Society of Toxicologic Pathology Guideline

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Best Practices Guideline: Toxicologic Histopathology

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1.0 INTRODUCTION

Histopathology is the study of the structural manifestations of disease at the light-microscopic level. The microscopic examination of a tissue specimen is an evaluation of a 2-dimensional image of a complex 3-dimensional biologic structure fixed in time. Therefore, histopathology is necessarily a largely descriptive and interpretive science. The trained and experienced toxicologic pathologist must be able to distinguish normal histological variation and spontaneous natural disease processes in tissues from those changes that may arise as a result of the administration of test article to a particular experimental subject. As histopathology is a critical part of the toxicologic and risk assessment of foods, drugs, chemicals, biologics, and medical devices, it is important that the approach to histopathologic examination satisfy both the regulatory demands for unbiased observations while facilitating the sensitive and efficient evaluation of large amounts of microscopic information. Much debate has occurred within and outside of the scientific literature about the manner in which the microscopic examination of toxicologic specimens should take place.

2.0 OBJECTIVE

This document is intended to identify and define the fundamental elements of histopathological examinations that are necessary for the best practice of histopathology along with appropriate techniques to minimize observational bias. When these practices are applied as integral components of safety/toxicology studies, it is believed the resulting data and information will have high inferential value for evaluation by regulatory agencies. This guideline should serve pathologists and nonpathologists by providing an understanding of the procedures involved in the practice of histopathology as it is used in toxicology.

3.0 SCOPE

This guideline covers the elements of toxicologic histopathology used in support of studies to be submitted to regulatory agencies for their review. This guideline identifies, clarifies, and explains the processes leading to our recommendations. The purpose of this document as envisioned by the Society of Toxicologic Pathology is to promote excellence in the practice of histopathology and to make the process transparent.

4.0 REQUIREMENTS OF THE PATHOLOGIST

4.1 The pathologist is responsible for the histopathologic evaluation of slides, which includes the identification and interpretation of tissue abnormalities. In order to carry out such duties, which concern the well-being of both animals and humans, the pathologist must be qualified and have credentials that document a high level of education, training, experience,

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[1]*Abbreviations*: EPA, Environmental Protection Agency; FDA, Food and Drug Administration; GLP, Good Laboratory Practices; NOAEL, No Observable Adverse Effect Level; NOEL, No Observable Effect Level; PWG, Pathology Working Group.

and expertise in toxicologic pathology. These abilities and the integrity of the pathologist are vitally important factors in the assurance of high-quality histopathology.

4.2 Histopathology is a descriptive and interpretive science. As such, there is an element of subjectivity to the science of histopathology. No two pathologists independently evaluating the same study could be expected to produce identical findings for every tissue, organ, or animal. However, as pathologists evaluate tissues, they apply their training and experience as consistently as possible to highly variable biological systems to avoid the introduction of artificial differences to the findings. Because all tissues within a study are generally evaluated by 1 pathologist with consistent standards for detecting, naming, and grading tissue changes, the effect of a single perspective consistently applied to each of the tissues in a study results in the uniform scrutiny of all tissues sufficient for the detection of differences induced by treatment. When properly done, histopathologic evaluations of the same study by any qualified pathologist should identify the same treatment-related findings. The terminology used by different pathologists for the same lesions is expected to be similar, but may not be identical.

4.3 In order to achieve the best result, one pathologist should evaluate all tissues from a study. However, 2 or more pathologists are occasionally involved in evaluation of a study, depending on the amount of data to be generated or the urgency of specific timelines. If more than 1 pathologist is used for histopathologic evaluation, the utmost care must be taken to ensure that their observations are consistently applied to the task. They must communicate extensively, since nomenclature and severity grading systems used by different pathologists may vary and this variation could impede and/or complicate data review and interpretation. Standardized criteria and consistent terminology should be agreed upon for grading systems of common spontaneous and treatment-related findings. At the end of the process, additional discussions are required to refine certain elements of the data and to reach consensus on interpretation of the data. The use of a shared computer system that can define a study-specific lexicon for capturing data facilitates this process. Peer review by a single pathologist is considered to be essential in studies evaluated by more than one pathologist to ensure consistency of the histopathologic findings.

