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

ICCVAM
Interagency Coordinating Committee on the Validation of Alternative Methods

The 3Rs: Alternatives for Laboratory Animal Use in Testing

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2012 North Carolina Workshop in Laboratory Animal Medicine
North Carolina State University
May 17, 2011

Outline

- **Introduction to Alternatives**
- Laws, Regulation, and Policies
- Government Alternatives Organizations
- Animal Use in Testing
- Strategies for Avoiding or Minimizing Pain and Distress
- Examples of Accepted Alternative Methods
  - Toxicity Testing
  - Vaccine Testing
- Regulatory Science Research Initiatives

Disclaimer: This presentation does not necessarily reflect the official position of any U.S. Agency.

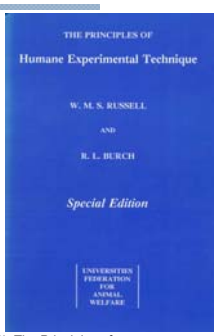
Alternatives in Laboratory Animal Medicine: Introduction

- How are animal use alternatives commonly defined?
- What two scientists published a book describing the concept of the 3Rs?
  - What was the name of the book?
  - When was it published?
- What is commonly referred to as the "Forgotten R"?

"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

Workshop Overall Recommendation:

"*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.

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Alternatives; Laws, Regulations, and Policies

- What Federal **law and implementing regulations** 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 vertebrate animal use is approved?
- What Federal Committee is responsible for reviewing and forwarding recommendations on alternative test methods to Federal agencies?

Alternatives: U.S. Legal and Statutory Mandates

- USDA Animal Welfare Act: 1985 Amendment
  - 1989 Implementing Regulations (9CFR)
- U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals
- Each 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:** conduct research to reduce, refine, and replace animal use, including lower species
  - **NIEHS**: develop and validate alternative methods for acute and chronic safety testing
  - **NIEHS**: 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 and respond to ICCVAM recommendations in 180 days

Legal/Statutory Mandates: Europe

- **7th Amendment to the Cosmetics Directive**
  - Bans animal testing of cosmetics, and marketing if tested on animals:
    - **2004:** ALL ***finished*** cosmetic products
    - March, 2009: All cosmetic **ingredients**
      - exceptions for repeat dose testing procedures: cancer, developmental and reproductive toxicities
    - **2013: Complete ban on ALL animal testing for all ingredients**
- **REACH Legislation**
  - **R**egistration, **E**valuation, and **A**uthorization of **CH**emicals
  - 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
- **Directive 2010/63/EU of the European Parliament, September, 2010**
  - Updates EU law for the protection of animals used for scientific purposes (revises Directive 86/609/EEC, 1986)

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Government Alternatives and Related Organizations: Describe the Acronyms

- NICEATM
- ICCVAM
- JaCVAM
- KoCVAM
- ECVAM
- ICATM
- NC3Rs
- OECD
- ICLAS

What is ICCVAM?

- The **I**nteragency **C**oordinating **C**ommittee on the **V**alidation of **A**lternative **M**ethods: *"Ich' vam"*

- Established by NIEHS in 1997
  - Response to Public Law 103-43
- ICCVAM Authorization Act of 2000
  - Permanent committee under NICEATM
- Members: 15 U.S. Federal regulatory and research agencies that require, use, generate, or distribute safety testing information
- **ICCVAM Duties (P.L. 106-545)**:
  - Advise on test method development and validation
  - Conduct technical reviews of new safety testing methods
  - Transmit formal recommendations to Federal agencies
  - Promote regulatory acceptance of valid methods
  - Foster national and international harmonization

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-CDC
- 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 **N**ational Toxicology Program (NTP) **I**nteragency **C**enter for the **E**valuation of **A**lternative **T**oxicological **M**ethods
  - ***"Nigh' see tum"***

