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The Concept of Humane Endpoints and their Identification: Application to Acute Toxicity

William S. Stokes, D.V.M., DACLAM
RADM, U.S. Public Health Service
Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS,NIH, DHHS
Research Triangle Park, North Carolina

Workshop on Acute Chemical Safety Testing: Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations
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Protecting, Promoting and Advancing the Health and Safety of the Nation

National Toxicology Program

U.S. Department of Health and Human Services
National Institutes of Health

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Outline

- Pain and distress in testing
- The concept of humane endpoints
- Identifying Humane Endpoints
- Examples of Humane Endpoints
- International Principles and Guidance
- Future progress

Why is there Pain And Distress In Safety Testing?

- Endpoints to identify potential toxicity often involve pain and/or distress when toxic effects occur
  - Associated with tissue damage, organ damage/failure
  - Examples: systemic toxicity, ocular injuries, skin irritation/corrosion, cancer, etc.

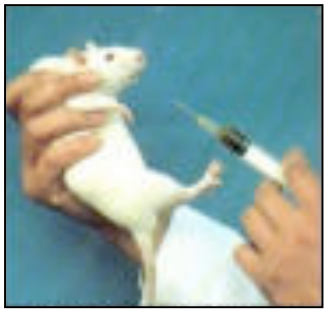
What Obligations are there to Minimize or Avoid Pain and Distress?
U.S. Regulations and Policies

- 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 to attain scientific objectives

- *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*

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
  - Death is not a required endpoint for toxicity 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

How might we further reduce unrelieved pain and distress in testing?

- Reconsider if analgesics, anesthetics, tranquilizers can be used, and still accomplish scientific objectives
- Consider other non-drug interventions to improve animal well-being
  - Supportive care, soft food, etc.
- Consider earlier more humane endpoints for studies

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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 reduce the duration and severity of pain and distress that would otherwise occur; **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*
- *Humane endpoints provide a systematic approach to refinement*

[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.

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

- Clinical signs
  - Abnormal behavior
  - Abnormal appearance
- Changes in objective clinical measurements
  - Body temperature
  - Body weight
  - Blood pressure
  - Heart rate; Heart rhythm
  - Respiratory Rate
  - Transcutaneous PO2 (using pulse oximeter)

Other Potential Biomarkers that may Serve As Earlier More Humane Endpoints

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

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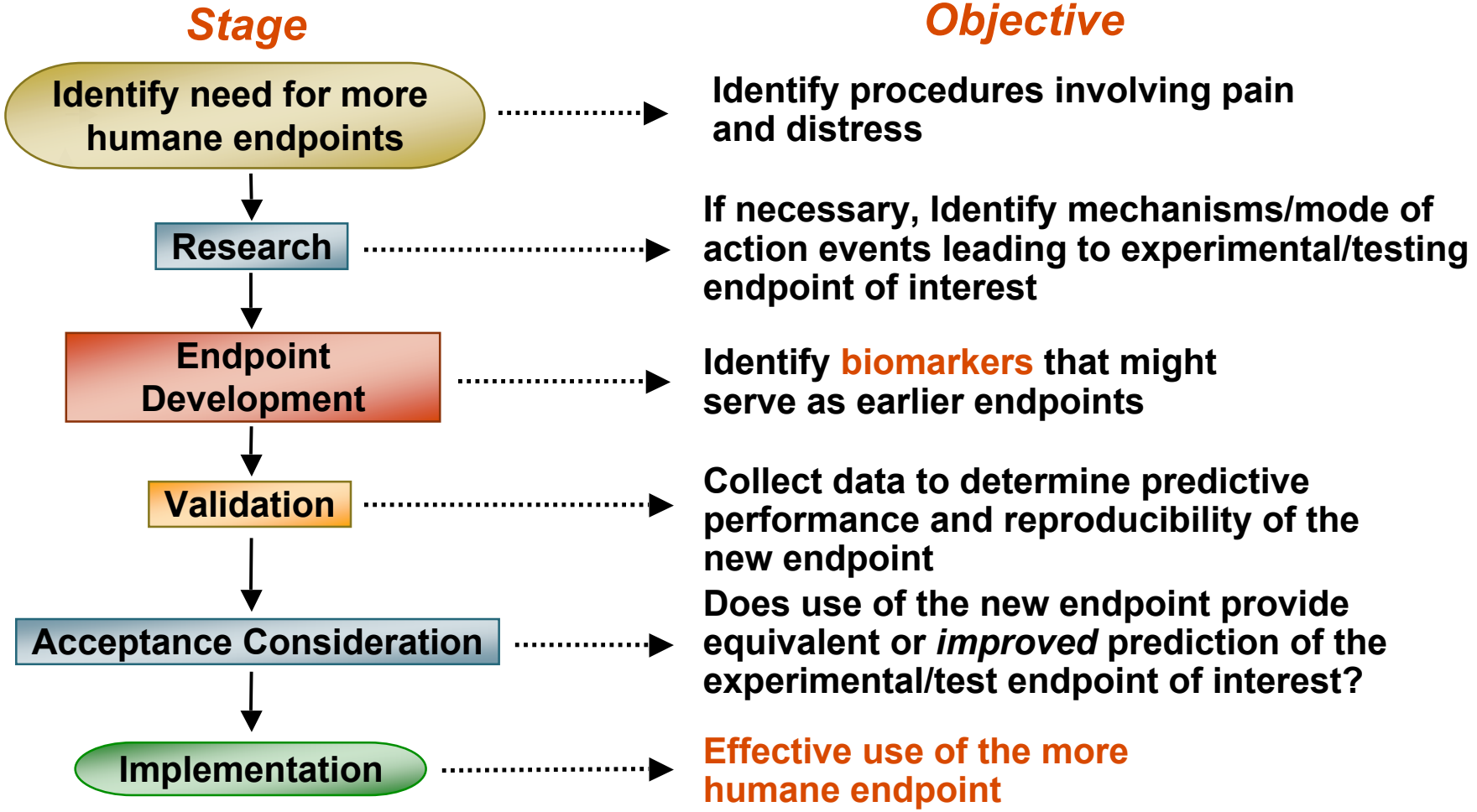
Process for Developing Humane Endpoints for Research and Testing



