - Initiatives underway to maximize non-animal testing procedures
- European Center for the Validation of Alternative Methods (ECVAM) established 1992

Government Alternatives and Related Organizations: Describe the Acronyms

- NICEATM
- ICCVAM
- JaCVAM
- ECVAM
- ICATM
- NC3Rs
- CAAT
- FRAME
- OECD
- ICLAS

Government Alternatives and Related Organizations: Describe the Acronyms

- NTP
- NICEATM
- ICCVAM
- JaCVAM
- ECVAM
- ICATM
- NC3Rs
- CAAT
- FRAME
- OECD
- ICLAS

What is ICCVAM?

- The **I**nteragency **C**oordinating **C**ommittee on the **V**alidation of **A**lternative **M**ethods
- Composed of representatives of the heads of 15 Federal regulatory and research agencies
  - that require, use, generate, or distribute safety testing information
- Duties include:
  - Conducting technical reviews of the scientific validation status of new, revised, and alternative safety testing methods
  - Forwarding formal recommendations to Federal agencies
  - Providing advice on test method development and validation
  - Fostering national and international harmonization
  - Promoting the acceptance of scientifically valid test methods

ICCVAM: 15 Member Agencies

Regulatory Agencies

- Consumer Product Safety Commission
- Department of Agriculture
- Department of the Interior
- Department of Transportation
- Environmental Protection Agency
- Food and Drug Administration
- Occupational Safety and Health Administration

Research Agencies

- Agency for Toxic Substances and Disease Registry
- National Institute for Occupational Safety and Health--CDC
- National Cancer Institute--NIH
- National Institute of Environmental Health Sciences--NIH
- National Library of Medicine--NIH
- National Institutes of Health, Office of the Director
- Department of Defense
- Department of Energy

What is NICEATM?

- The National Toxicology Program (**N**TP) **I**nteragency **C**enter for the **E**valuation of **A**lternative **T**oxicological **M**ethods
  - Established in 1998
  - Administers and provides scientific support for the **Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)**
  - A Center in the NTP, which is headquartered at NIEHS and coordinates toxicology testing programs across the federal government
  - A component of the National Institute for Environmental Health Sciences, one of the 27 Institutes and Centers of NIH
  - Located in Research Triangle Park, NC

ICCVAM's Mission[1]

- To *facilitate* development, validation and regulatory acceptance of new and revised regulatory test methods that:
  - Reduce, refine, and replace the use of animals in testing, and that
  - Maintain and promote scientific quality and the protection of human health, animal health, and the environment

[1]Adopted by ICCVAM February 2004

All of ICCVAM's activities are grounded in the U.S. Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples

What is Scientific Validation?

- A determination of the usefulness and limitations of a test method for a specific purpose.[1]
- The process by which the *reliability* and *relevance* of a test method are established for a specific purpose[1]
  - ***Reliability:*** A measure of the extent to which a test method can be performed reproducibly within and among laboratories over time
    - A mimimum of three labs are used in validation studies
  - ***Relevance:*** The extent to which a test method will correctly predict or measure the biological effect of interest
    - Includes determination of accuracy: sensitivity, specificity, false negative, and false positive rates
    - Involves testing of substances for which there is high quality reference data from animal testing and/or humans (where ethical)

[1]ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods;
NIH Pub. No. 03-4508, 2003, NIEHS, Research Triangle Park, NC.
http://iccvam.niehs.nih.gov/docs/guidelines/subguide.htm

Why is scientific validation important?