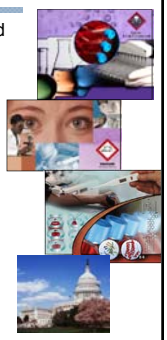
- A Center of the U.S. NTP
  - NTP headquartered at the National Institute of Environmental Health Sciences, NIH, DHHS
  - NIEHS: one of the 27 NIH Institutes and Centers
  - NTP: conducts and coordinates toxicology testing programs across the federal government
  - Research Triangle Park, North Carolina
- NICEATM functions:
  - Conduct international validation studies
  - Administer and provide scientific support for the **Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)**
    - Evaluate proposed new, revised, and alternative test methods

The Five ICCVAM Purposes (Public Law 106-545)

1. **Reduce, refine, or replace the use of animals in testing, where feasible**
2. Increase the efficiency and effectiveness of Federal agency test method review
3. Eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies
4. Optimize utilization of scientific expertise outside the Federal Government
5. Ensure that new and revised test methods are validated to meet the needs of Federal agencies

NICEATM and ICCVAM: Recent Progress

- Together with ICCVAM agencies, contributed to national and/or international regulatory acceptance of 51 alternative test methods:
  - 28 *in vitro* methods that **replace or reduce** animal use
  - 23 *in vivo* methods that significantly **reduce** animal use, or **refine** animal use by avoiding or reducing pain and distress
- Numerous International guidelines/guidances: OECD, ISO, ICH
  - Validation Guidance, OECD GD
  - Humane Endpoints, OECD GD



http://iccvam.niehs.nih.gov/

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 (e.g. animal testing and/or humans (where ethical))

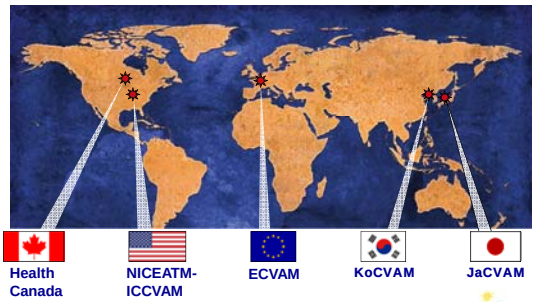
[1]ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods;
NIH Pub. No. 03-4508, 2003, NIEHS, Research Triangle Park, NC.
http://iccvam.niehs.nih.gov/docs/guidelines/subguide.htm

Why is scientific validation necessary?

- 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***

ICATM: International Cooperation on Alternative Test Methods

ICATM is a **voluntary** international cooperation of national organizations:
Currently Canada, European Union, Japan, Republic of Korea, and United States



Health Canada | **NICEATM-ICCVAM** | **ECVAM** | **KoCVAM** | **JaCVAM**

ICATM Validation Organizations

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

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Questions: Animal Use in Testing

- **Based on <u>available</u> US and EU data:**

1. Are more animals used for research or for testing?

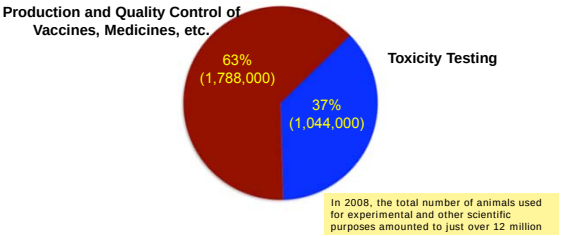
2. Testing encompasses two major areas of animal use:
   - Vaccine/biologics potency and safety testing
   - Drug/chemical/product safety and efficacy testing

   a. Which uses more animals (based on available data)?

   b. Which involves more unrelieved pain and distress (i.e. USDA column E listing)?

Animals Used for Testing by Major Categories (EU 2010)

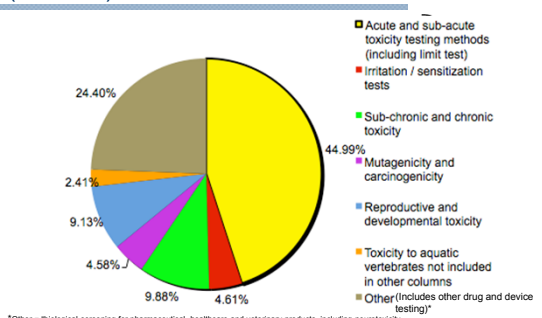
Testing accounts for 24% of total EU animal use (2.8M/yr)



In 2008, the total number of animals used for experimental and other scientific purposes amounted to just over 12 million

Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union COM(2010) 511

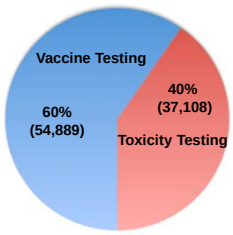
Animals Used for Toxicity Testing by Test Type (EU 2010)



*Other = "biological screening for pharmaceutical, healthcare and veterinary products, including neurotoxicity, toxicokinetics, testing of biological evaluation of medical devices"

Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union COM(2010) 511

Unrelieved Pain and Distress (No Pain Relievers): Animals by Testing Type Reported to USDA (2010)



- **Testing accounts for 95% of all animals reported with unrelieved pain and distress: 91,997 animals**
- **Research: 5% (5,126)**
- **Total: 97,123 (8.6% of total animals)**
- Note: RATS, MICE, and BIRDS are NOT included; account for an estimated 95% of animal use
  - **Est. total animals with unrelieved pain and distress: 1.84 million**

Toxicity Testing	% (# Animals)
Unspecified Procedures	
Safety Testing and Drug Efficacy Testing	73% (27,249)
Specified Procedures	
Dermal Sensitization	12% (4,454)
Acute Systemic Toxicity	11% (4,071)
Dermal Irritation	3% (1,004)
Other Specified Uses	< 1% (330)

Data for all states with all animal data for Column E of APHIS Form 7023; USDA. 2010. Annual Report - Animal Usage by Fiscal Year. United States Department of Agriculture. Animal and Plant Health Inspection Service. Available at: http://www.aphis.usda.gov/animal_welfare/efoia/7023.shtml

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

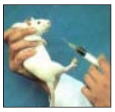
- What regulatory <u>toxicity</u> testing procedures require death as an endpoint?
- What is a humane endpoint?
- Name an in vivo alternative test method for allergic contact dermatitis (skin sensitization) testing that avoids potential pain and distress by using an earlier predictive mechanistic endpoint?
- What humane endpoints are approved by the USDA for early termination of rodents used in rabies vaccine potency testing?

Avoiding and Minimizing Pain and Distress: Four **Refinement** Strategies

1. Identify humane endpoints that can be used to end studies *before* the onset of pain and distress
   - *LLNA*
2. Identify humane endpoints for earlier termination of studies in order to shorten the duration and/or severity of pain and distress
   - *Rabies vaccine challenge test; ocular safety testing*
3. Use appropriate analgesics and/or anesthetics
   - *Ocular safety testing; Rabies IC injections*
4. Provide appropriate supportive veterinary and nursing care
   - *All animal testing*

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
- 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.

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.

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

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**

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Alternative Methods for Toxicity Testing: Q&As-1

- 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 toxicity test, and should be used where determined appropriate?
- Name two in vitro tests approved for ocular irritation testing?
  - When can the results be used for hazard classification?
  - Are animals still required for ocular safety testing?

Immunotoxicity: Allergic Contact Dermatitis

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;updated 2010
- http://iccvam.niehs.nih.gov/llna.htm

Alternative Test Method for Allergic Contact Dermatitis Testing

	GPMT[1]	LLNA
Time to perform:	32+ days	7 days
Number of animals:	30+	12-20
Dermatitis induced:	Yes	No
Adjuvant required:	Yes	No

- LLNA Advantages over guinea pig tests for ACD
  - Refinement: Elimination of pain and distress
  - Reduction: 33-60% fewer animals
  - Efficiency: 75% reduction in time to perform
  - Added value: Dose-response information

[1] Guinea Pig Maximization Test

LLNA Humane Endpoint: Earlier Predictive Tissue Biomarker for Allergic Contact Dermatitis Testing