5.0 Identification of Information Necessary for the Pathologist Before the Microscopic Evaluation Commences

5.1 The nature of the test substance and known activities of this class of compounds.

5.2 The results of any previous toxicity studies with this test substance should be available to the pathologist before evaluation of the tissue slides begins. Whether in the same species or different species, knowledge of target organs and tissues and the types of changes previously encountered facilitates the evaluation of tissues and provides for the consistent use of terminology. Previous knowledge of target tissues should be utilized during the protocol development to determine whether special pathology procedures should be used in obtaining, fixing, processing, or staining of sections.

5.3 Knowledge of the experimental design must be made available to the pathologist in order to adequately evaluate and interpret results. These include, but are not necessarily limited to: study protocol, including amendments and relevant deviations, species, strain, and age of animals, route, doses, and duration of dosing.

5.4 Metabolic, pharmacokinetic, or toxicokinetic information may be necessary for understanding patterns of change and interpreting differences in species responses.

5.5 In-life data (i.e., clinical signs, body weight changes, food consumption, etc.) from animals may help greatly in the identification of target organs and in understanding mechanisms of toxicity.

5.6 Hematology, clinical chemistry, and urinalysis results add necessary perspective for the identification of target organs and may contribute to an understanding of the mechanism of action. Results of special assays, such as hormone concentrations or enzyme induction, are equally important in locating morphologic changes and in the understanding of their significance.

5.7 Necropsy (gross) findings for individual animals must be available to the pathologist for lesion tracking and correlation with histopathology findings. The pathologist should be aware of organ weight changes. Often histomorphologic correlates of altered weights can be identified.

5.8 All of the above-mentioned data, if available, should be provided to the pathologist at the time of the initial slide evaluation. If this information is not available initially, selected tissues may need to be re-evaluated to ensure accurate diagnoses and interpretations.

6.0 Histopathology Specimen Processing and Quality

High-quality tissue specimens are necessary for histopathologic evaluation. Tissues must be promptly and appropriately fixed by immersion (commonly in neutral-buffered formalin (Bancroft and Cook, 1994; Luna, 1968; Thompson and Hunt, 1966), or other appropriate fixative for eyes, testes, etc.) or by perfusion or other special technique. Protocol-required specimens should be obtained from standard locations within organs to reduce potential variability. Specimens should be

reduced in size, if necessary, to allow for optimal fixation. Lesions in non-standard locations should be saved as additional specimens. Adequate fixation time is necessary before tissue processing commences. Standardized uniform trimming techniques must be used. Tissues should be processed in a manner that reduces the potential for variation to be introduced among groups, i.e., proportional numbers of slides from control and treated groups should be processed per day as opposed to processing all controls on 1 day and all tissues from treated animals on another day. Special stains should be used where appropriate. All specially stained tissues should be accompanied by a positive control specimen that affirms the proper functioning of the technique. Slides should be identified with the study number, animal number, and slide (block) number.

7.0 The Process of Histopathologic Evaluation

7.1 The pathologist should ensure that standard sections of tissues and organs are present on the slides to be evaluated. The intention of requiring standard sections is to provide comparable samples among all groups, thus reducing inconsistency. It is the pathologist's responsibility to assure that appropriate sampling of the tissues has been done. If this is not feasible, such issues should be taken into account in the interpretation of the data and discussed in the pathology narrative.

7.2 If missing tissues or partially missing tissues (medulla of adrenal, pars distalis of pituitary, mucosa of intestine, etc.), impair the pathologist's ability to detect or evaluate treatment-related effects, it is the pathologist's responsibility to obtain recuts, to the extent possible, of those missing or inadequate tissues necessary for identification and interpretation of treatment-related effects.

7.3 The pathologist should use standardized nomenclature and diagnostic criteria (such as Standardized System of Nomenclature and Diagnostic Criteria. Guides For Toxicologic Pathology). Terminology should be chosen to clearly communicate the nature of the tissue change. Where concise terminology is inadequate to convey lesion complexity, detailed free text descriptions should be used to define the diagnostic term used for tabulation.

7.4 Tissues may be evaluated animal by animal or organ by organ as the preference of the pathologist dictates. The animal by animal technique affords an encompassing overview of an animal's complete health status. The organ by organ technique allows more focused attention to changes and aids in the consistent grading of changes in a particular organ.

