Recommended ("Best") Practices for Determining, Communicating, and Using Adverse Effect Data from Nonclinical Studies

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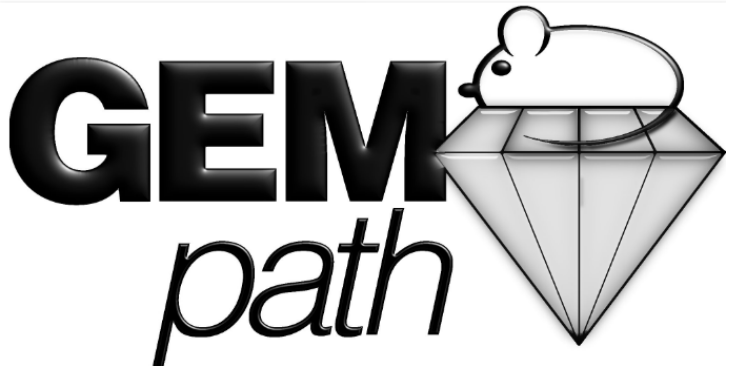
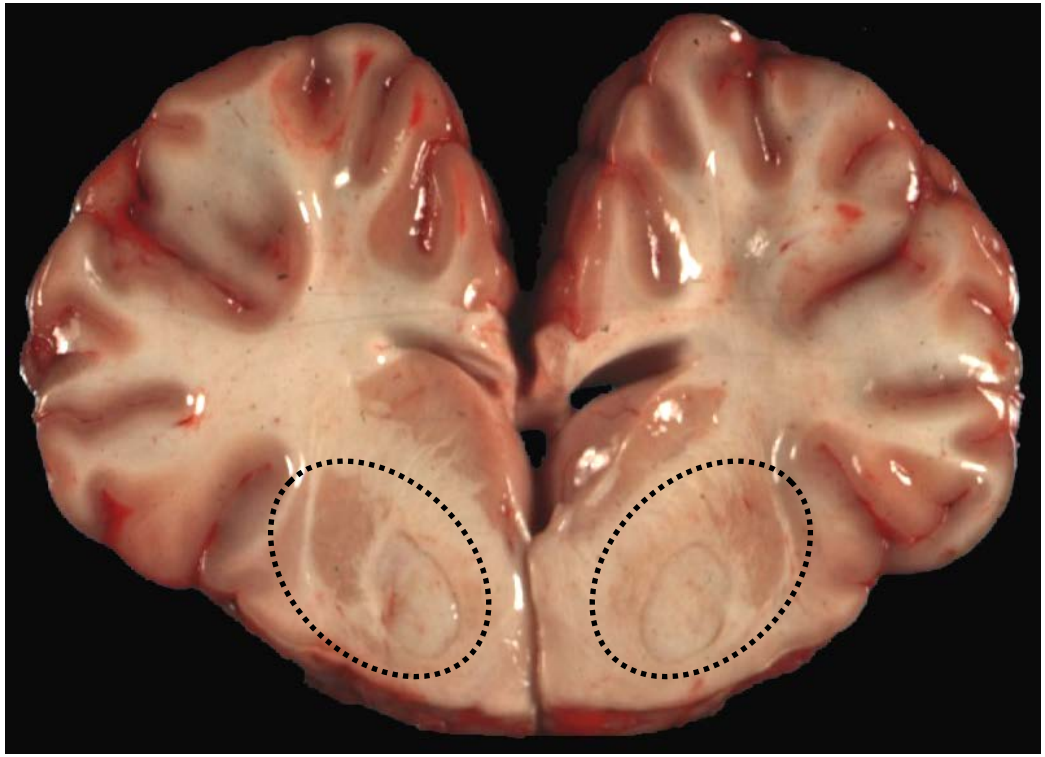