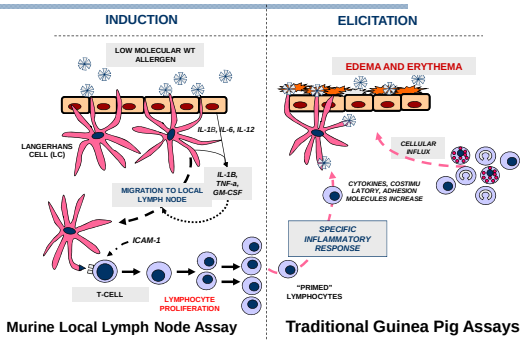


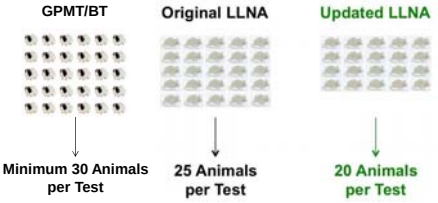
*Illustration by D. Sailstad

Alternative Methods for Allergic Contact Dermatitis: The Updated Murine Local Lymph Node Assay

- ICCVAM recommendations: 2009
  - Updated LLNA
    - 20% reduction in animal use
  - Reduced LLNA (rLLNA)
    - Further reduces animal use by 40%
  - 2 non-radioactive LLNA test methods
  - Acceptance, US Agencies - 2010
  - International Adoption-2010

Reduction and Refinement for Updated ICCVAM Local Lymph Node Assay Protocol, 2010

- 20% **reduction** in animal numbers (4 vs. 5/group)

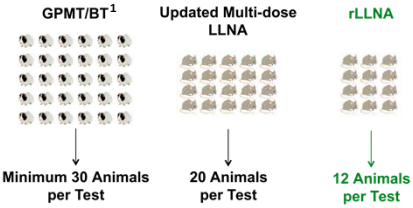


- **Refinement**
  - Avoids pain and distress associated with guinea pig tests:
    - Avoids the elicitation phase involving pruritis, erythema, edema
    - Avoids the need for irritating adjuvants (i.e., GPMT)

Abbreviations: GPMT/BT = guinea pig maximization test/Buehler test

Further Reduction of Animal Use: Reduced LLNA (rLLNA); Using *in vitro* data to inform test selection

- *In chemico* and *in vitro* tests can be used to screen for potential ACD activity; negatives can be confirmed in the rLLNA with only 12 mice
- rLLNA reduces animal number by 40% for each test vs. multi-dose LLNA



[1] GPMT/BT = Guinea Pig Maximization Test/Buehler Test

Dermal Corrosivity and Irritation

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

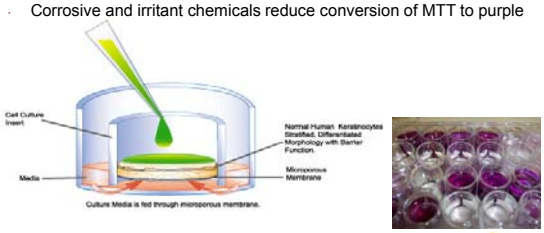
- Five in vitro methods accepted
  - Corrositex, 1999
  - Epiderm, Episkin, Rat skin TER, 2002, SkinEthic, 2004
- Can identify most corrosive substances without the need for animals
- International acceptance as 3 OECD test guidelines
- No animals required when positive results; negative results require 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**

In Vitro Test Methods for Skin Irritation and Corrosion Testing

- Test methods approved for skin corrosion testing:
  - Corrositex: measures skin corrosion potential by testing if the substance can pass through a biobarrier
  - Transcutaneous electrical resistance (TER): measures skin corrosion potential by testing if the substance alters electrical resistance in isolated rat skin
  - Reconstructed Human Epithelial (RhE) models: measures skin corrosion potential by testing if the substance reduces cell viability
    - Uses multilayer culture of NHK cells
- RhE test methods also available for skin irritation testing

Reconstructed 3D Human Epithelial Models: Skin Irritation and Corrosivity Testing

- Human cultured skin cells grown on a membrane and exposed to test chemicals
- Health of exposed cells measured by incubation with MTT dye
- Healthy cells convert MTT to a purple color
- Corrosive and irritant chemicals reduce conversion of MTT to purple



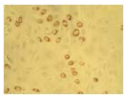
Toxicity Testing
Acute Systemic Toxicity

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*
- February 6-7, 2008 Workshop:
  - *Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations*

