

THE VETERINARY PATHOLOGIST'S ROLE IN THE 3Rs

Renee R. Hukkanen, Sean Troth, and Niraj Tripathi



Webinar Overview:

- Working Group Introduction
 - Drivers for effort
- Areas of relevance for the pathologist
 - Influence on regulatory guidance
 - Study design
 - Optimizing sample collection
 - Improving animal welfare
 - Real-world Examples
- Looking to the Future
- Q&A



**Discussion is based on the collaborative efforts of a
Working Group chartered by the Scientific and Regulatory
Policy Committee (SRPC) of the Society for Toxicological
Pathology**

RENEE HUKKANEN, EISAI INC.

SEAN TROTH, MERCK

NIRAJ TRIPATHI, COVANCE LABORATORIES INC.

NOEL DYBDAL, GENENTECH

PATRICIA V. TURNER, CHARLES RIVER LABORATORIES

ADVISED BY:
THE EXECUTIVE COMMITTEE OF THE STP
LYN M. WANCKET, NAMSA



The Toxicological Pathologist Works within a Regulatory Environment

- Guidelines define:

- Group Size
- Dose Levels
- Endpoints



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH



- Pathologists may not be actively involved during the study design or in-life phase

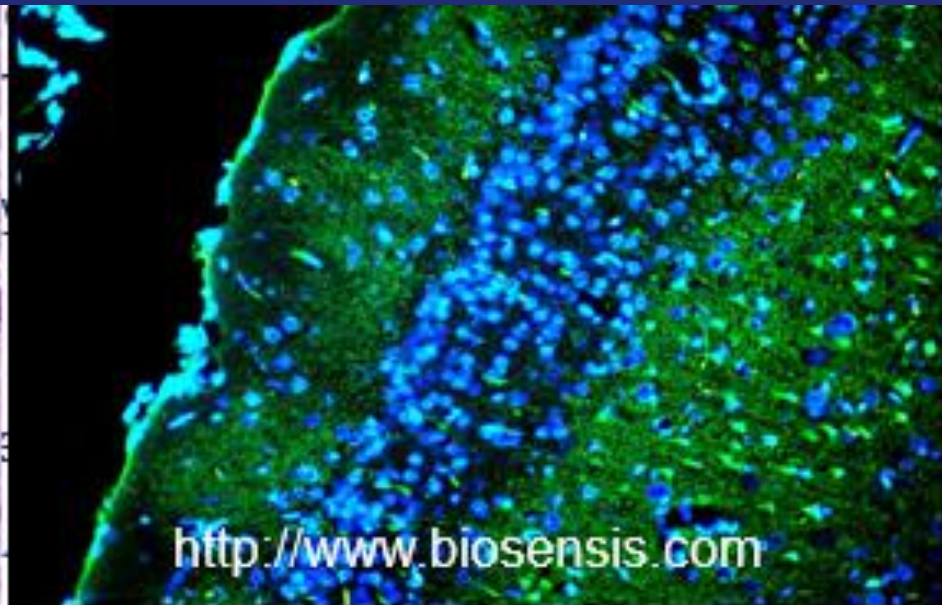
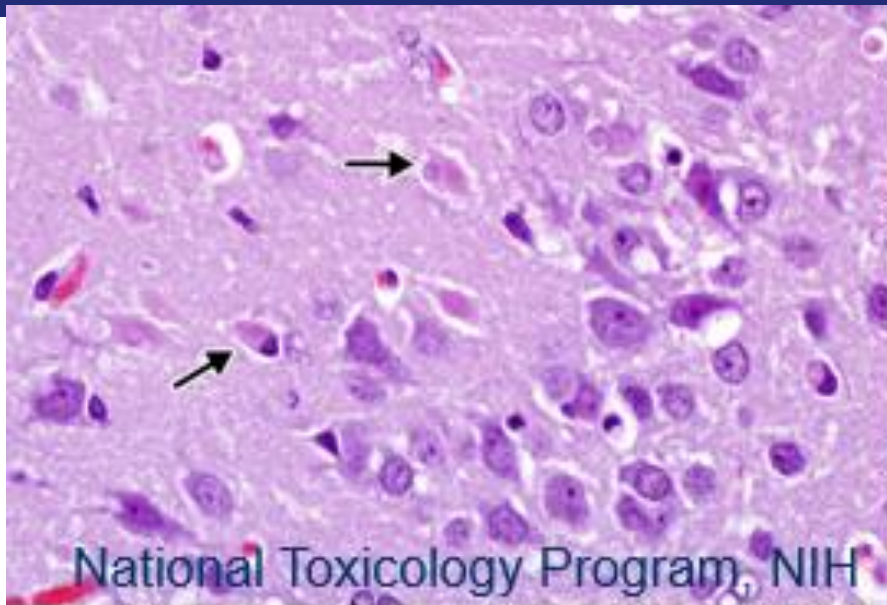
Where can we find relevance?

