# Adversity in Nonclinical Reporting: Myths, Legends, and Reality

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# Outline

## Adversity in Nonclinical Reporting: Myths Legends and Reality

- Introduction
- Problem statement, and history
- The Society of Toxicologic Pathology response
- Best practices/recommendations for Adversity/NOAEL
- Case studies and examples

# Adversity and the NOAEL
# The Problem Statement

- Determination of whether a test article effect is “adverse” in nonclinical reports has too frequently resulted in confusion and misunderstandings over the past 40 years

- Standardization of basic principles related to “adversity” and the consequent designation of the “No Adverse Effect Level (NOAEL)” could vastly improve the communication of nonclinical study results to clinicians and regulators

# What are the consequences of inconsistent use of the terms “Adverse” and “NOAEL”

- Reports may be difficult to understand
  - Summary Documents may have to deal with inconsistently applied adverse calls between subreports and main report
  - May be hard to summarize and to logically articulate test article effects with respect to dose, exposure and organ systems affected

- Differences in approaches and opinions related to using adverse effect data and NOAEL may affect the assessment of human risk by regulators who may apply more conservative approaches to human dosing than otherwise necessary with better prepared discussion and application of the concepts of adversity

# Literature and Historical View of Adversity and NOAEL

- Multiple and sometimes conflicting definitions of "adversity" have been provided in the literature (references included in the STP publication)

- Decisions about "adversity" are predicated on:
  - Objective assessment of data, including statistics
  - Subjective evaluation of data (consideration of broader knowledge of biology including tissue, cellular and molecular understanding of responses to xenobiotics)
  - Professional judgment relying on either or both objective and subjective assessment of data

# What Do You Mean by "Professional Judgment"

- Isn't this just an excuse to say what you want in order to favor the drug and progress development?
  - No!!
    - Determination of "adverse" or nonadverse" effects MUST be accompanied by clear and compelling logic regarding the basis on which the decision was made

- For example, a slight, but statistically significant change in a parameter with a wide dynamic range (e.g. WCC in nonhuman primates), and which is inside normal parameters for that effect is likely not to be considered adverse – in fact it may not even be a test article effect
  - But the reasoning must be explained clearly in the text
  - Another example may be that all the controls were unusually low giving apparently elevated values for test article animals

# “Professional Judgment” (continued)

- Similarly, a non statistically significant effect may be considered test article related and even adverse
  - If the effect is very rarely seen in historical controls, but the low “n” created a difficulty in allowing a test article effect to reach statistical significance
  - An example may be a low platelet count with petechiae in two animals out of 6, but with a wide range in controls that prevented statistical significance.
    - Would this change your opinion if the compound had a precedented effect on platelet number and function?
    - What about if there was NO effect on platelets known about for this drug target??
    - Other considerations

- A “gut feeling” is not enough to discount the application of adversity to a test article effect
  - Outline your logical argument

# The Society of Toxicologic Pathology (STP) initiative began in 2013

- To establish the current “best practice” around the assessment of adversity and then develop recommendations for identifying, communicating, and utilizing adverse effects in nonclinical studies

- The intent was to improve communication with regulators and clinicians through more effective and efficient reporting of nonclinical data and interpretations

# Society of Toxicologic Pathology
# Adversity Working Group

| | |
|---|---|
| Brad Bolon | GEMpath Inc. |
| John Burkhardt | Abbvie (Pfizer, from Jan 2016) |
| Sabine Francke | U.S. Food and Drug Administration |
| Peter Greaves | Leicester Royal Infirmary (UK) |
| Roy Kerlin (Chair) | Pfizer |
| Vince Meador | Covance |
| James Popp | Stratoxon |

# STP Scientific and Regulatory Policy Committee

## Recommended ("Best") Practices for Determining, Communicating, and Using Adverse Effect Data from Nonclinical Studies *(Toxicologic Pathology 2016, 44(2):147-162)*

- E-Published in December, 2015
- Sponsored and supported by the Society of Toxicologic Pathology,
- The published manuscript is also endorsed by:
  - The American College of Veterinary Pathologists,
  - The American Society for Veterinary Clinical Pathology,
  - Société Française de Pathologie Toxicologique" (French Society of Toxicologic Pathology),
  - The British Society of Toxicologic Pathology,
  - The European Society of Toxicologic Pathology,
  - The Japanese Society of Toxicologic Pathology,
  - The American College of Toxicology.