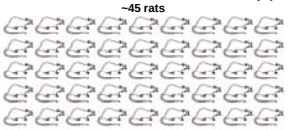
Innovative Strategies: Acute Oral Toxicity

- First, use cytotoxicity data to estimate starting doses for animal studies
  - can reduce and refine animal use
  - OECD Guidance Document
- 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



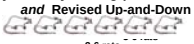
Reduction Test Method for Acute Oral Systemic Toxicity Testing: >70% Fewer Animals

OECD Test Guideline 401: Acute Oral Toxicity (1981)
~45 rats



OECD Test Guideline 425: Acute Oral Toxicity – Up-and-Down Procedure (2001)
6-9 rats

***In Vitro* Cytotoxicity Testing (OECD Guidance Document, 2010)**
***and* Revised Up-and-Down Procedure**



3-6 rats

Ocular Toxicity Testing

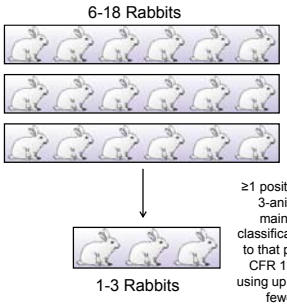
Before Ocular Safety Testing Requirements: The Lash-Lure Tragedy (1933)

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

Using Fewer Animals to Identify Chemical Eye Hazards

16 CFR 1500.42 (CPSC 2010): Requires 6 animals per test and may require up to three sequential tests for each substance, thereby requiring 6, 12, or 18 animals to reach a hazard decision



≥1 positive animal in a 3-animal test will maintain hazard classification equivalent to that provided by 16 CFR 1500.42, while using up to 50% to 83% fewer animals

Sequential testing recommended

In vitro Methods for Ocular Toxicity Testing: Recent Progress

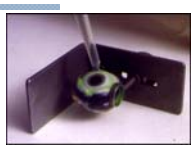
- ICCVAM recommended two in vitro methods for identifying severe and irreversible eye damage from chemicals without the use of animals:
  - BCOP: Bovine Corneal Opacity and Permeability Assay
  - ICE: Isolated Chicken Eye Assay
- The first scientifically valid alternative methods to gain international regulatory acceptance for ocular safety testing that do not use live animals.
  - 2008: accepted by U.S. Federal regulatory agencies
  - 2009: OECD TG 437 (BCOP) and OECD TG 438 (ICE) adopted
  - 2010: ISO 10993-10:2010 Biological evaluation of medical devices

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, weight-of-evidence approach
  - Positives: No further testing (no animals used)
  - Negatives: requires additional testing, currently in vivo in most cases
- Advantages
  - Uses intact eyes; slit-lamp biomicroscope
  - Chicken cornea most similar to human
- Limitations
  - Not highly predictive for alcohols, surfactants and solids
  - For all other substances, false negative rate of 30% and false positive rate of <10%



Avoiding and Minimizing Pain and Distress: Using Analgesics and Anesthetics for Ocular Safety Testing

- Balanced preemptive pain management should *always* be provided during ocular safety testing
- Pain management should include:
  - **Topical anesthetic and systemic analgesic** *prior to* test substance administration (TSA)
    - proparacaine; tetracaine: 5 min prior
    - Buprenorphine- 1 hr prior
  - **Systemic analgesics** after TSA ; rescue doses if necessary
    - Buprenorphine q12hr (or SR); meloxicam q24hr
  - Scheduled evaluation/ recording of all clinical signs, especially those indicative of pain and/or distress;
  - **Humane endpoints:** frequent evaluation and recording of all eye injuries for nature, severity, and progression;
- Adopted by U.S. agencies: March, 2011; OECD Test Guideline approved, Mar 12;

Pyrogen Testing

Pyrogen (Fever) Detection Methods

Rabbit Pyrogen Test

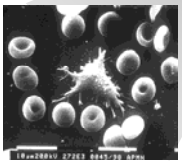
Bacterial Endotoxin Test
•Uses hemolymph from horseshoe crabs
•Limulus amoebocyte lysate (LAL) test