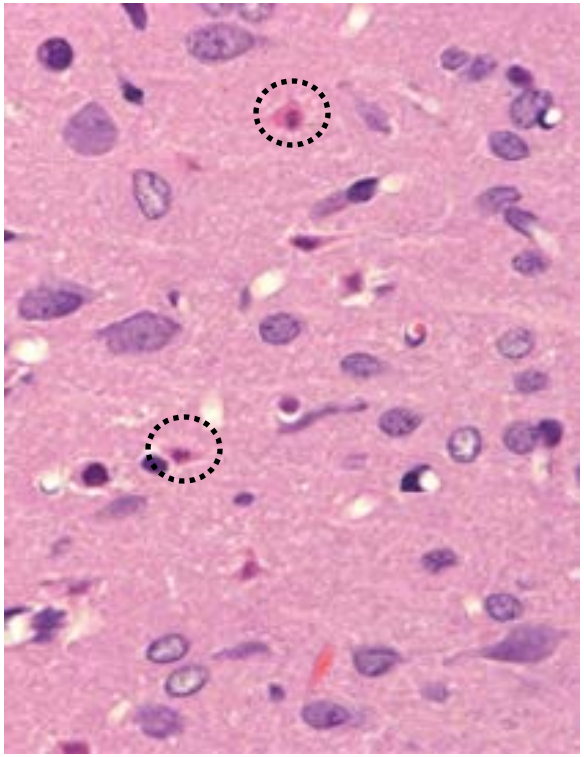
Obvious Interpretations: The Road is Clear