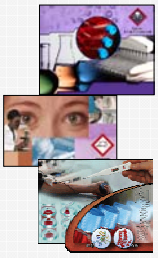
- To determine if a test method protocol will accurately predict whether a substance is hazardous or safe
  - *Must minimize false negative results:*
    - *the test predicts it is safe, when it is actually hazardous*
- To ensure that similar results can be obtained in different laboratories when the same test method protocol is used
- ***Adequate validation of a new test method is required before a test method can be recommended for regulatory testing by U.S. Federal agencies (P.L. 106-545).***
- ***Acceptance requires determination that use of the new test method will provide for equivalent or improved protection of people, animals, or the environment,depending on its purpose***

Drivers for New Alternative Methods

- U.S. laws and policies
  - Animal Welfare Act; PHS Policy on Humane Care and Use of Laboratory Animals
  - NIH Revitalization Act; ICCVAM Authorization Act
  - NICEATM-ICCVAM Five Year Plan
- Advances in science and technology
  - NAS Report: Toxicology in the 21st Century: A Vision and Strategy
    - Long-term vision: few or no animals
- International
  - UN GHS implementation worldwide
  - EU REACH implementation
  - EU Cosmetics Directive
    - 2004 ban on testing cosmetic products
    - 2009 testing ban on ingredients
- Public interest in animal welfare

ICCVAM Progress

- 23 alternative methods accepted/endorsed by U.S. regulatory agencies since 1999
  - 18 non-animal methods
  - 5 animal methods (refinement & reduction)
  - ICCVAM contributed to all 23; 15 based on ICCVAM comprehensive technical evaluations
  - Impact: Alternatives are being used, including alternatives for the four most commonly performed product safety tests
- Recommendations provided for R&D, translation, and validation activities to further advance methods
- International guidances developed
  - Validation and acceptance
  - GLPs and *in vitro* testing
  - Humane endpoints
- Performance standards
  - To expedite validation and regulatory acceptance
- International partnerships: ECVAM, JaCVAM, and Health Canada



International Cooperation on Alternative Test Methods (ICATM)

Memorandum of Cooperation Signed, April 27, 2009

ICATM Validation Organizations

- JaCVAM: Japanese Center for the Validation of Alternative Methods
- ECVAM: European Centre for the Validation of Alternative Methods
- ICCVAM: U.S. Interagency Coordinating Committee on the Validation of Alternative Methods
  and NICEATM: National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, NIEHS, NIH, DHHS
- Health Canada: Environmental Health Science and Research Bureau

International Cooperation on Alternative Test Methods

Goals:

- *To establish international cooperation in three critical areas to ensure that alternative methods/strategies are more readily accepted worldwide:*
  - *validation studies*
  - *independent peer review*
  - *development of harmonized recommendations*
- *To establish international cooperation necessary to ensure that new alternative test methods/strategies adopted for regulatory use will provide:*
  - *equivalent or improved protection for people, animals, and the environment*
  - *while replacing, reducing, or refining animal use whenever scientifically feasible.*

Examples of Accepted Alternative Test Methods

Alternative Methods for Toxicity Testing: Q&As

- What in vivo alternative test is accepted for allergic contact dermatitis testing? What advantages does it offer in terms of the 3Rs?
- Name the three alternative test methods that replaced the traditional LD50 test for acute oral toxicity.
- Which of these three tests does not use moribund condition as the endpoint?
- What in vitro test should always be considered before performing an in vivo acute oral toxicty test, and should be used where determined appropriate?
- What two in vitro tests are approved for ocular irritation testing?
  - When can the results be used for hazard classification?
  - Are animals still required for ocular safety testing?
- Name at least three in vitro tests that can be used to identify skin corrosives?

Alternative Methods for Toxicity Testing: Q&As

- Name two in vitro alternative tests for pyrogenicity testing?
- Name the current limitation of these tests that does not allow complete replacement of the rabbit pyrogenicty test?

Alternative Methods for Allergic Contact Dermatitis: The LLNA

- ICCVAM recommendations: 1999
  - Acceptance, US Agencies - 1999
  - International Adoption-2002
- Advantages
  - Avoids pain and distress
  - Uses fewer animals per test
  - Valid substitute for most traditional guinea pig testing
- A reduction and refinement success
- 2008 ICCVAM Evaluations
  - Reduced LLNA (rLLNA)
    - Further reduces animal use by 40%
  - Performance standards
  - 3 non-radioactive methods