In Vitro Pyrogen Test Methods
• Uses human mononuclear cells

Slide provided by Dr. Thomas Hartung

In Vitro Pyrogen Test Methods: Based on Human Fever Reaction

Test article → Exogenous Pyrogen →
1. Whole blood
2. Monocytes
3. Cell lines
4. Cryopreserved whole blood



→ Release of Endogenous Pyrogens (IL-1, IL-6) → Fever / ELISA

Slide provided by Dr. Thomas Hartung

2008 ICCVAM Report on *In Vitro* Pyrogen Methods

- ICCVAM TMER:
  - Validation Status of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products
- Can detect Gram-negative endotoxin
- 2009: Accepted by FDA, following product-specific validation
- 2009: European Pharmacopoeia 2.6.30 adopted: Monocyte-activation test (MAT)

Outline

- Introduction to Alternatives
- Alternatives: Laws, Regulation, and Policies
- Government Alternatives Organizations
- Animal Use in Testing
- Strategies for Avoiding or Minimizing Pain and Distress
- **Examples of Alternative Methods**
  - Toxicity Testing
  - **Vaccine Potency and Safety Testing**

Disclaimer: This presentation does not necessarily reflect the official position of any U.S. Agency.

Alternative Methods for Vaccines and Other Biologics

- Rationale for Priority:
  - Vaccine testing accounts for more animal use than toxicity testing (at least 60% of animal use in testing)
  - Vaccine testing accounts for the largest number of animals experiencing significant unrelieved pain and distress
- Public Health Significance
  - Biologics include vaccines, toxins, blood and blood components, tissues, antibodies, materials used in gene therapy, and recombinant therapeutic proteins

http://iccvam.niehs.nih.gov/docs/5yrPlan/NICEATM5YR-Final.pdf

2010 International Workshop on Alternative Methods for Vaccine Potency and Safety Testing: Key Outcomes

- Workshop Proceedings published December 2011: *Procedia in Vaccinology Vol. 5, pp. 1-266*
  - 26 Manuscripts:
    - State of the science
    - Recommendations for using alternative methods
    - Priorities for future work to advance alternatives
- Highest Priorities for future work
  - Human vaccines
    - Rabies
    - Diphtheria and tetanus toxoids (Clostridials)
    - Pertussis Vaccines (whole cell and acellular)
  - Veterinary vaccines
    - Rabies
    - Leptospira
    - Clostridials

Workshop Proceedings available at http://www.sciencedirect.com/science/journal/1877282X

International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing

- October 2011: Organized by NICEATM-ICCVAM with ICATM partners
  - National Centers for Animal Health, Ames, Iowa
  - Sponsors: NICEATM, USDA, ECVAM
- 80 participants, 12 countries
- Regulatory requirements for rabies vaccine potency testing: "NIH challenge test"
  - Intracranial injection of live rabies virus
  - Unprotected mice develop clinical rabies and die
- Workshop addressed:
  - Refinement (less pain and distress)
  - Reduction
  - Replacement
- Public health significance
  - 70,000 human deaths annually worldwide
  - 15 million patients treated with post-exposure vaccines annually

2011 International Workshop on Rabies Vaccine Potency Testing: Highlights

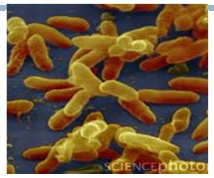
- **Refinement and Reduction** of the NIH challenge test:
  - Anesthesia: should always be used for IC injections
  - Analgesics: should be provided to all mice; studies needed to ensure no interference with test objectives
  - Earlier humane endpoints: should be immediately incorporated in all testing regulations (where not already included)
  - Reduction: Evaluate potential for fewer dilutions, fewer mice per dilution, and possible deletion of duplicate testing on each lot
- **Replacement** of the NIH Challenge Test
  1) Serological Methods: Serum virus neutralization test (SNT)
     - Eliminates the need for challenge with live rabies virus: complete refinement
     - Sufficiently standardized per recent int'l study; manufacturers should conduct and submit product-specific validation studies, in consultation with regulators
  2) *In vitro* antigen quantification methods: complete animal replacement
     - Requires use of monoclonal antibodies specific for the trimeric glycoprotein G
     - Validation should proceed once Mab identified; must discriminate sub-potent lots
- 2012: Workshop report to be published in *Biologicals*