Trail Ridge Road, Rocky Mountain National Park, Colorado

Seeing is Believing



Equine – Yellow Star Thistle Toxicity



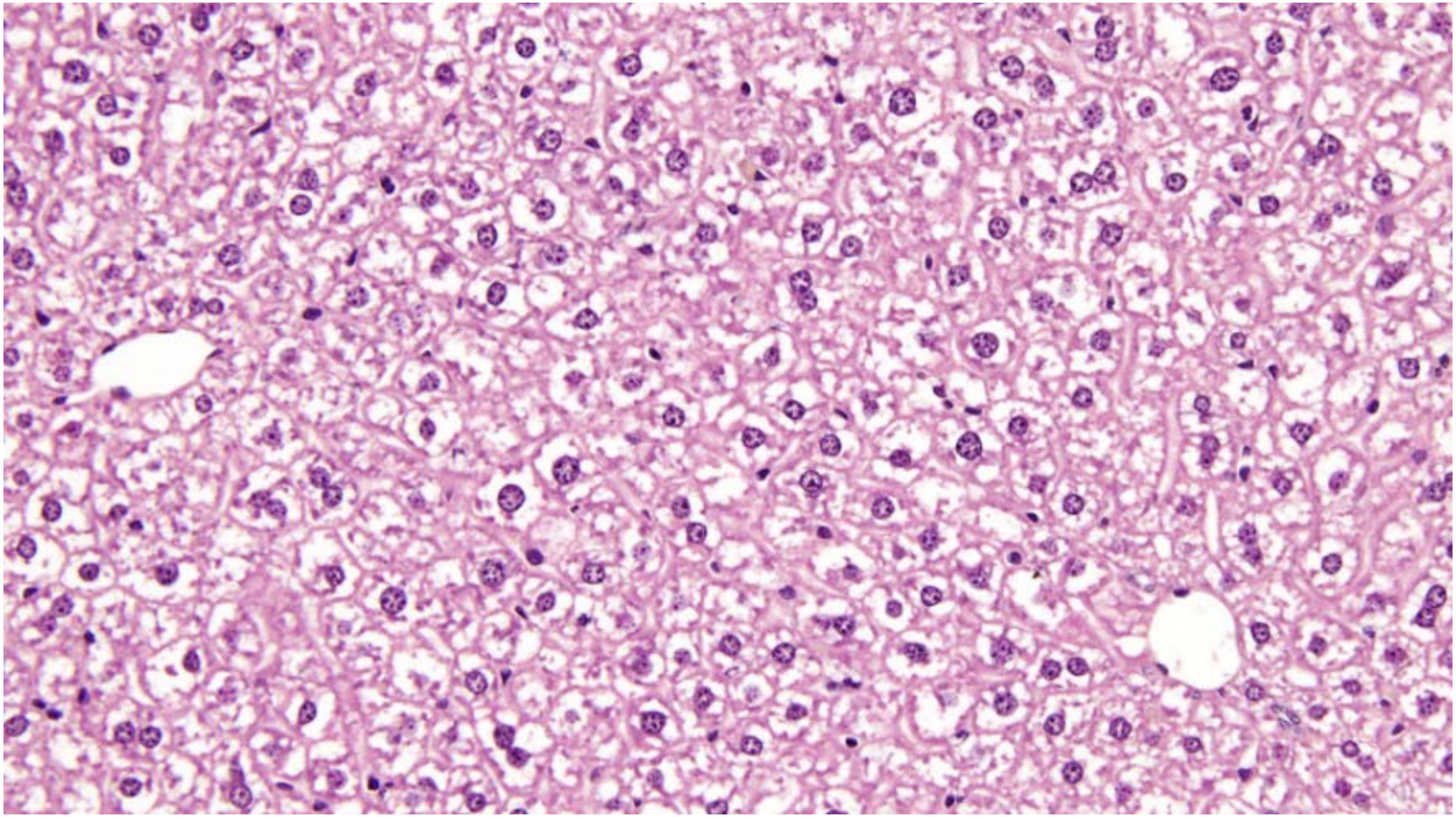
Acute Neuronal Necrosis

Noah's Arkive **(University of Georgia), Image Nos. 4942**

Shadowed Interpretations: Dangerous Curves Ahead

Mount Washington, New Hampshire

Is What We See Real?



Dog – Hepatocyte Vacuolation

Is What We Measure Real?

Species : Adult Canine
Patient :
Client :

Ver: 8.03B
Date : 04-Jun -2005 12:01

Test	Results	Reference Range	Indicator (LOW / NORMAL / HIGH)
ALB	= 2.9 g/dl	2.2 - 3.9	
ALKP	= 136 U/L	23 - 212	
ALT	= 48 U/L	10 - 100	
AMYL	= 887 U/L	500 - 1500	
BUN	= 13 mg/dl	7 - 27	
Ca	= 9.9 mg/dl	7.9 - 12.0	
CREA	= 0.9 mg/dl	0.5 - 1.8	
GLU	= 123 mg/dl	74 - 149	
LIPA	= 613 U/L	200 - 1800	
PHOS	= 3.0 mg/dl	2.5 - 6.8	
TBIL	= 0.3 mg/dl	0.0 - 0.9	
TP	= 6.2 g/dl	5.2 - 8.2	
GLOB	= 3.3 g/dl	2.5 - 4.5	

Dog – “Normal” Serum Chemistry Panel


LOST
CONFUSED
UNSURE
UNCLEAR
PERPLEXED
DISORIENTED
BEWILDERED

Seminar Objectives

- **A brief introduction to the recently published "best practice" recommendations for determining, communicating, and using "adversity" and the "no observed adverse effect level" (NOAEL)**
- **"Adversity" and the "NOAEL"**
  - **Why are these concepts problematic?**
  - **How can we most simply and reliably mitigate the problems?**
- **Determining "adversity" and "NOAEL"**
- **Communicating "adversity" and "NOAEL"**
- **Other factors to consider in decisions regarding adversity**

A Brief Introduction to the 2016 STP "Best Practice" Recommendations for Determining, Communicating, and Using Adversity and the NOAEL

Scope of the STP "Best Practice" Recommendations on Adversity

- The concepts in this talk represent final recommendations formulated by the Society of Toxicologic Pathology's (STP) Adversity Working Group
- The recommendations were formulated based on input of numerous toxicologic pathologists belonging to the STP and allied toxicologic pathology societies around the globe
- These recommendations were endorsed by the STP; the societies of toxicologic pathology from Britain, Europe, France, and Japan; the American College of Veterinary Pathologists (ACVP); the American Society for Veterinary Clinical Pathology (ASVCP); and the American College of Toxicology (ACT)

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Scientific and Regulatory Policy Committee: Recommended ("Best") Practices for Determining, Communicating, and Using Adverse Effect Data from Nonclinical Studies

Roy Kerlin[1], Brad Bolon[2], John Burkhardt[3], Sabine Francke[4], Peter Greaves[5], Vince Meador[6], and James Popp[7]