Opportunities for the Pathologist to Maximize Animal Value:

- Influence on regulatory guidance
- Study design
- Optimizing sample collection
- Improving animal welfare
 - Euthanasia
 - Communication
- Become involved in innovative efforts



Societal and Consortia Advocacy Efforts Can Influence Guidelines:



- Example:
 - FDA is evaluating the need to improve detection sensitivity of transient necrotizing events
 - Sensitivity of experimentally induced CNS changes in toxicological studies can be improved by:
 - interim sacrifices?
 - immunohistochemistry, stereology, fluorescence microscopy?

Best Practice Position Publications to Proactively Craft Guidance **Before** it is Written:

Scientific and Regulatory Policy Committee

STP Best Practices for Evaluating Clinical Pathology in Pharmaceutical Recovery Studies

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Lindsay Tomlinson¹, Lila Ramaiah², Niraj K. Tripathi³,
Valerie G. Barlow⁴, Allison Vitsky⁵, Florence M. Poitout-Belissent⁶,
Denise I. Bounous⁷, and Daniela Ennulat⁸

Society of Toxicologic Pathology Position Paper on Best Practices on Recovery Studies: The Role of the Anatomic Pathologist

RICK PERRY¹, GEORGIA FARRIS², JEAN-GUY BIENVENU³, CHARLES DEAN JR.⁴, GEORGE FOLEY⁵,
CHUCK MAHRT⁶, AND BRIAN SHORT⁷

¹*Pfizer, Groton, Connecticut, USA*

²*Merck Research Laboratories, West Point, Pennsylvania, USA*

³*Charles River Laboratories, Senneville, Quebec, Canada*

⁴*Amgen Inc., Seattle, Washington, USA*

⁵*AbbVie, North Chicago, Illinois, USA*

⁶*Lilly Research Laboratories, Indianapolis, Indiana, USA*

⁷*Allergan, Inc., Irvine, California, USA*



Study Design: Champion 3R's Principles While Preserving Scientific Integrity

- **Animal Number**

- Recovery Groups
- Multiple species for juvenile studies
- Parallel vs Cross Over Study Design

- **Risk Assessment and Read-Across**

- **Endpoint Integration**

Application of a novel integrated toxicity testing strategy incorporating “3R” principles of animal research to evaluate the safety of a new agrochemical sulfoxaflor

Study Design: Endpoint Determination

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition



Members of the Panel on Euthanasia

Steven Leary, DVM, DACLAM (Chair); Washington University, St. Louis, Missouri
Wendy Underwood, DVM (Vice Chair); Eli Lilly and Company, Indianapolis, Indiana
Raymond Anthony, PhD (Ethicist); University of Alaska Anchorage, Anchorage, Alaska
Samuel Cartner, DVM, MPH, PhD, DACLAM (Lead, Laboratory Animals Working Group);
University of Alabama at Birmingham, Birmingham, Alabama
Douglas Corey, DVM (Lead, Equine Working Group); Associated Veterinary Clinic, Walla Walla, Washington
Temple Grandin, PhD (Lead, Physical Methods Working Group); Colorado State University, Fort Collins, Colorado
Cheryl Greenacre, DVM, DABVP (Lead, Avian Working Group); University of Tennessee, Knoxville, Tennessee
Sharon Gwaltney-Brant, DVM, PhD, DABVT, DABT (Lead, Noninhaled Agents Working Group); ASPCA Poison
Control Center, Urbana, Illinois
Mary Ann McCrackin, DVM, PhD, DACVS (Lead, Companion Animals Working Group); Virginia Polytechnic
Institute and State University, Blacksburg, Virginia
Robert Meyer, DVM, DACVA (Lead, Inhaled Agents Working Group);
Mississippi State University, Mississippi State, Mississippi
David Miller, DVM, PhD, DACZM (Lead, Reptiles, Zoo and Wildlife Working Group); Loveland, Colorado
Jan Shearer, DVM, MS, DACAW (Lead, Animals Farmed for Food and Fiber Working Group);
Iowa State University, Ames, Iowa
Roy Yanong, VMD (Lead, Aquatics Working Group); University of Florida, Ruskin, Florida

● AVMA Guidelines for the Euthanasia of Animals

The AVMA Guidelines for the Euthanasia of Animals are intended for use by members of the veterinary profession who carry out or oversee the euthanasia of animals. The overriding commitment of these Guidelines is to provide veterinarians guidance in relieving pain and suffering of animals that are to be euthanized.