How Can We Identify Biomarkers to serve as Humane Endpoints?

1. Develop detailed observation logs of most relevant potential biomarkers
    - Clinical signs, objective measures, etc.
2. Record detailed observations on all animals during entire study
3. Analyze data to determine if any of the biomarkers are predictive of the study outcome at an earlier timepoint

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*

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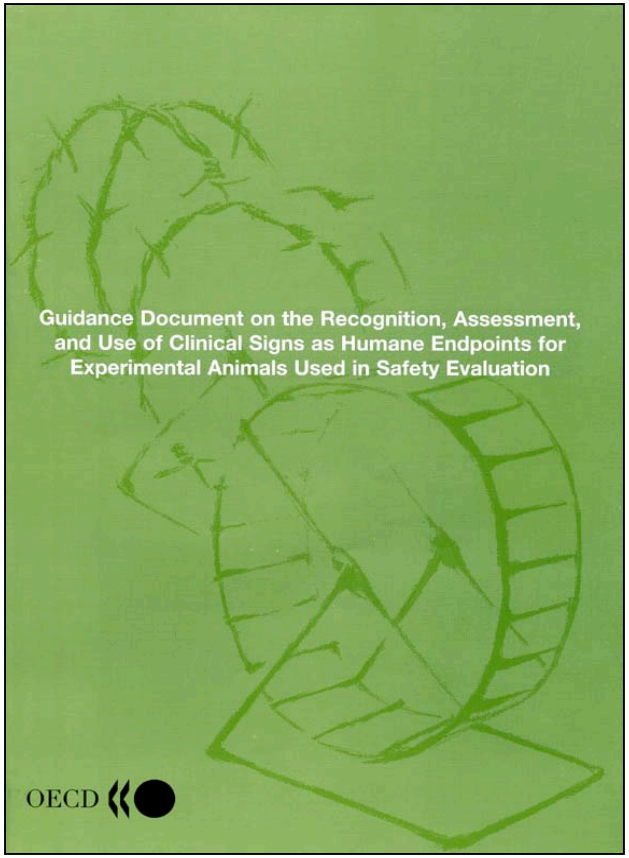
Selected Guidance on Humane Endpoints

- *Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing,* CCAC, 1998.
- *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
- 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 Safety Evaluations: International Current Best Practices

- OECD 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
- Largely provides guidance and criteria for humane euthanasia to avoid spontaneous deaths



[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 Endpoints
  - U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Research, Testing, and Education

[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 - 1

- *There is strong evidence that animals experience pain and distress in situations comparable to those that cause pain and distress 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 - 2

- *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*.

The Way Forward:
Developing and Applying Humane Endpoints -1

- Identify and collect potential biomarker data during in vivo studies that involve pain and distress
  - At a minimum this should include collection of detailed clinical signs, and data for objective biomarkers that are candidates for earlier humane endpoints
  - Periodically analyze data to determine if any biomarkers are sufficiently predictive as earlier more humane endpoints
- Routinely consider humane endpoints, *prior to* the use of animals, whenever unrelieved pain and distress is anticipated or will occur.
  - Incorporate where appropriate

The Way Forward:
Developing and Applying Alternative Methods -2

- Consider and use new science and technology
  - New sensitive biomarkers
  - Remote sensing devices
  - Telemetry
- Involve the entire research team

Summary

- Humane endpoints *can* reduce the *duration* and *severity* of pain and distress experienced by animals.
- Humane endpoints can coexist with research and toxicology studies
- Advances in science and technology provide opportunities for greater development and use of humane endpoints.
- Commitment and cooperation by all stakeholders will expedite progress!

Thank You For Your Attention!