Abstract

Recommendations (best practices) are provided by the Society of Toxicologic Pathology's Adversity Working Group for making consistent interpretations of test article–related effects as "adverse" and assigning a "no observed adverse effect level" (NOAEL) in nonclinical toxicity studies. Adverse is a term indicating "harm" to the test animal, while nonadverse indicates lack of harm. Adverse findings in the study reports should be defined in relation to effects on the test species used and within the context of the given study. Test article–related effects should be described on their own merits, and decisions to consider them as adverse or non-adverse should be justified. Related effects may be discussed together; in particular, markers of toxicity that are not in and of themselves adverse ideally should be discussed in conjunction with the causal toxicity to determine adversity. Adverse findings should be identified in subreports (clinical data, pathology data, etc.) if sufficient information is available, and/or in the final study report as individual or grouped findings, but study NOAELs should be established at the level of the overall study report. Interpretations such as "not biologically relevant" or "not toxicologically important" should be avoided unless defined and supported by scientific rationale. Decisions defining adverse findings and the NOAEL in final study reports should combine the expertise of all contributing scientific disciplines. Where possible, use of NOAELs in data tables should be linked to explanatory text that places them in context. Ideally, in nonclinical summary documents, NOAELs from multiple studies are considered together in defining the most important adverse responses in the most sensitive species. These responses are then considered along with an understanding of their likely mechanisms, as well as other information such as variability in species sensitivity, comparative pathology, reversibility and progression, kinetics, and metabolism of the test substance to help assess human risk.

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Characterizing "Adversity" of Pathology Findings in Nonclinical Toxicity Studies: Results from the 4th ESTP International Expert Workshop

Xavier Palazzi[1], John E. Burkhardt[2], Henri Caplain[1], Vicki Dellarco[3], Pierluigi Fant[4], John R. Foster[5], Sabine Francke[6], Paul Germann[7], Sibylle Gröters[8], Takanori Harada[9], Johannes Harleman[10], Kosei Inui[11], Wolfgang Kaufmann[12], Barbara Lenz[13], Hirofumi Nagai[14], Gabriele Pohlmeyer-Esch[15], Agnes Schulte[16], Mikala Skydsgaard[17], Lindsay Tomlinson[2], Charles E. Wood[18], and Midori Yoshida[19]

Abstract

The identification of adverse health effects has a central role in the development and risk/safety assessment of chemical entities and pharmaceuticals. There is currently a need for better alignment regarding how nonclinical adversity is determined and characterized. The European Society of Toxicologic Pathology (ESTP) therefore coordinated a workshop to review available definitions of adversity, weigh determining and qualifying factors of adversity based on case examples, and recommend a practical approach to define and characterize adversity in toxicology reports, to serve as a valuable prerequisite for future organ- or lesion-specific workshops planned by the ESTP.

Adversity and the No Observed Adverse Effect Level (NOAEL)

The Problem with Adversity and the NOAEL

- **No universally acknowledged, one-size-fits-all definition of "adversity" exists among all stakeholders**
- **Accordingly, no common meaning can be defined for the term "no observed adverse effect level" (NOAEL)**
- **Inconsistent definitions prevent clear communication, and therefore impede data interpretation and risk assessment**

STP ‘Best Practice’ Recommendation No. 1

“Adversity” is a term indicating “harm” to the test animal.

Rationale:

- **Not all changes are harmful (i.e., capable of detrimentally impacting an animal’s performance or lifespan, either under normal conditions or if challenged)**
- **Only harmful changes are adverse**
- **Test article-related changes that are observed but not harmful are “non-adverse”**

Determining
“Adversity” and the “NOAEL”

STP 'Best Practice' Recommendation No. 2

The decision about whether test article-related effects (or a group of related effects) in a nonclinical study are considered "adverse" or "non-adverse" should be unambiguously stated and justified in sub-reports and/or the study report.