7.5 For common lesions in a species or strain of animal, it is important to know if the experimental treatment alters severity. The pathologist should use a severity grading system that allows for an appropriate severity classification, as treatment may affect incidence or severity. Toxicologic lesions are found in a continuous spectrum of severity and are often on a borderline in any classification system. Therefore, severity grading systems should be: 1) definable, 2) reproducible, and 3) meaningful. A description of each of the various grades should be included in the narrative for target lesions where severity is critical to interpretation of the data. This gives the reviewer a mental image of the differences noted among the groups of animals. Photomicrographs may be helpful in conveying the severity differences for the grading system used. Well-defined severity grading systems greatly aid the pathology peer-review process.

7.6 For carcinogenicity studies, it is the pathologist's responsibility to distinguish between hyperplasia, dysplasia, and neoplasia and to classify tumors, where applicable, as 1) benign or malignant, and 2) primary or metastatic. The pathologist may also provide an evaluation as to the cause of death for individual animals (The Society of Toxicologic Pathology's Recommendations on Statistical Analysis of Rodent Carcinogenicity Studies, 2002). This evaluation may be important in the overall interpretation of the study.

8.0 Procedures Used to Enhance the Accuracy and Consistency of Histopathology

8.1 Informal re-evaluation of specific tissues for specific changes by the study pathologist aids in promoting consistency of nomenclature and severity grading. Pathologists may seek the counsel of colleagues or recognized experts to ensure proper nomenclature is used.

8.2 Pathologists are aware of the phenomenon of "diagnostic drift." Drift refers to a gradual change in nomenclature or severity grading of lesions within a single study. Drift is more of a problem in large studies with many animals and tissues requiring evaluation over a prolonged period of time. It is a source of inconsistency that can negatively impact detection of treatment-related lesions and changes or the determination of no-effect-levels. When a pathologist becomes aware of drift in his/her selection of terminology or severity grading, he/she must re-evaluate the tissue(s) involved. The re-evaluation may be done with the use of a "blinding" or "masking" technique, where appropriate.

8.3 Appropriate techniques to minimize the introduction of observational bias into the histopathologic evaluation may be considered before, during, and after the microscopic evaluation of tissues by the pathologist. One such technique is *masked evaluation*. Masked evaluation (also known as *blinded evaluation*) in this section refers to the evaluation of microscopic material by the pathologist without prior knowledge of treatment group, which could include untreated or other control groups. As

previously described in Section 5, the study pathologist should have access to in-life clinical observations, clinical pathology data, and macroscopic findings of the postmortem examination for each animal in addition to complete information about the experimental design, signalment and husbandry of the study population, and physical chemistry of the test compound. Examples of other techniques used to minimize observational bias are discussed in 8.4 and 8.5.

There is a considerable consensus of opinion among toxicologic pathologists that implementation of masked evaluation during the initial evaluation of tissues can have a negative impact on both the time it takes to accomplish the microscopic evaluation as well as the quality of the information obtained from the study (Editorial SOTP, 1986; Goodman, 1988; House et al., 1992; Iatropoulos, 1984; Newberne and de la Iglesia, 1985; Prasse et al., 1986). There is a concern that masked evaluation makes the task of separating treatment-related changes from normal variation more difficult. In addition there is concern that masked review during the initial evaluation may result in missing subtle lesions. It is felt that an awareness of the treatment group assignment, particularly knowledge of which animals have been assigned to the untreated or other control group, allows the pathologist to intensely focus the histopathologic evaluation and to find important, and sometimes subtle, differences between the tissues of treated and untreated animals. Overall, toxicologic pathologists feel that an awareness of treatment group favors the finding of all treatment-related effects and enhances the accuracy of the histopathological evaluation.

Because it has the potential to limit the pathologist's awareness of normal variation and effects of the experimental design, *masked evaluation* has traditionally been reserved for reevaluation of findings in specific tissues (targeted masked review). Also, in evaluations where a known toxic syndrome with a defined spectrum of lesions exists, it may be beneficial for the pathologist to be unaware of treatment groups for the evaluation of target lesions. An example would be certain studies in a target species such as those conducted as target animal safety studies for the FDA[1] Center for Veterinary Medicine, where a known toxic syndrome with a defined spectrum of lesions exists or is likely to exist.