Murine Local Lymph Node Assay

- Virtually eliminates pain and distress for allergic contact dermatitis testing
  - the ideal humane endpoint!
- Incorporates a predictive mechanistic humane endpoint:
  - Lymphocyte proliferation in draining lymph nodes during *induction phase*
- Eliminates progression to *elicitation phase*
  - Avoids overt ACD erythema and edema
- *ICCVAM recommended in 1999 as valid substitute for GPMT*
- Regulatory Acceptance
  - FDA, EPA,CPSC- 1999
  - OECD Test Guideline 429- 2001
- http://iccvam.niehs.nih.gov/llna.htm

Murine Local Lymph Node Assay (LLNA)

	GPMT[1]	LLNA
Time to perform:	32+ days	7 days
Number of animals:	32-43	20
Dermatitis induced:	Yes	No
Adjuvant required:	Yes	No

[1] Guinea Pig Maximization Test

ICCVAM Evaluation of *In Vitro* Methods for Skin Corrosivity Testing

- Five in vitro methods accepted
  - Corrositex, 1999
  - Epiderm, Episkin, Rat skin TER, 2002, SkinEthic, 2004
- Can identify most corrosive substances without the need for animals
- International acceptance as 3 OECD test guidelines
- No animals required when positive results; negative results require assessment of irritation potential (except for DOT) and to detect false negatives
- **Always consider before using animals for skin corrosivity/ irritation testing; use where determined appropriate**

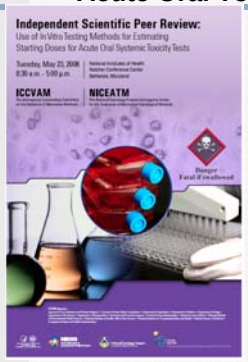
Why are in vitro methods not yet complete replacements for corrosivity testing?

- They have false negative rates of 13-21%
  - Corrosives cause permanent skin damage from chemical burns
  - If a corrosive product is not correctly identified and properly labeled as a corrosive, permanent injuries(scarring, blindness) or death could result
- Not all substances can be tested in all methods

Innovative Strategies: Acute Oral Toxicity

- First, Use cytotoxicity data to estimate starting doses for animal studies
  - can reduce and refine animal use
- Then use one of 3 accepted alternative methods to the rodent LD50 test
  - Up-and-Down Procedure
    - OECD TG 425
    - EPA, CPSC, DOT approved
  - Acute Toxic Class Method
    - OECD Test Guideline 423
  - Fixed Dose Procedure
    - OECD Test Guideline 420
    - Evident toxicity is the endpoint, rather than moribund condition

Innovative Strategies: Acute Oral Toxicity



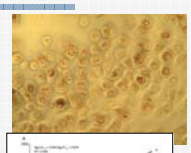
- ICCVAM International Scientific Peer Review: May, 2006
- 2 cytotoxicity methods recommended for estimating in vivo LD50
- Starting closer to the actual LD50 will reduce animals up to 50%;
  - fewer deaths for highly toxic materials
- In vitro protocols and recommendations available at: http://iccvam.niehs.nih.gov

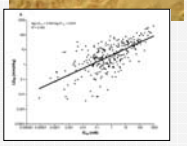
Alternative Methods for Acute Oral Toxicity Testing ("LD50" Testing)

- Revised Up-and-Down Procedure, 2001
  - Reduces animal use by 70%
  - 3-9 vs. 25-45 animals
- *2008: In vitro* methods recommended to further reduce animal use up to 50% per test, 2001 and 2008
  - *Should always be considered before using animals for acute oral toxicity, used where appropriate*
  - *Endorsed by Federal Agencies, 2008*
  - *OECD Guidance Document in preparation*
- February 6-7, 2008 Workshop:
  - *Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations*

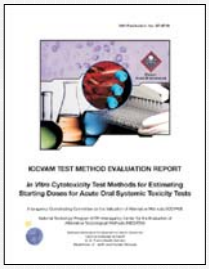
In Vitro Methods for Acute Oral Systemic Toxicity

- ICCVAM evaluated two *in vitro* cytotoxicity test methods to estimate starting dose for LD_{50} tests
- Tests measure neutral red dye uptake of normal human keratinocytes (NHK) or BALB/c 3T3 mouse fibroblasts
- Test methods were evaluated using 72 reference substances
- Study confirmed correlation between in vitro cytotoxicty and in vivo acute systemic toxicity