# Recommendations

- Recommendations of the STP Adversity Working Group are presented in the order they apply in the course of performance and communication of results from a nonclinical study
- Three subsections of recommendations
  - Determining “Adversity” and “NOAEL”
  - Communicating “Adversity” and “NOAEL”
  - Using “Adversity” and “NOAEL” in assessing human risk

# Recommendation 1
## (Determining "Adversity" and "NOAEL")

***"Adversity" is a term indicating "harm" to the test animal***

within the constraints of a given study design (dose, duration etc.)

- The inference is that not all test article-related effects are harmful
- Only harmful changes are adverse
- Test article-related changes that are not harmful are "non-adverse"

# Recommendation 1 (continued)
## (Determining "Adversity" and "NOAEL")

- Some suggest that if an effect is reversible it is nonadverse. However, many reversible effects can be adverse, such as necrosis in a parenchymal organ without damage to infrastructure
  - Therefore, logic dictates that reversibility *per se*, cannot establish whether a finding is adverse or nonadverse

- However, reversibility data can be useful for confirming an assessment of adversity
  - For example a lack of reversibility may indicate infrastructure damage that was not evident in the initial examination

# Recommendation 1 (continued)

**(Determining "Adversity" and "NOAEL")**

- Note that human risk assessment is not a part of adversity determination
  - Something that leads to mortality in rats may be shown subsequently to be due to a species specific effect that does not occur in other species tested, and thereby unlikely to occur in humans. However it is still considered to be adverse in the rats (i.e. mortality is harmful)

- Human risk relevance can certainly be important discussion points within a study report to help inform risk assessments made in overview documents to support an IND. For example, reversibility may impact the subsequent assessment of human risk.
  - But this cannot enter into the decision about whether the test article effect was adverse in the species being tested

# Recommendation 2
**(Determining "Adversity" and "NOAEL")**

***The decision about whether or not 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***

The decision about determination of adversity is an interpretation based on both objective and subjective evaluation

- The decision should be explained clearly, concisely, accurately and completely

# Recommendation 2 (continued)
## (Determining "Adversity" and "NOAEL")

- Although there should be no ambiguity in a report about whether or not any given effect is related to the test article administration, or whether it is adverse or not, there are some important aspects to this communication
  - Each test article effect does not need to be separated out and explained in isolation, when it really belongs to a spectrum of effects due to a single cause
    - Example, liver necrosis with effects on liver weight, histo observations, AST. ALT, Alk Phos etc….
  - Small effects of unknown cause can be lumped together and explained away: For example:
    - As small changes in electrolytes associated with minor nonadverse body weight loss and inanition at the end of a 1 month study
    - Or alternatively as small effects on electrolytes associated with adverse diarrhea

# Recommendation 2 (continued)
## (Determining "Adversity" and "NOAEL")

Clinical Pathology endpoints are worthy of further discussion

- Some changes in clin path endpoints can be adverse in and of themselves
  - Large decreases in erythroid mass
  - Large decreases in platelets

- Other clin path end points are not adverse themselves, but are markers of a potentially adverse effect.
  - Circulating ALT, AST, etc., are not adverse chemicals *per se*, but are markers of cellular damage, and could, for example indicate an adverse effect on hepatocytes. In these cases, the assignment of adversity is to the proximate effect, while clearly associating the markers

# Recommendation 3

**(Determining "Adversity" and "NOAEL")**

***"Adversity" as identified in a nonclinical study report should be applied only to the test species and under conditions of the study***

- As indicated in a previous slide, study report should be addressing the observations only in regards to the potential harm to the species in the study and is not predicated on extrapolation to potential for human risk, which is dealt with using a broader data set in overview documents

- Extrapolation to potential for greater effects in future studies should not be used to assess adversity in the current study
  - For example, hyperplasia should not be called adverse simply because of the possibility that it may lead to neoplasia in a future study. Such considerations may be critical to decisions in drug development, but are not appropriate for driving adversity decisions.

# Recommendation 3 (continued)
## (Determining "Adversity" and "NOAEL")

- Some effects in treated animals may represent exacerbations of species-specific background lesions
  - The issue of species specificity of an effect may be discussed in a report, but should not be used to define whether or not an effect is adverse. This call should be made on the basis of whether or not the effect is harmful to the animal species under test

- Why should a species specific effect be considered adverse if it is not relevant to humans?
  - Because in most cases we really don't know whether a particular lesion or group of findings are due to the species specific effect, or if they are really a *de novo* effect of the test article that mimics this
    - Is it really rat cardiomyopathy or is it primary cardiotoxicity that in rats is expressed like the background rat lesion?