Comment on
this policy

(AVMA Members Only)

interested in
**AVMA
POLICY
CHANGES?**

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Sample Collection Can Influence Animal Use

Sample collection drives animal numbers for rodents

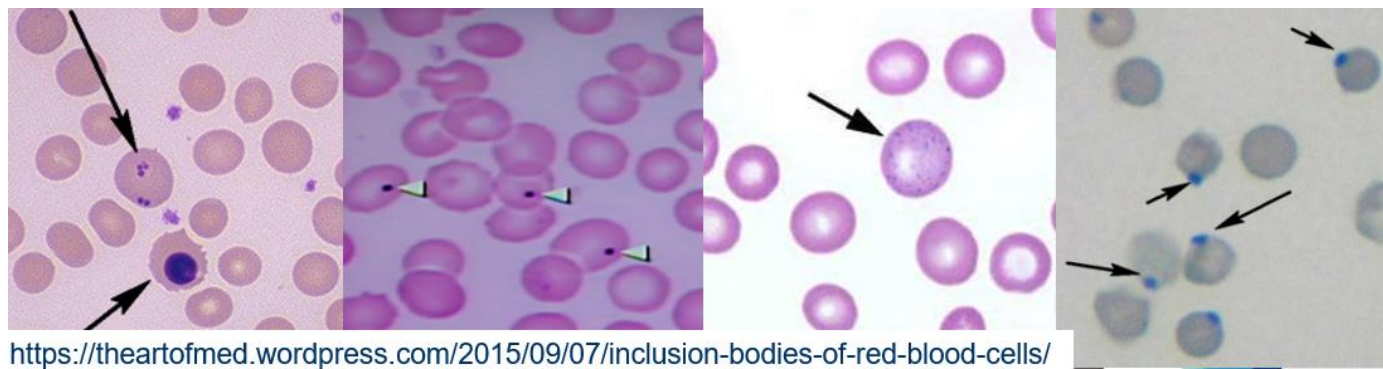
Toxicokinetic (TK)

Pharmacokinetic/pharmacodynamics (PK/PD)

Clinical pathology



- Reduce volume requirements via:
 - **Microsampling**
 - Serial sampling can avoid terminal (destructive sampling) collections
 - Improve data quality by reducing inter-animal variability
 - Large animal refinement using smaller gauge needle/smaller vessel
 - **Removal of redundant sample volume** for “back-up” when re-bleeds can be performed
 - **Use of pediatric coagulation tubes**
- Consider sampling main study animals for rats
 - Complications with exaggerated hematotoxicity



<https://theartofmed.wordpress.com/2015/09/07/inclusion-bodies-of-red-blood-cells/>

Sample Banking May Replace Animal Use

- Preservation of terminal blood and tissues requires specialized preservation and storage
 - IHC
 - Biomarker
 - EM
 - Genomics



Causes | Preventions | Treatments | Cures

- GLP Regulations should not hamper the 3R's
 - Reporting responsibilities for using tissues from GLP studies
 - Memorandum of understanding between FDA/NIH

Communication Can Bridge In-Life & Post-Mortem

- Ensure humane euthanasia
 - Communicate and advocate for best practices
 - Research community understands this to be an area of influence for the Veterinary Pathologist
- Improve husbandry and care
 - Communicate post-mortem findings
 - Mitigating confounding conditions to improve study data
 - Reduction of background and secondary lesions within tissue sections



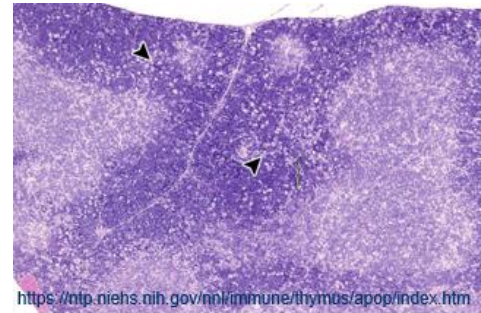
<https://www.sciencedaily.com/releases/2012/03/120330/>



<http://www.immunosystem.co.kr/sub/2products/index71.html>

“Closing the Communication Circle”

- Observation can facilitate advocacy for less invasive study procedures or improvements to housing and husbandry above standard regulatory requirements
- Examples of histological findings that should be communicated:
 - Stress
 - Sub-optimal restraint
 - Bleeding or dosing techniques
 - Husbandry-related injuries or concerns