In Vitro Cytotoxicity Test Methods Endorsed by U.S. Agencies, 2008



- In vitro methods may be used in a weight-of-evidence approach to determine the starting dose for current acute oral toxicity protocols; however, they are not accurate enough to predict acute oral toxicity for regulatory hazard classification
- **Should always be considered, and used where determined appropriate, before acute oral toxicity testing is conducted using animals**
- Use of these methods will reduce the numbers of animals needed for testing, and may also reduce the number of animals that die or need to be humanely killed during testing

The Lash-Lure Tragedy (1933)

The Ad read:
"The New and Improved Eye Brow and Eye Lash Dye
Lash Lure
Radiates Personality"

The Actual Effects:
Allergic reactions
Severe pain
Blindness
Death

Tiered Testing Strategy: Ocular Toxicity Testing

- **Consider all available information and data at each level or tier**
  - Stop testing when sufficient information to determine hazard category
- **No animal testing may be necessary if:**
  - Existing human and/or animal information sufficient for a hazard decision
  - Known skin corrosive (in vitro or in vivo data)
  - pH ≤ 2 or > 11.5., especially if significant buffering capacity
  - Valid and accepted QSARs to predict hazard
  - Valid and accepted in vitro methods: positive results
- **If animals required, perform sequential testing**
  - Test 1st animal; Stop if Category 1: serious damage to e
  - Test 2nd animal: Stop if Cat. 1
  - Test 3rd animal if necessary

)

Alternatives for Ocular Toxicity Testing: Agency Acceptance of ICCVAM Recommendations

- *ICCVAM Test Method Evaluation Report: In Vitro Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives-2007*
  - Two methodsconsidered useful for regulatory hazard classification testing
  - BCOP: Bovine Corneal Opacity and Permeability Assay
  - ICE: Isolated Chicken Eye Test
  - **Positive substances can be classified as ocular corrosives or severe irritants *without the need for animal testing***
- U.S. Regulatory Agency Acceptance-2008
  - These are the first validated *in vitro* alternative test methods accepted for regulatory use for ocular toxicity testing

ICCVAM Recommendations: *In Vitro* Ocular Safety Testing Methods

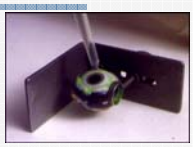
- *In accordance with USDA Animal Welfare Act regulations*
  - *These alternative methods should always be considered before using rabbits for ocular safety testing, and used where determined appropriate*
- Animal Welfare Act responsibilities
  - *Principal Investigators:*
    - *Must provide narrative discussion of alternatives consideration in animal study protocols*
  - *Institutional Animal Care and Use Committees:*
    - *Must review the consideration of alternatives, and approve animal use*

BCOP Assay - Uses and Limitations

- Accepted as a screening test to identify corrosives and severe irritants
  - In a tiered-testing strategy
  - Part of a weight-of-evidence approach
  - Positive results do not require testing in animals
  - Negatives require additional testing, currently in vivo in most cases
- Limitations
  - Not highly predictive for alcohols, ketones and solids
  - For other substances: false negative rate of 12% and false positive rate of 16%

ICE Assay - Uses and Limitations

- Accepted as a screening test to identify corrosives and severe irritants
  - In a tiered-testing strategy
  - Part of a weight-of-evidence approach
  - Positives do not require testing in animals
  - Negatives require additional testing, currently in vivo in most cases
- Limitations
  - Not highly predictive for alcohols, surfactants and solids
  - For all other substances, false negative rate of 30% and false positive rate of <10%

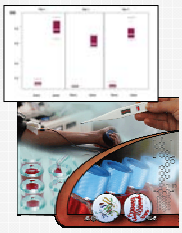


Use of Pretreatment Topical Anesthetics During Ocular Safety Testing

- Evaluated use of 0.5% tetracaine hydrochloride pretrateon irritancy classification in *in vivo* testing
  - No significant impact on hazard classification category, variability of response, or days required for lesion to clear
  - Reduces pain and distress from initial application of test articles
  - Should always be considered before performing
  - Pretreatment recommended by U.S. Consumer Product Safety Commission since 1984