2012 SOT Poster on 2011 Rabies Workshop available at: http://iccvam.niehs.nih.gov/meetings/SOT12/sotabst.htm

Using Clinical Signs as Humane Endpoints: Rabies Vaccine Potency Challenge Testing

- Potency of human and veterinary vaccines currently requires challenge with live rabies virus IC
- Control and inadequately protected vaccinates die from rabies infection
- Earlier humane endpoints now adopted for use:
  - Paresis, paralysis, and/or convulsions
  - Results in euthanasia 2-3 days before death from infection
- 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*

Pertussis (Whooping Cough)



- Highly contagious disease caused by the bacterium *Bordetella pertussis*
- Characterized by violent coughing accompanied by deep "whooping" sound
- Over the past 5 years, between 10,000 and 27,000 cases have been reported annually in the U.S.[5]
- Whole cell vaccine introduced in the 1940s and replaced by an acellular vaccine (aP) over the last 20 years
  - Reduced side effects

[5] http://www.cdc.gov/pertussis/

Murine Histamine Sensitization Test (HIST)

Graphic provided by Arciniega

- Safety test for aP
- Measures residual Pertussis toxin (PT) in vaccine preparations[6]
- PT induces histamine sensitivity in mice
- 20 mice/group injected with aP vaccine; 5 days later mice are challenged IP with histamine. Mouse death within 24 hours is recorded.
- Large number of animals are used; involves significant unrelieved pain and distress
- Highly variable tests; require frequent repeats

[6] Arciniega et al 2011

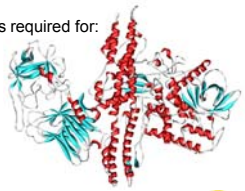
3Rs Alternatives to the HIST Assay

- Biochemical tests:
  - Carbohydrate Binding Assay: High sensitivity, easy to standardize; nonspecific reactions possible
  - eHPLC: High sensitivity; quantitative measurement
- Cell based Assays
  - Functional, holistic system
  - Sensitivity needs to be improved
- Current Status: International spiked vaccine working group established to test alternative *in vitro* methods using standardized aP vaccines and pertussis toxin
  - 13 international laboratories involved in the study
- November 28-29, 2012: International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccine Safety Testing, NIH, Bethesda, Maryland
  - Organized by NICEATM and ICCVAM with international ICATM partners

Botulinum Toxin Testing

Botulinum Neurotoxin

- Produced by *Clostridium botulinum*
- Botulism: flaccid paralysis, death by respiratory failure
- Seven major BoNT serotypes (A through G)
- Severity and duration of botulism depends on the serotype
- Affects humans, other mammals, birds, and fish
- Development of BoNT antitoxin and improved clinical practice reduced mortality rate to ~10%
- BoNT potency and detection tests required for:
  - Foodborne botulism
  - Pharmaceuticals
  - Cosmetics
  - Bioterrorism threat



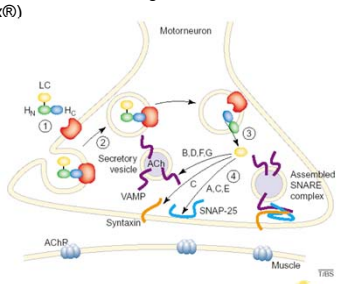
Botulinum Toxin Mouse LD50 Potency Assay

- Requires up to 300 mice to perform one potency test[1]
- Results in significant unrelieved pain and distress
  - Death due to respiratory failure due to paralysis of the respiratory muscles
  - At the highest doses, 90% of animals die
- Global pharmaceutical potency testing estimated at 600,000 mice per year[2]
- NICEATM-ICCVAM Workshop on Alternatives for the Mouse Botulinum Workshop, 2006
  - Reviewed the state of the science and provided recommendations for future progress

[1]Adler et al., 2010
[2]Bitz et al, 2010

Replacement Model for Mouse LD50 Botulinum Toxin Potency Bioassay, 2011

- Allergan Inc. BoTox® Cell-based Potency Assay[3]
  - Approved by the FDA for the testing of botulinum A products (BoTox®)
  - First-of-its-kind
- Will reduce use of animals for BoTox testing by 95%