Example 1

| Species: Study Type | In-life Observation | Histological Observation or Correlate | Impact to Study | Proposed 3Rs Improvement | Impact |
|-------------------------------------|---|--|---|--|--|
| Mouse: Sub- Chronic 90 day | Pruritis, secondary to auricular lesions | Ulcerative dermatitis and cervical lymphadenopathy | Spleen weight Clinical pathology changes confounded interpretation of treatment-related change | Instituting routine nail trims and additional enrichment | Reduced incidence and severity of skin excoriations and secondary inflammatory changes |

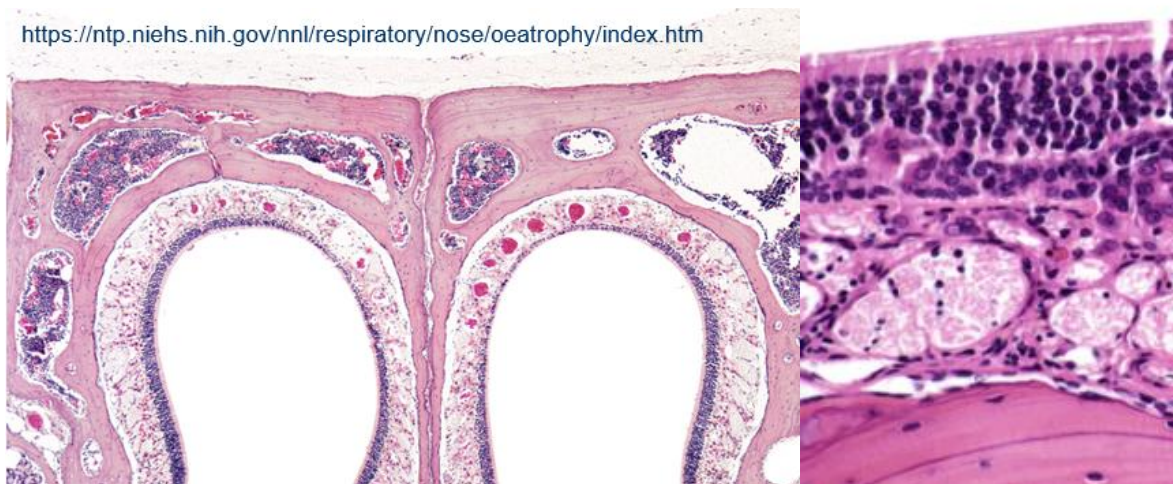


<https://med.stanford.edu/news/all-news/2016/01/toenail-trim-saves-lab-mice-from-life-threatening-skin-condition.html>



Example 2

| Species: Study Type | In-life Observation | Histological Observation or Correlate | Impact to Study | Proposed 3Rs Improvement | Impact |
|--|------------------------|---|---|---|---|
| Rat: Investigational Reproductive Study | None | Necrosis of the olfactory epithelium | Quarantine of animal room; restriction on shipment to co- investigator | Increased floor space allotted to breeding females, thereby reducing ammonia levels within the cage | Normal olfactory epithelium in subsequent studies |



Example 3

| Species: Study Type | In-life Observation | Histological Observation or Correlate | Impact to Study | Proposed 3Rs Improvement | Impact |
|------------------------|---------------------------------|---|--|--|--|
| Mouse: 7 day | Sudden death and weight loss | Esophagitis (food material within connective tissues) | Early termination and loss of study animals | Communicate finding to study-director | Retraining of technical staff reduced future incidence |