# Recommendation 3 (continued)
## (Determining "Adversity" and "NOAEL")

- An example is a study where there appears to be an exacerbation of Murine Chronic Progressive Nephropathy due to the test article. A report may dismiss this as due to the effect of general debilitation at the high dose causing an increased frequency or severity of this change
  - But is this really the case, or could the compound be a primary tubular toxin, which has a minor effect in rats, but which results in an exacerbation of CPN as a **rat-specific response** that may really be a risk to humans at high doses
  - This may be refuted or given support depending on the effects in a second species, reinforcing the need to deal with such speculation in overview documents, and maintain the independence of each separate study report
  - Importantly many reports dismissing "species specific" effects fail to consistently document the many studies with similarly debilitated rats which do NOT show an exacerbation of CPN (or other 'species specific change)

# Recommendation 4
## (Determining "Adversity" and "NOAEL")

***Toxic effects on cells, tissues, organs, or systems within the test animal should be assessed on their own merits***

- Adversity decisions should be based on actual observations and not speculation. Examples of issues regarding this are
  - Possible pathogenesis - primary, secondary or tertiary effects
  - Exaggerated efficacy
  - Adaptive effects

# Recommendation 4 (continued)
## (Determining "Adversity" and "NOAEL")

- Possible pathogenesis - primary, secondary or tertiary effects
  - Speculative pathogenesis can be "wishful thinking" and may be wrong
  - Better to just assess each major effect independently in regard to adversity

- For example, is the neuronal necrosis due to low glucose secondary to the test article effect being developed for diabetic glucose control? Or is the compound also a primary neurotoxicant?
  - The speculative pathogenesis is important to articulate clearly in a report, but the call of adversity in respect to neuronal necrosis is independent of that

# Recommendation 4 (continued)
**(Determining "Adversity" and "NOAEL")**

## Exaggerated Efficacy

- Should exaggerated efficacy ever be considered to be adverse? After all it is what we expect to see at high doses
  - Yes! Humans may manifest this also. What are the unanticipated sequelae to exaggerated efficacy? Previous example of glucose decrease resulting in neuronal necrosis – clearly adverse!
  - Maybe humans are more sensitive than animals to these effects?

- In all cases it is best to simply ask if the presumptive pharmacologic effect caused "harm" to the animal, and thereby determine adversity

# Recommendation 4 (continued)
## (Determining "Adversity" and "NOAEL")

### Adaptive effects

- As in the previous example, should an adaptive effect ever be called adverse?
  - Again – yes!
  - The adaptive effect may or may not occur in humans, but if it did, a 'harmful' effect may be the result
  - Maybe we are wrong, and the adaptation is actually a manifestation of an otherwise occult toxicity expressed in the animal species.
    - An example may be a mild hepatotoxin that is also an hepatic P450 enzyme inducer. This may cause enlarged hepatocytes with scattered foci of degeneration that could be 'written off' as a consequence of marked adaptive hypertrophy
- Similar to exaggerated efficacy, it is best to simply determine if the effect caused "harm" to the animal, and thereby determine adversity

# Recommendation 5
## (Communicating "Adversity" and "NOAEL")

***Communication of what is considered "adverse" and assignment of the NOAEL in the overall study report should be consistent with, and supported by, the information provided in the study sub-reports***

- All test article-related changes should be documented in the sub-reports and study report
  - Whether deemed adverse or non-adverse
  - Regardless of presumed pathogenesis or human relevance
  - Overall study report is then reflective of the compiled subreports

# Recommendation 5 (continued)
**(Communicating "Adversity" and "NOAEL")**

- NOAEL should be identified in the study report based on all study data, but a NOAEL should not be identified in sub-reports
  - It may be that subreports document a number of effects, none of which alone would be adverse, but when compiled together constitute a total effect that IS adverse. This should be clearly articulated
- Ambiguous statements (e.g. "not biologically relevant" and "not toxicologically significant") should not be used unless the scientific rationale is presented
  - Such terms may be used to escape the necessity of determining if an effect is related to the test article or is adverse or not.
  - These only should be used where a specific need is determined and the reasoning is clear.
  - These are generally discouraged for the reasons above