In Vitro Pyrogenicity Test Methods

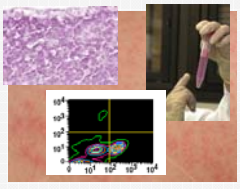
- Evaluated five *in vitro* test methods proposed as replacements for the rabbit pyrogen test (RPT)
- Methods measure increased cytokine levels released by human blood cells or cultured human cells using ELISA tests
- ICCVAM recommends consideration of these methods to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis subject to product-specific validation
- Not yet validated to identify non-endotoxin pyrogens; therefore not a valid replacement for all rabbit testing
- Recommendations endorsed by U.S. Federal agencies, April, 2009
- Limulus Amoebocyte Lysate Test; another in vitro test pyrogen test accepted by FDA since 1986; also detects endotoxin pyrogens.(Do not detect non-endotoxin pyrogens



Other Workshops and Test Method Evaluations

- 2006 Workshop on Alternatives for Botulinum Toxin Testing - HSUS nomination
- 2000 Evaluation of FETAX
  - -- EPA nomination
  - Frog Embryo Toxicity Assay-Xenopus

Addressing Other Agency Test Method Priorities



- *In Vitro* Methods for EPA's Endocrine Disruptor Screening Program
  - Provided guidance for validation: essential protocol components, 78 reference chemicals
  - International validation study: NICEATM-ICCVAM, ECVAM and JaCVAM, 2007-
  - Performance standards will be provided in 2009

National Academy of Sciences Report - 2007

- Sponsored by U.S. EPA
- Envisions a future in which virtually all routine toxicity testing would be conducted in human cells or cell lines *in vitro*
- Based on evaluating perturbations of cellular responses in a suite of toxicity pathway assays
- Will require high-throughput robotic-assisted methodologies

NTP High Throughput Screening Program

- The first NTP "1408" compound set
  - 1353 unique compounds, 55 in duplicate to evaluate assay reproducibility
  - 1206 with NTP test data
  - 147 are ICCVAM reference substances recommended for the validation of alternative in vitro test methods (e.g., dermal corrosion, acute toxicity, endocrine activity).
- Selection based on solubility in dimethyl sulfoxide (DMSO) at 10 mM, while avoiding excessive volatility.
- NTP compound library available to other Centers using different HTS technologies
- Robots can conduct thousands of assays each day

Alternatives in Laboratory Animal Medicine: Refinement Alternatives, Q&As

- When is an animal listed in Column E on the USDA Annual report?
- Approximately what % of animals reported to USDA are in Column E: 1%; 5%; 10%; 25%; 45% ?
- What regulatory toxicity testing procedures require death as an endpoint?
- How do you define moribund condition?
- What is a humane endpoint?
- Name an alternative test method that avoids potential pain and distress by using an earlier predictive mechanistic endpoint?

Alternatives in Laboratory Animal Medicine: Refinement Alternatives, Q&As

- What humane endpoints are approved by the USDA for early termination of rodents used in rabies vaccine potency testing?
- What humane endpoint is predictive of death for rodents used in Pertussis vaccine potency testing?

How Common Are Unrelieved Pain And Distress In Research And Testing?

- 9% of the total animals reported to USDA experience unrelieved pain and distress
  - > 100,000/year
  - > 1 million (est), with rats and mice
- 75% justified by *regulatory testing requirements*
  - Vaccine potency testing
  - Rabbits: ocular, dermal
  - Guinea Pigs: sensitization
- 25% justified for research uses

Why Do We Still Have Pain And Distress In Research And Testing?