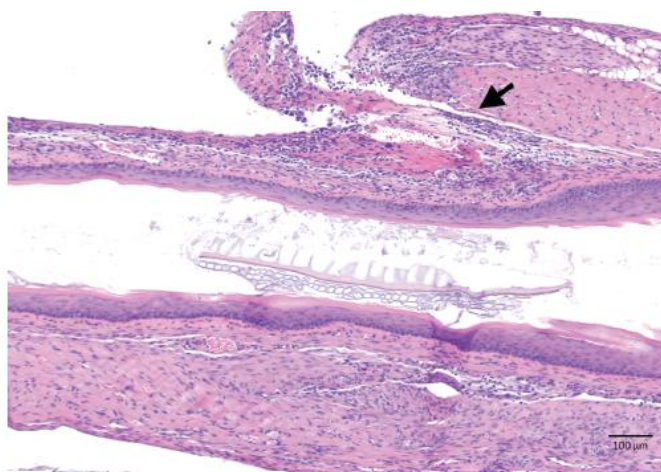
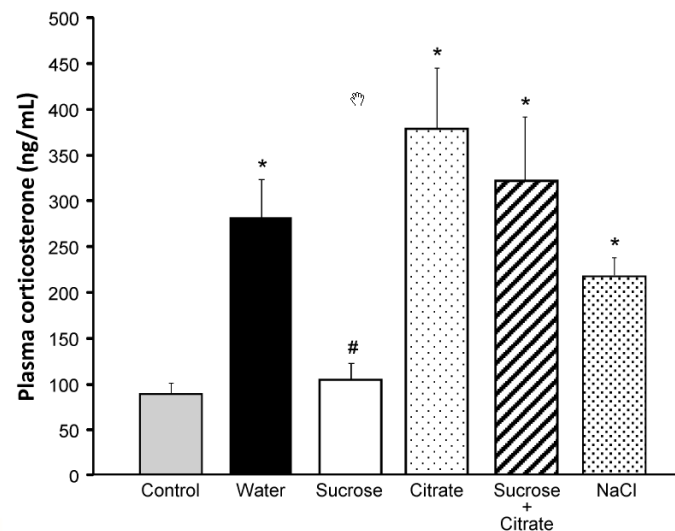


Figure 3. Esophageal histopathology of a mouse in the awake gavage group, with marked periesophagitis within the esophageal wall (arrow) suggestive of a healed, partial esophageal tear. Hematoxylin and eosin stain; magnification, 10 \times .



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Evaluation of Mice Undergoing Serial Oral Gavage While Awake or Anesthetized

Carissa P Jones,^{*} Kelli L Boyd, and Jeanne M Wallace

A Spoonful of Sugar Helps the Medicine Go Down: A Novel Technique to Improve Oral Gavage in Mice

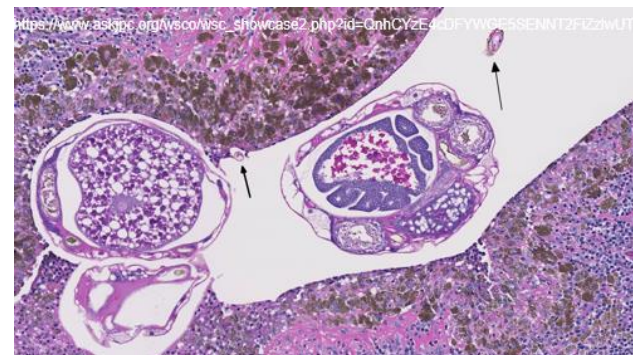
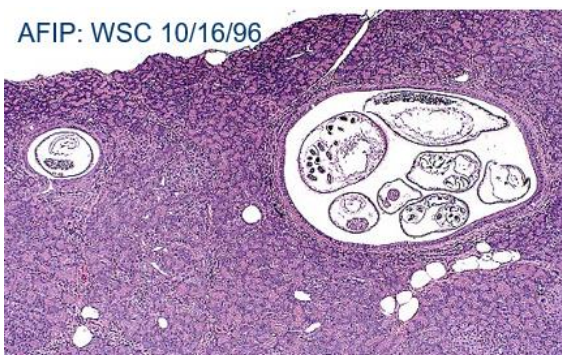
Amber F Hoggatt,^{1,2*} Jonathan Hoggatt,² Meghan Honerlaw,³ and Louis M Pelus²

Slide 16



Example 4

| Species: Study Type | In-life Observation | Histological Observation or Correlate | Impact to Study | Proposed 3Rs Improvement | Impact |
|------------------------|------------------------|---|--|---|----------------------------|
| Monkey: 28 day | None | Protozoa and nematodes were observed histologically in treated animals | Underlying test- article immune- suppression identified | Suggestions for improvement to anthelmintic protocols | Colony-wide prophylaxis |



An effective anthelmintic program includes histological evaluation!

Working for Improvements Today, While Keeping Tomorrow in Mind!

- Ultimate goal of reducing test materials entering *in vivo* testing paradigms
 - Tox21 Program
 - Toxicology Roadmap



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 - Eisai, Inc
- The audience for interest in the subject!



Back-up



Example 4

| Species: Study Type | In-life Observation | Histological Observation or Correlate | Impact to Study | Proposed 3Rs Improvement | Impact |
|------------------------|--|---|---|---|---|
| Dog: Chronic | Animals to be unwilling or uninterested in cage-front interactions | Pododermatitis | A test-article association could not be ruled-out | Improved cage flooring reduced chronic exposure to moisture; change from USDA to EU housing | Subsequent studies did not result in pododermatitis |