# Recommendation 6
## (Communicating "Adversity" and "NOAEL")

***Communication of adverse findings and the NOAEL should include direct interaction between staff within different contributing scientific disciplines***

- A single toxicity may manifest in different ways to scientists in distinct disciplines and thus be presented uniquely in the various sub-reports
  - To an in-life technician or safety pharmacologist, syncope may simply reflect a decreased sympathetic tone resulting in lower blood pressure and fainting. However this same effect may prompt a pathologist to review the brain and heart more carefully, or perhaps to look at the echocardiogram.
  - A holistic viewpoint requires the various experts get together and share their findings, making connections where possible

# Recommendation 6 (continued)
## (Communicating "Adversity" and "NOAEL")

- A complete view of a test-article related effect requires integration of all perspective within the study report

- Study components are an artificial division usually without biological reality
  - For example, changes in an ECG in a single animal may not be important unless coupled with histopathology evidence of an infarct in the heart, increase in troponin levels, or changes in AST or other clinical evidence of cardiac insufficiency.
    - Each component alone may or may not be important, but in this case may reflect the cardiac necrosis. However, even this may be better put into context by the clin path changes reflecting a clotting abnormality caused by the test article in all high dose animals, that in a single animal resulted in a cardiac infarct

# Recommendation 7
**(Communicating "Adversity" and "NOAEL")**

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

- Integration across studies is necessary because a NOAEL identified in one study may be discounted as irrelevant within an overview document based on data from another study
  - An increase in CPN in debilitated rats at a high dose may be irrelevant if data from monkeys shows no effect on kidneys
  - However if yet another study shows that rats express receptors for the test article on renal tubules, but monkeys do not, this may elevate the interest in the increased CPN as reflecting potential risk in humans

# Recommendation 7 (continued)
## (Communicating "Adversity" and "NOAEL")

- Selection of the NOAEL in the most sensitive species requires analysis of data from all available studies
  - Simply looking at the NOAEL in the most sensitive species is the wrong approach and may lead to disaster

- As an example, The most sensitive species may be the rat, with liver toxicity driving the NOAEL
  - But if there is retinal toxicity at a much higher dose and exposure in monkeys, it may be far more important to monitor than liver enzymes
  - Human sensitivity may not mimic animal sensitivity to any given effect

- Understanding ALL the animal data and the science underlying them is critical for safe dosing in humans

# Recommendation 8
**(Communicating "Adversity" and "NOAEL")**

***In order to place them in appropriate context, the use of NOAELs in data tables should be referenced to explanatory text***

- Rationale provided in text provides critical insight regarding the basis for the NOAEL, as well as important scientific perspectives regarding the importance or even the relevance to humans (remembering that this does not influence adversity or the NOAEL)

- Use of the NOAEL without an understanding of the science and relevance of effects observed in nonclinical studies can lead to inappropriate drug development decisions

# Recommendation 9

**(Using "Adversity" and "NOAEL" in assessing potential human risk)**

***Nonclinical scientists, including toxicologists, pathologists, and other contributing subject matter experts who interpret data from nonclinical studies, should be active participants in assessing and communicating human risk***

- Individuals who generate sub-reports are best qualified to explain the data set and its interpretation
  - This is the reason that in the GLP regulations, raw data for Pathology is defined as both the study tables AND the signed Pathology Report
    - The data tables could be interpreted a number of ways, but should only be assessed in the way that the study pathologist who reviewed the specimens interpreted them. They created them specifically to impart a view of the Pathology effects that they observed and interpreted

# Recommendation 9 (continued)
## (Using "Adversity" and "NOAEL" in assessing potential human risk)

- Nonclinical scientists from multiple disciplines provide valuable insight in
  - Assisting the study director to weigh the evidence to set the NOAEL
  - Advising the clinical research team with respect to setting the initial dose

- Note that if the original study personnel are not available, someone else from the same discipline should be used to assess the material and provide perspective about the interpretation of the reports
  - Not all adverse effects are equally important, and it may not be evident without an expert weighing in to provide this perspective

# Recommendation 10

## (Using "Adversity" and "NOAEL" in assessing potential human risk)

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

- Experimental studies designed to understand the pathogenesis of a nonclinical study finding may profoundly influence the human risk profile
- Assessment of human risk should be based on all available data
  - Nonclinical studies
  - Clinical studies
  - Literature of structurally related or similar acting agents