- Safety testing
  - Endpoints to identify potential toxic effects
    - Acute lethality, ocular hazards, cancer, etc.
- Vaccine testing
  - Challenge tests with infectious agents
  - Toxin potency
- Biomedical research
  - Models of human disease and injury

What Are Humane Endpoints for Research and Testing?[1,2]

- Criteria that can be used to end an animal study:
  - Following the onset of pain and distress, in order to avoid further pain and distress; or *ideally*,
  - *Prior* to the onset of potential pain and distress, such that more than minimal pain and distress is completely avoided.
- *Humane endpoints must be consistent with attainment of research or testing objectives*

[1] Stokes, W.S. 2000. Humane Endpoints for Laboratory Animals Used In Toxicity Testing. In: Progress in the Reduction, Refinement, and Replacement of Animal Experimentation. Balls, M., van Zeller, A.M., Halder, M. (eds.): Amsterdam: Elsevier Sciences.

[2] Stokes, W.S. 2000. Reducing Unrelieved Pain and Distress in Laboratory Animals Using Humane Endpoints. ILAR J 41:59-61.

Humane Endpoints: IACUC Guidance

- ARENA/OLAW Institutional Animal Care and Use Committee Guidebook
  - Produced by the Applied Research Ethics National Assn. and the NIH Office of Laboratory Animal Welfare
  - 2nd Edition, 2002 (replaces 1st edition, 1992)
  - Increased emphasis:
    - Application of the 3Rs
    - Humane Endpoints
      - Especially where pain-relieving drugs cannot be used
      - Emphasis on clarifying endpoints when studies can be terminated
      - Encourages development of earlier humane endpoints
      - Avoiding death as an endpoint
  - Available at: http://grants.nih.gov/grants/olaw/

Guidance on Humane Endpoints

- Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing, CCAC, 1998.
- Proceedings of the 1st International Conference on Humane Endpoints in Animal Experiments for Biomedical Research, November 22-25, 1998, Hendriksen, C.F.M. and Morton D.B., eds. 1999.
- Humane Endpoints for Animals Used in Biomedical Research and Testing, ILAR Journal, 41(2); 2000
- Proceedings of the ICLAS/CCAC International Symposium on Regulatory Testing and Animal Welfare, ILAR Journal, 43(Suppl.), 2002

Humane Endpoints For Safety Evaluations: Current Best Practices

- Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluations[1]
- Applicable to all OECD test guidelines

[1] OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 19, OECD, Paris, France, 2000. http://www.oecd.org/ehs/test/monos.htm

International Principles for Establishment of Humane Endpoints[1]

- Developed by the International Council on Laboratory Animal Science (ICLAS)
  - Working Group on Harmonization of Guidelines
- Based on:
  - OECD Guidance Document on Humane Endpoints
  - CCAC Guidelines on Choosing Appropriate Endponts

[1] Demers g, Griffin G, De Vroey G, Haywood JR, Zurlo J, Bedard M. (2006) Harmonization of Animal Care and Use Guidance. *Science* 312: 700-701. www.sciencemag.org/cgi/content/full /312/5774/700

International Principles for Establishment of Humane Endpoints

- There is strong evidence that animals experience pain and distress in situations comparable to those that cause pain and dstress for humans
- Death or severe pain and distress should be avoided as endpoints.
- The earliest possible endpoint should be used that is consistent with the scientific objectives.
- Studies should be designed to minimize any pain or distress, likely to be experienced by the animals, while meeting the scientific objectives.

International Principles for Establishment of Humane Endpoints

- The duration of studies involving pain and distress should be kept to a minimum.
- Pilot studies should be encouraged as means of determining morbidity, time course of events, and frequency of observations required to set an earlier endpoint.
- Before commencing the experiment, agreement should be reached on: 1)appropriate endpoints for the study, and 2) the persons or persons to be responsible for making the judgment that the endpoint has been reached.

International Principles for Establishment of Humane Endpoints-3

- A team approach should be used, employing the professional judgment of the scientist, veterinarian, animal care staff, and ethics committee to agree on the appropriate endpoint for the study.
- Research and animal care staff must be adequately trained and competent in recognition of species-specific behavior and, in particular, species specific signs of pain, distress, and moribundity.
- Animals should be monitored by means of behavioral, physiological, and/or clinical signs at an appropriate frequency to permit timely termination of the experiment once the endpoint has been reached.