Rationale:

- **A decision regarding a designation of adversity represents an interpretation**
- **Accordingly, the reasons for the interpretation need to be explained logically and thoroughly**

STP ‘Best Practice’ Recommendation No. 3

“Adversity” as identified in a nonclinical study report should be applied only to the test species and under the specific conditions (dose, duration, etc.) used in the study.

Rationale:

- **Studies are conducted in a particular species**
- **Some effects that can be modulated by treatments actually represent exacerbations of incidental background changes that have no known counterpart in humans**

STP 'Best Practice' Recommendation No. 4

Effects on cells, tissues, organs, or systems within the test animal should be assessed on their own merit.

Rationale:

- **Adversity decisions should be based on actual observations and not speculation concerning:**
  - **their possible pathogenesis**
  - **the potential relevance of changes in predicting risk to humans**
- **The conservative approach is to fully disclose the complete spectrum of biological changes:**
  - **Supra-physiologic (on target) effects – often deemed "non-adverse"**
  - **Toxic (off target) effects – generally considered adverse**

Communicating “Adversity” and “NOAEL”

STP 'Best Practice' Recommendation No. 5

Communication of "adversity" and assignment of the "NOAEL" in the overall study report should be consistent with, and also supported by, information in the study sub-reports.

Explicate test article-related changes as follows:

- **Document them in detail, in sub-reports and the study report**
  - **whether or not they are deemed adverse or non-adverse**
  - **regardless of their presumed pathogenesis or human relevance**
- **Assign the NOAEL for the whole study, and not sub-reports**
- **Use ambiguous statements ("not biologically relevant" or "not toxicologically important") only if explained in detail**

STP ‘Best Practice’ Recommendation No. 6

Communication of adverse findings and the NOAEL should include direct interaction among staff in different scientific disciplines.

Rationale:

- **A single toxicity may manifest in different ways to scientists in distinct disciplines, and thus be presented uniquely in the various sub-reports**
- **The complete picture of a test article-related effect requires integration of these various views within the study report**

STP 'Best Practice' Recommendation No. 7

The NOAEL for a test article should be communicated in an overview document based upon data from multiple studies.

Rationale:

- **Integration is necessary because a NOAEL identified in one study may be discounted as irrelevant within an overview document based on data from another study**
- **Selection of the NOAEL in the most sensitive species is made using data from many studies in at least two species**

STP 'Best Practice' Recommendation No. 8

In order to place them in proper context, the use of NOAELs in data tables should be linked to appropriate explanatory text.

Rationale:

- **Nuances in the text often provide critical insight regarding the NOAEL**
- **Placement of NOAEL values inside tables without such access to nuances may obscure the reasons used to set them**

Assessing Potential Human Risk

STP 'Best Practice' Recommendation No. 9

Nonclinical scientists, including pathologists, toxicologists, and other contributing subject matter experts who interpret data from nonclinical studies should actively participate in assessing and communicating human risk.

Rationale:

- **Individuals who generate sub-reports are best equipped to explain the data set and its interpretation**
- **Nonclinical scientists from many disciplines provide valuable insights in**
  - **helping the Study Director weigh the evidence and set the NOAEL**
  - **advising the clinical research team with respect to initial dose setting**

STP ‘Best Practice’ Recommendation No. 10

All available data from all nonclinical studies must be evaluated together to define potential toxicities and predict human risk.

Rationale:

- **Experiments designed to understand the pathogenesis of a finding in nonclinical studies may profoundly influence the risk profile of a test article**
- **Assessments of human risk should be based on the entire portfolio (nonclinical and clinical data from all studies, literature for structurally related or similar acting agents)**

Other Factors to Consider in Decisions Regarding Adversity

What Other Parameters may Affect an Adversity Decision?