# Summary

- These recommendations are intended to produce a more consistent approach to determining, communicating and using information about adverse effects noted in nonclinical studies
- Consistency of approaches will minimize misunderstandings related to the nonclinical effects and the implications of these effects for indicating potential human risk

Identification of an effect as adverse and the resultant NOAEL designation will continue to be based on good science, skilled communication, and prudent decisions

# Case Examples

- Cases are completely fictitious and not related to any situations or studies, past or present, at Pfizer, or any other company
- Examples were created in order to exemplify common situations in the assignment of adversity or the use of the NOAEL in nonclinical studies
- Multiple answers may exist for any of these, and the author does not intend for these to be used by anyone as an example of the “correct” application of the recommendations, but rather as a means of illustrating how to consider these in real situations

# Case 1: Rats administered a compound that causes induction of P450 enzymes

- At high dose, ALT and AST were 10-15x and 4-5x control values, respectively. Livers were 50% heavier than controls. Histologically, very marked centrilobular hepatocellular hypertrophy with multiple foci of single cell or aggregated (5-10 cells) necrosis and moderate lipid accumulation

- At intermediate dose, ALT was 2-3x control value and AST was normal. Livers were 10% increased in weight. Centrilobular hypertrophy was minimal to mild and there was rare individual cell necrosis

- Low dose livers were significantly increased in weight but only 4%. No significant increase in liver enzymes. Histology was normal

- How would you establish adversity?

# Case 1. Salient points to be expressed in a report

- At the low dose, there is a small test-article effect of increased liver weight, but this is nonadverse since histology and liver enzymes were normal. This is consistent with adaptive increase in P450 enzyme induction. If other data shows this and especially if thyroid weights increased and thyroid follicular cell hypertrophy was observed, this is discussed in the text with references. Clearly this test article effect is not adverse at this dose level

- At the intermediate dose there is also a test article effect. Livers were mildly increased in weight with a small increases in ALT. Changes of this magnitude can be seen with enzyme induction (see above) and the centrilobular hepatocellular hypertrophy with an occasional individually necrotic cell is consistent with that etiopathogenesis. This is an adaptive change, and does not cause harm to the animal and is therefore not adverse at this dose level

# Answer to Case Example 1. (continued)

- At the high dose there is also a test article effect. Liver weights were quite high and liver enzymes were also high. The presence of centrilobular hypertrophy confirms the effects at the lower two doses and is consistent with very significant adaptive enzyme induction

- However the effects at the high dose differentiate from the lower two doses due to the presence of quite a large amount of necrosis, both individual cell, but also with aggregates of dead cells. When combined with the high levels of liver enzymes, and accumulation of lipid (indicating a potentially degenerative effect) it seems clear that this is an adversely affected dose

# Answer to Case Example 1. (continued)

- Is this only an extreme example of an adaptive effect? That scenario is possible. However this could also represent an additive effect of adaptation plus an "occult" toxic effect on hepatocytes that is only manifest at the high dose. In either case, it is clear that rats are harmed and the change at this dose must be deemed an adverse effect

  - Further studies in other species, or *in vitro* may help to define whether or not there is a hepatocellular toxicity, and whether rats are an appropriate species to define human risk.

# Case 2. Rats in a 3-month repeat-dose toxicity study

| Sprague Dawley Rat Toxicity Study | | Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|---|
| Number Evaluated | | 10 | 10 | 10 | 10 |
| Mortality/morbidity | | - | - | - | Yes, attributed to renal disease |
| Histologic Change (incidence): | Severity Grade[a] | | | | |
| Chronic Progressive Nephropathy | 1 | 2 | 5 | 4 | 1 |
| | 2 | 1 | | 3 | |
| | 3 | | | | 2 |
| | 4 | | | | 4 |
| | 5 | | | | 2 |
| Tissue Mineralization | | No | No | No | Yes |