[3]http://www.allergan.com/index.htm

Outline

- **Introduction to Alternatives**
- Laws, Regulation, and Policies
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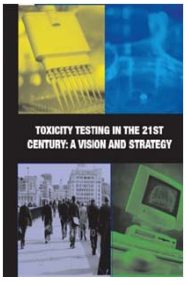
- Examples of Accepted Alternative Methods
  - Toxicity Testing
  - Vaccine Testing
- Regulatory Science Research Initiatives

Toxicity Testing in the 21st Century: NAS Report - 2007

- 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



Applying Innovative Science and Technology: The Tox21 Consortium

- NIEHS NTP, EPA, NIH NCATS, and FDA (est. 2009)
  - Goal is to characterize toxicity pathways: Expected to identify *in vitro* models/biomarkers predictive of adverse biological responses
  - New 10,000-chemical Phase II library: includes >900 NICEATM nominated chemicals; testing initiated December, 2011
  - NICEATM soliciting assay nominations for the Tox21 HTS
  - First NICEATM nominations: BG1 Luc ER TA agonist and antagonist assays adapted for testing; results under evaluation

http://iccvam.niehs.nih.gov/docs/annrpt/BiennialRpt2010-508r.pdf

Developing Future Test Methods: NIH-FDA Regulatory Science Program

- NIH and FDA partnership to foster regulatory science, announced in 2010
- Goal: To accelerate development and use of new tools, standards and approaches to efficiently develop products and to more effectively evaluate product safety, efficacy and quality
- Four grants announced September 27, 2010
  - NIH and FDA co-funded
  - $9.34M US awarded over 3 years
  - Includes grant to support an *in vitro* test battery for eye injury assessment (MB Research Labs)

Francis S. Collins, M.D., Ph.D.
NIH Director

Margaret Hamburg, M.D.
FDA Commissioner

http://commonfund.nih.gov/regulatoryscience/

Advancing Innovative Science and Technology

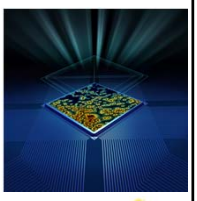
- NIH National Center for Advancing Translational Sciences (NCATS):

  Formally established in December, 2011 to catalyze innovative methods and technologies to enhance development, **testing**, and implementation of diagnostics and therapeutics across a wide range of diseases and conditions

http://commonfund.nih.gov/regulatoryscience/fundedresearch.aspx
F.S. Collins. 2011. Reengineering Translational Science: The Time is Right, *Science Translational Medicine* 3(90):1-6
http://ncats.nih.gov/index.asp

Advancing Innovative Science and Technology: Integrated 3D Multi-organ Platforms

- NIH and DOD Grants: 5 year, $140M USD ($70M each)
  - NIH partnership with Defense Advanced Research Project Agency and FDA; first awards in 2012
  - Develop "human on a chip" to screen for safe and effective drugs before tested in humans
    - 3D Micro-system organs connected by microfluidics
  - *Integrated Microphysiological Systems for Drug Efficacy and Toxicity Testing in Human Health and Disease*
  - *Stem/Progenitor Cell-Derived Human Micro-organs and -tissues*
  - *Engineering platforms and biological proof-of-concept: Microphysiological Systems*



http://commonfund.nih.gov/regulatoryscience/fundedresearch.aspx

Summary

- U.S. and International Laws and Policies require the consideration of 3Rs alternatives before animal use can be approved, and their use when determined scientifically appropriate
- NICEATM and ICCVAM provide a coordinated interagency translational infrastructure to advance new science and technology from the bench to accepted regulatory testing methods.
  - Facilitate assay standardization, optimization, validation, and regulatory acceptance
- Many new alternative test methods have now been accepted that can significantly **reduce, refine**, and **replace** animal use for safety assessments (N=51)
- The application of innovative science and technology is expected to provide improved models and integrated strategies that will further improve safety assessments and refine, reduce, and replace animal use.

Thank you for your attention.

For more information visit our website:

http://iccvam.niehs.nih.gov/

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