- Adverse ("harmful") effects may be reversible (liver necrosis) or irreversible (neuronal necrosis), so reversibility alone is not a sufficient reason to dismiss an otherwise harmful finding as non-adverse

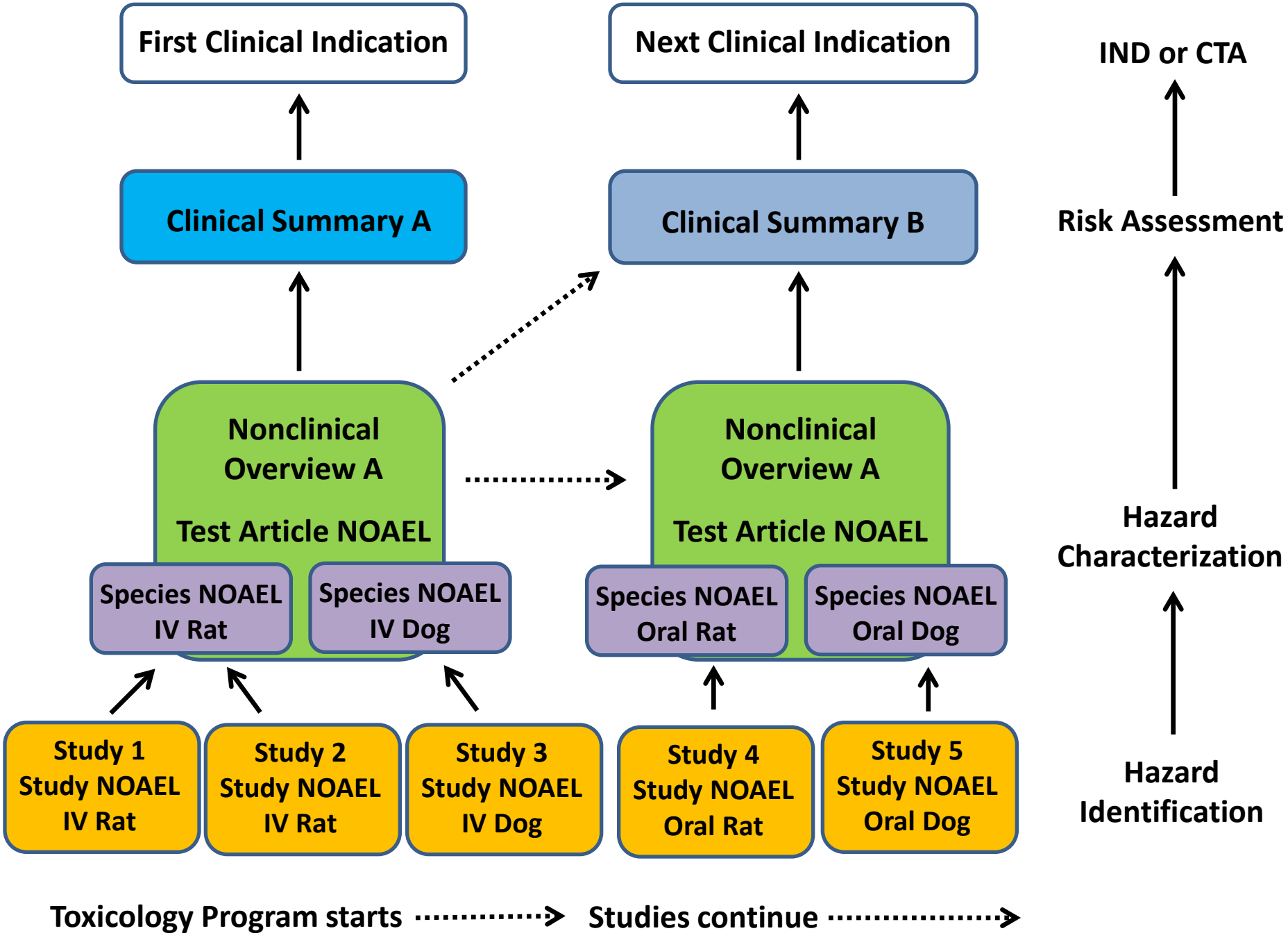
- The presence of premonitory biomarkers for an adverse finding (e.g., high serum activities of hepatocyte cytosolic enzymes) typically should be associated with the actual adverse finding (hepatocyte necrosis) to establish adversity in a study report

The Regulatory Perspective on Adversity Decisions

- **Regulatory agencies will make their own decisions regarding adversity and/or the NOAEL, using a sponsor's interpretation for guidance**