***What is the No-observed effect level?***
***What is the No-observed-adverse-effect level?***
***What are key points supporting your assignment of the NOAEL?***

a Severity Scale: 1=minimal, 2=slight, 3=moderate, 4=marked, 5= severe

# Answer to Case 2. Salient points to be expressed in a report

- A test article effect is established for all doses due to an increase in incidence or severity or both of CPN
- The top dose group is considered adversely affected because mortality was observed and nephropathy caused uremia and secondary calcification
- The low dose is NOT adversely affected, since although the incidence is slightly higher, the severity is no different to controls, and incidence is lower than is often seen in controls in this facility
- The mid dose is NOT adversely affected, since although the incidence and the severity are higher than concurrent controls, both incidence and severity are no greater than can be seen in controls in this facility
- Note that lack of relevance to humans is NOT used to determine the call of adversity. However, such a statement may be used in a report to position the findings and help to subsequently determine relevance in the NCO

# Case 3. Rats in a 1-month toxicity study administered a test article for diabetes treatment

| | Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|
| Number Evaluated | 10 | 10 | 10 | 10 |
| Mortality | - | - | - | 5 |
| Clinical Signs: Lethargy, Tremor, Convulsion | 0 | 0 | 0 | 10 |
| Clinical Pathology: Decreased Serum Glucose | - | ↓ | ↓ | ↓↓ |
| Histology: Incidence of Pancreatic Islet Enlargement, increased cellularity | - | 4 | 7 | 10 |
| Brain: Neuronal Necrosis | - | - | - | 7 |

***What is the No-observed effect level?***
***What is the No-observed-adverse-effect level?***
***What are key points supporting your assignment of the NOAEL?***

# Answer to Case 3. Salient points to be expressed in a report

- Histological increase in Islet size and cellularity is not adverse on its own merit (no evidence of any structural damage, atypia or neoplasia)
  - Note that although this has the potential to progress to neoplasia (as with any hyperplasia) in longer studies, and may affect drug development decisions, there was NO evidence in this study

- Small decrease in glucose levels at the low and mid dose are not adverse. At the high dose at levels low enough to cause neurological effects (lit should be cited) this would be considered adverse and reported as such in its own right

# Answer to Case 3. Salient points (continued)

- Mortality, tremors, lethargy and convulsions, along with neuronal necrosis are adverse at the high dose. Whereas we can speculate that these may be caused by the low glucose, they may be independent, and the compound may also be a neurotoxin

- Note that a presumptive pathogenesis should be outlined in the report – i.e. islet B cell hyperplasia – insulin secretion – glucose drop – neuronal effects and mortality. However, this cannot be discounted as an expected pharmacologic effect and therefore not adverse
  - Humans may get the same effect so it is valid to report as such
  - Until there is more proof, the pathogenesis is only speculation, albeit well-founded

# Case 4. Rats in a 1-month toxicity study

| Sprague Dawley Rat Toxicity Study | | Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|---|
| Number Evaluated | | 10 | 10 | 10 | 10 |
| Histologic Finding | Severity Grade | | | | |
| Testes: Degeneration and loss of spermatids, occasional vacuoles[a] | 1 | 2 | 1 | 8 | 0 |
| | 2 | 0 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 | 2 |
| | 4 | 0 | 0 | 0 | 8 |
| Epididymis: luminal cellular debris | 1 | 0 | 0 | 2 | 1 |
| | 2 | 0 | 0 | 0 | 9 |

a Grade 1 testes lesion involved approximately 5% of seminiferous tubules. Grade 4 lesions involved approximately 50% of the seminiferous tubules

***What is the No-observed effect level?***
***What is the No-observed-adverse-effect level?***
***What are key points supporting your assignment of the NOAEL?***

# Answer to Case 4. Salient points to be expressed in a report

- Testis findings at the high dose are considered to be adverse
  - Reasoning is that the degree of degeneration and necrosis at this dose would be harmful in and of itself. Note that the reasoning does not involve speculation about fertility which WAS NOT assessed in this study (talking point). This would be expected to reverse, since there is no structural damage

- Testis findings at the mid dose are treatment-related, but NOT considered adverse
  - Reasoning is that this severity of change (only 1/20 tubular sections affected with other tubules normal) may be occasionally seen spontaneously in animals of this age

- NOAEL would be the mid-dose in this study. All findings would be expected to reverse, and are monitorable through examination of semen. Neither of these reasons should be used to rule in or out the assignment of adversity