What Types Of Biomarkers Can Serve As Earlier More Humane Endpoints?

- Clinical signs
  - Abnormal behavioral
  - Abnormal appearance
- Changes in objective clinical measurements
  - Body weight
  - Blood pressure
  - Heart rate
  - Respiratory Rate
  - Body temperature
  - Transcutaneous PO2

Other Potential Biomarkers that may Serve As Earlier More Humane Endpoints

- Serum Biomarkers
  - Hematology
  - Serum Chemistry
- Urinary biomarkers
- Molecular biomarkers in serum or tissues
- Imaging biomarkers

Process for Identifying Candidate Biomarkers as Potential Humane Endpoints

- Develop detailed observation logs of most relevant potential biomarkers
  - Clinical signs, objective measures, etc.
- Record detailed observations on all animals during entire study
- Retrospectively assess if any of the biomarkers are predictive of the study outcome at an earlier timepoint

Implementing Humane Endpoints in Research and Testing Studies

- Adopt clearly defined endpoints for removal of animals from a study
- Incorporate endpoints into study protocol
  - Establish appropriate observation schedules
  - Establish procedures for decisions on termination
- Provide education/training
  - Animal care staff
  - Study Directors

How are Pain, Distress, and Death Addressed in Safety Testing?

- Analgesics and tranquilizers rarely used
  - GLPS: Only if no interference with the study [1,2,3]
- However, nearly all testing regulations allow humane euthanasia if:
  - Severe pain and distress
  - Moribund condition
  - Some exceptions for human biologics/toxin potency testing

[1] EPA Good Laboratory Practice Standards, 1998.
[2] FDA Good Laboratory Practice for Non-clinical Laboratory Studies, 1999.
[3] OECD Good Laboratory Practice in the Testing of Chemicals, 1998

What are Current Humane Endpoints in Safety Testing Guidelines[1,2]?

- Clinical signs of severe pain and distress
- Moribund condition
- Chronic studies:
  - Clinical signs indicative of discomfort, pain, distress, or rapidly deteriorating condition
- Death is not a required endpoint for toxicity testing

[1] OECD Test Guidelines Program; http://www.oecd.org/ehs/test/index.htm
[2] EPA OPPTS Test Guidelines; http://www.epa.gov

Avoiding Death as an Endpoint: Using Moribund Condition as a Humane Endpoint

- Moribund: "in the state of dying; at the point of death"
- Moribund conditions:
  - prolonged inability to reach food or water
  - excessive weight loss/extreme emaciation
  - absence of voluntary response to external stimuli
  - difficult labored breathing
  - prolonged inability to remain upright
  - not likely to survive until next scheduled observation
- Goal: Minimize or avoid spontaneous deaths

Humane Endpoints: Chronic Studies

- Rapid and/or excessive weight loss
  - Rapid weight loss, e.g., >20 % in < l week
  - Most sensitive indicator of severe disease
  - Causes: chronic systemic disease, cancer, anorexia
- Excessive tumor burden and large masses
  - Consider body condition scoring rather than weight loss as endpoint
  - Interference with ambulation
  - Reduced ability to eat and/or drink
  - Ulcerations; Infections
- Goals: minimize spontaneous deaths

Humane Endpoints: Testing of Biological Products

- USDA Center for Veterinary Biologics Notice No. 04-09, April 1, 2004
- Provides policy for humane endpoints in animal challenge potency tests
- Animals may be treated or humanely destroyed if illness has progressed to a point where death is certain to occur
- *Moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power may be humanely euthanized and considered as deaths as outlined in 9CFR 117.4*

Humane Endpoints: Vaccine Potency Testing

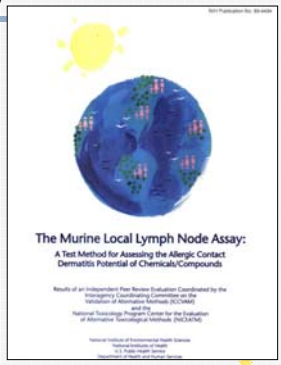
Vaccine	Endpoint	References
Pertussis	**Hindlimb paralysis Body temperature <34.5°C**	**Calver et al., 1999 Cussler, Morton, Hendriksen, 1999**
Rabies	**Weight loss; Neurologic signs**	**Cussler, Morton, Hendriksen, 1999**