- **Accordingly, regulatory agencies prefer large data sets for which full explanations and justifications are recorded in unambiguous fashion giving the rationale for the sponsor's interpretation and risk assessment**

Adversity and the NOAEL in Practice


First Clinical Indication
Next Clinical Indication
IND or CTA
Clinical Summary A
Clinical Summary B
Risk Assessment
Nonclinical Overview A
Test Article NOAEL
Nonclinical Overview A
Test Article NOAEL
Hazard Characterization
Species NOAEL IV Rat
Species NOAEL IV Dog
Species NOAEL Oral Rat
Species NOAEL Oral Dog
Study 1 Study NOAEL IV Rat
Study 2 Study NOAEL IV Rat
Study 3 Study NOAEL IV Dog
Study 4 Study NOAEL Oral Rat
Study 5 Study NOAEL Oral Dog
Hazard Identification
Toxicology Program starts
Studies continue

Establishing Adversity and the NOAEL in a Nonclinical Study Report

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A nonclinical study in which nonhuman primates were given a monoclonal antibody exhibited the following pattern of test article-related changes:

- **High dose – diffuse mammary gland atrophy in all animals**
- **Mid dose – multifocal lobular atrophy in some animals**
- **Low dose – multifocal lobular atrophy in a few animals**
- **Control – rare lobular atrophy in rare animals**

How would you establish adversity?

Reporting Adversity and the NOAEL in a Nonclinical Study Report

- High dose – diffuse mammary gland atrophy = adverse
  - A profound and highly unusual structural change
  - So extensive that function would be predicted to be altered
- Mid dose – multifocal lobular atrophy = non-adverse
  - The incidence of the finding is elevated, indicating that it is related to test article administration
  - The extent of the finding is limited, suggesting that function will not be altered
- NOTE: Unsupported speculation discounting the change as a secondary effect of test article-induced hormonal fluctuations is conjectural and thus should not affect the decision regarding whether or not the effect is adverse

Establishing Adversity and the NOAEL in a Nonclinical Overview Document

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A series of nonclinical studies for a novel monoclonal antibody revealed the following pattern of test article-related changes:

- **Mouse – mammary gland atrophy is dose-dependent**
  - **High dose – diffuse mammary gland atrophy in all animals**
  - **Mid dose – multifocal lobular atrophy in some animals**
  - **Low dose – multifocal lobular atrophy in a few animals**
  - **Control – rare lobular atrophy in rare animals**
- **Monkey – mammary gland atrophy is extremely limited**
  - **Incidence – rare (occurs sporadically at all doses)**
  - **Severity – minimal (at all doses)**

How would you establish adversity?

Reporting Adversity and the NOAEL in a Nonclinical Overview Documents

- Mouse – mammary gland atrophy = adverse
  - Limited to the high dose, where the change is diffuse and occurs in all animals
  - The interpretation applies to the mice in this study
- Monkey – mammary gland atrophy = non-adverse
  - Occurs rarely at all doses, and is of minimal degree
  - The interpretation applies to the monkeys in this study
- In the overview document, mammary gland atrophy may be speculated to be a possible risk in humans, but its lack in monkeys may render it a lower matter of concern

What About Adaptive Responses?

- **Adaptation = the process whereby a cell or organism responds to a xenobiotic so that it will survive without impaired function (*Toxicol Sci* 126: 291-297, 2012)**

- **Common examples**
  - **Increased production of cytochromes P450**
  - **Parenchymal hyperplasia**

- **Adaptation may lead to other changes that may be adverse**
  - **Decreased β-oxidation leading to lipid accumulation in hepatocytes (a consequence of increased cytochromes P450 production—but also of chronic over-nutrition or longstanding under-nutrition)**
  - **Parenchymal metaplasia / dysplasia (a possible outcome of long-term epidermal hyperplasia)**

Adaptation vs. Adversity: Drawing the Line

A nonclinical study in which rats were given a small molecule exhibited the following pattern of test article-related changes:

- **High dose**
  - **ALT and AST activities higher by 10x and 3x control values, respectively**
  - **Liver weights increased by 45%**
  - **Marked centrilobular hepatocyte hypertrophy with moderate centrilobular lipid accumulation and scattered single cell necrosis and occasional focal necrosis**
- **Mid dose**
  - **ALT activity higher by 3x, but AST activity within normal limits**
  - **Liver weights increased by 8%**
  - **Minimal to mild centrilobular hepatocyte hypertrophy with minimal lipid accumulation and rare single cell necrosis**
- **Low dose**
  - **ALT and AST activities within normal control limits**
  - **Liver weights increased by 3%**
  - **No significant histopathologic changes**

Establishing Adversity and the NOAEL in the Nonclinical Study Report

- **Low dose**
  - **Slightly increased liver weight (consistent with increased P450 enzyme induction)**
  - **Non-adverse since liver histology and liver enzyme activities were normal**
- **Mid dose**
  - **Mildly increased liver weight with a small rise in ALT activity (consistent with increased P450 enzyme induction)**
  - **Non-adverse because the degree of centrilobular hepatocellular hypertrophy (minimal to mild) and rare single cell necrosis is consistent with the pathogenesis and does not cause harm to the animal**
- **High dose**
  - **Marked increases in liver weights and ALT and AST activities (consistent with increase in P450 enzyme induction)**
  - **Related findings include moderate lipid accumulation (a potential degenerative effect) and scattered presence of focal necrosis (definitive evidence of lethal cell damage)**
  - **Adverse because, whether this pattern represents extreme adaptation or an additive effect of adaptation with low-level hepatotoxicity, the test animals are harmed**

Relevance of Clinical Pathology

A nonclinical study in which rats were given a small molecule exhibited the following pattern of test article-related changes:

- **High dose**
  - **BUN and creatinine levels higher by 5x and 3x, respectively, relative to control values**
  - **Urine specific gravity is 1.010 (isosthenuria = inability to concentrate urine)**
  - **Mild to occasionally moderate epithelial necrosis affecting proximal tubules in cortex**
- **Mid dose**
  - **BUN and creatinine levels higher by 2x and 1.5x, respectively, relative to control values**
  - **Urine specific gravity is 1.020**
  - **No significant histopathologic changes**
- **Low dose**
  - **BUN and creatinine levels within normal control limits**
  - **Urine specific gravity is 1.025**
  - **No significant histopathologic changes**

Establishing Adversity and the NOAEL in the Nonclinical Study Report

- **Low dose – non-adverse**
  - **Clinical pathology values (BUN, creatinine, urine specific gravity) were normal**
  - **Renal histology was unremarkable**
- **Mid dose – subject to interpretation**
  - **Minimally increased BUN and creatinine levels with "normal" urine specific gravity**
  - **Renal histology was normal**
- **High dose – adverse**
  - **Substantially higher BUN and creatinine levels with reduced urine specific gravity**
  - **Mild to sometimes moderate renal tubular necrosis**

Exacerbation of Background Findings May be Considered Adverse in Some Cases

- **<u>Exacerbation</u> = increase in incidence and/or severity of an age-related and/or strain-specific background change that often may be seen in control animals**

- **Situations in which exacerbation might be deemed adverse:**
  - **The increase is a biologically plausible primary or secondary effect that follows test article exposure**
  - **The increase shows a clear dose-response**
  - **The increase falls outside the historical control range**

The Future of Adversity Determination?

- **The Tox21 initiative is seeking to move away from animal testing in favor of measuring molecular pathway changes**
- **Molecular methods have yet to be validated in most cases, so adversity decisions will continue to rely on data gotten from conventional animal testing for the foreseeable future**

Basic Messages

- **Decisions about adversity and the NOAEL in a nonclinical study report should depend on effects seen in the test species**
- **Descriptions regarding potential relevance to humans or based on a speculative pathogenesis are not needed to interpret the data set for a single nonclinical study but may be included to position the findings in a nonclinical overview document**
- **Whatever the interpretation, the sponsor should articulate the justification for the decision clearly (generally in a nonclinical overview document) so that clinicians and regulators will be able to unambiguously understand the rationale**

If confusion is the first step to knowledge, I must be a genius….

Larry Leissner