# Case 5. Rats administered a compound with known pharmacology producing hepatic lipid accumulation

| | | Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|---|
| **Number Evaluated** | | 10 | 10 | 10 | 10 |
| **Liver Weight (g)** | | 22 | 21 | 25.6* | 34.1* |
| **ALT (U/L)** | | 63 | 59 | 151* | 945 |
| **AST (U/L)** | | 153 | 144 | 243* | 2448* |
| **Histologic Finding** | **Severity Grade** | | | | |
| **Liver: increased vacuolation, lipid**[a] | 1 | 2 | 3 | 7 | 0 |
| | 2 | 1 | 0 | 1 | 1 |
| | 3 | 0 | 0 | 0 | 1 |
| | 4 | 0 | 0 | 0 | 8 |

a Hepatocellular vacuolation occurred in the absence of histologic evidence of degeneration and/or necrosis.
*=statistically significant
Severity Scale: 1=minimal, 2=slight, 3=moderate, 4=marked, 5= severe

***What is the No-observed effect level?***
***What is the No-observed-adverse-effect level?***
***What are key points supporting your assignment of the NOAEL?***

# Answer to Case 5. Salient points to be expressed in a report

- At the mid dose there is a test article affect that is not adverse. Livers were only slightly heavier with an increase in incidence but not severity of fat (cf controls) and small increases in ALT/AST. Changes of this magnitude can be seen with adaptive change (such as enzyme induction) and the severity grade of fat accumulation can be seen in control animals

- At the high dose there are two possible arguments that may be made
  - One option says that there is no necrosis or cellular damage so despite the high ALT and AST levels, this would not be an adverse effect
  - Another opinion is that this dose IS adversely affected. The liver is 50% increased in weight. This would be a friable liver that would be easily fractured. The ALT and AST are increased to such an extent that it is clear that cellular cytoplasm is being lost to the plasma. Even if there is no overt necrosis or degeneration, this is evidence of very significant cytoplasmic loss
- Note that although the fat accumulation is likely a pharmacologic effect, this cannot be discounted as *prima facia* evidence of no adversity. It may be that the effects on the liver may be the result of additional toxicity unrelated to the fat accumulation

# Case 6. Monkeys administered a monoclonal antibody

| Female Cynomolgus Monkey | | Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|---|
| Number Evaluated | | 4 | 4 | 4 | 4 |
| Histologic Finding | Severity Grade[c] | | | | |
| Mammary Gland: Diffuse Lobular Atrophy[a] | 4 | 0 | 0 | 0 | 4 |
| Mammary Gland: Multifocal lobular atrophy[b] | 1 | 0 | 0 | 2 | 0 |

a In diffuse atrophy all lobules were affected
b In multifocal lobular atrophy only sporadic lobules were affected and intervening lobules were normal
In no case was there evidence of inflammation or alteration of overall mammary gland architecture
c Severity Scale: 1=minimal, 2=slight, 3=moderate, 4=marked, 5= severe

***What is the No-observed effect level?***
***What is the No-observed-adverse-effect level?***
***What are key points supporting your assignment of the NOAEL?***

# Answer to Case 6. Salient points to be expressed in a report

- At the high dose, diffuse mammary atrophy is considered to be adverse. This is a quite profound and highly unusual change, distorting the architecture and adjacent connective tissues
  - An argument could be made that this change did not show degeneration or necrosis and therefore might not be adverse. However, the counter argument is that this is not consistent with any normal atrophy that may be physiological in nature, and would likely have functional consequences (although not tested in this study!!)

- At the mid dose the change is considered to be non-adverse. There is a lobular atrophy present that is treatment related due to incidence, but is similar to that very occasionally seen in controls. In addition, the magnitude of the change did not alter tissue architecture or affect adjacent tissues

- Any argument discounting this change on the basis that it may be a hormonal change secondary to something expected by the compound is completely conjecture and should not affect the decision on adversity. Furthermore, although some might argue that this could affect lactation, this would need to be supported by literature linking such changes with a functional effect since this WAS NOT tested in the current study

# Conclusion

- Determination of adversity in nonclinical toxicology studies is a difficult issue and, until recently, there has been no consistent guidance about how to do this consistently

- The Society of Toxicologic Pathology has just published a position paper of “best practices” containing 10 recommendations to help align Industrial Toxicologists, Pathologists and Government Regulators applying or using the concept of adversity in nonclinical reports and overview documents

- Although this document was developed to create consistency, each case must be assessed within these new rules using good judgment and clear communication of the reasons used to apply or not apply this important term to effects caused by test articles in nonclinical toxicology studies

# Thank you for your participation in the American College of Toxicology Webinar!

*We hope to see you at the 37th Annual Meeting of the American College of Toxicology.*