Humane Endpoints: Testing of Rabies Vaccine

- USDA Center for Veterinary Biologics Notice No. 04-09, April 1, 2004
- *Animals exhibiting paresis, paralysis, and/or convulsions may be humanely euthanized and considered as deaths as outlined in 9 CFR 117.4*

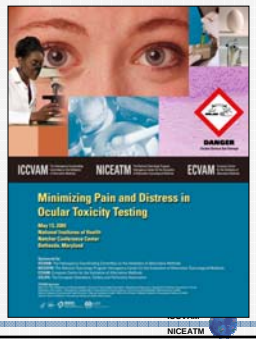
The Murine Local Lymph Node Assay

- Alternative method for assessing allergic contact dermatitis (ACD)
- An example of using a mechanistic earlier endpoint to eliminate pain and distress
- Accepted internationally as valid *substitute* for guinea pig tests (GPMT, BA)
- Should always be considered for regulatory testing for ACD; justification provided if not used



Humane Endpoints: Ocular Safety Testing

- Symposium on Minimizing Pain and Distress in Ocular Safety Testing
- May 13, 2005
- Organized by ICCVAM, NICEATM, and ECVAM
  - Open to the public
  - 56 participants
- Invited Experts
  - Veterinary and human ophthalmology, anesthesia
  - Ophthalmic researchers
  - Toxicologists
- http://iccvam.niehs.nih.gov



Focus of the Symposium

- Can earlier humane endpoints be identified and used to terminate studies to avoid or minimize pain and distress from ocular injuries?
- Can pre-application topical anesthetics be used routinely without interfering with the ocular hazard classification?
- Can pain and distress from induced eye injuries be routinely treated, as with human injuries, without interfering with the hazard classification?

Current "Humane" Endpoints in Regulatory Test Guidelines

- *Animals showing severe and enduring signs of pain and distress may be euthanized*
- *Animals with the following eye lesions post-instillation should be humanely killed because such lesions generally are not reversible (OECD):*
  - Corneal perforation or significant corneal ulceration including staphyloma (protrusion)
  - Blood in the anterior chamber of the eye
  - Grade 4 corneal opacity which persist for 48 hours
  - Absence of a light reflex(iridal response grade 2) which persist for 72 hours
  - Ulceration of the conjunctival membrane
  - Necrosis of the conjunctivae or nictitating membrane
  - Sloughing

Recommended Additional Humane Endpoints for Ocular Safety Testing

- Endpoints listed in current regulations, plus:
- Vascularization of the corneal surface (pannus)
- Greater than 75% of the limbus destroyed
- Flourescein staining indicating:
  - The site of injury is not healing after 2-3 days; and/or
  - Depth of injury is increasing in the days after the test substance is applied
- Other potential endpoints should also be considered

Identifiying Earlier Humane Endpoints for Ocular Safety Testing

- Collect quantitative objective data from ongoing testing
  - Slit lamp biomicroscopy with staining
  - Depth of injury
  - Pachymetry (corneal thickness)
  - Photo-documentation
  - Altered tear production
  - Postmortem exams
    - Histopathology
    - Live/Dead cell assays
- These data will also provide useful data applicable for development and validation of in vitro methods

Summary

- Humane endpoints and strategies can reduce the *duration* and *severity* of pain and distress experienced by animals.
- Humane endpoints can coexist with research and toxicology studies
- Humane endpoints have now been accepted by regulatory authorities
- Advances in science and technology now provide new opportunities for progress in humane endpoints.
- Commitment and cooperation by all stakeholders will expedite progress!

Good Science for Good Decisions!

Thank you for your attention.

For more information visit our website:
http://iccvam.niehs.nih.gov/

or contact

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