Therefore during the initial review of microscopic material in preclinical toxicology studies for new chemical entities, novel uses, or uses of previously studied compounds in new species, it is recommended that the pathologist be aware of treatment group during the histopathologic evaluation. Following the recognition of treatment-related findings, masked evaluations of target tissues may be beneficial to ensure consistent use of diagnostic criteria and terminology, uniform application of severity scores, and appropriate determination of a NOEL (no-observable-effect-level).

8.4 Peer review increases confidence in the accuracy of the histopathology findings from a study. The peer-review pathologist usually reviews a defined subset of animals and tissues as well as the study interpretation. A number of procedures can be used for peer review (Eighmy, 1996; Frantz, 1997; Mann, 1996; McCullough et al., 1997; Peters, 1996; Position of the Society of Toxicologic Pathologist: Documentation of Pathology Peer Review, 1997; Sahota, 1997; The Society of Toxicologic Pathologists, Peer Review in Toxicologic Pathology: Some Recommendations, 1991; Ward et al., 1995). Peer reviews are generally prospective in that they are included in the protocol and are conducted prior to the issuance of the study report (Ward et al., 1995). This procedure is then part of the process that leads to finalizing diagnoses and interpretations. Therefore, changes resulting from a prospective peer-review require no formal documentation as long as the study and peer-review pathologists agree on the results in the study report. If the study protocol specifies that a prospective peer-review will be done, documentation of the peer review must be a part of the data file. Retrospective peer reviews are undertaken after the issuance of the study report. Any changes required by a retrospective peer-review result in generating a report amendment, and as such, must be documented as required by Good Laboratory Practices.

The objectives of a formal histopathology peer review (prospective or retrospective) are several: 1) determine accuracy and consistency of nomenclature i.e., survey for the presence of incorrectly diagnosed or inaccurately described treatment-related lesions, 2) determine completeness; i.e., survey for the presence of undiagnosed treatment-related lesions, 3) determine the appropriateness of the NOEL or NOAEL by reviewing all target tissues and organs, and 4) review the correctness of the textual interpretations derived from those data. The intention of the peer review is not the corroboration of every detail of every histologic finding; rather it is to ensure that treatment-related findings are properly identified, consistently diagnosed, and correctly interpreted.

8.5 A Pathology Working Group (PWG) may be formed to review, revise and/or interpret diagnoses (Mann, 1996; Peters, 1996; The Society of Toxicologic Pathologists, Peer Review in Toxicologic Pathology: Some Recommendations, 1991). PWGs generally consist of experts in the tissues or lesions being reviewed and their report reflects the consensus opinion of those experts. PWGs can be formed to resolve differences between the study pathologist and the peer-review pathologist, to assure sponsors of the correctness of the findings, to change diagnoses once the report has been submitted (required by EPA), or

to answer questions from regulators or other interested parties. A PWG is formed when a substantive issue arises from study data that has an impact on the determination or interpretation of treatment-related findings and/or a NOEL. Results of PWGs are always formal and are documented in a report, since these findings are considered definitive for the issue and study reviewed.

9.0 Recording of Histopathologic Findings

9.1 A variety of methods may be used to record, store, and publish histopathology findings. These methods include written hard copy, transcribed and typed hard copy, voice recognition computer storage, or computer programs designed to manage pathology data. Regardless of the methods used in the recording, storing, and producing of individual animal reports and summary data, it is the pathologist's responsibility to ensure the integrity and accuracy of the data. Where manual tabulation of histopathology data is performed, the pathologist is the only person who can determine the correctness of these data, even if others have been delegated to assist in this tabulation. Proper interpretation of the study findings is completely reliant on accurate summation of individual animal results. The best histopathologic evaluations are of no value if improper or error-prone procedures are used to produce tabular summaries of the results. Since many pathologists use computerized systems to process histopathologic data, validation of computer pathology systems for GLP studies is mandatory.

9.2 Once the histopathologic findings have been recorded and individual animal and tabular summary data have been generated, it is the pathologist's responsibility to write or approve of a narrative text that accurately and completely describes and interprets the changes encountered in the study. An adequate pathology evaluation requires a written report, not just presentation of data. The narrative discussion should include correlations with other relevant findings, the nature and significance of the any treatment-related changes, the identification of no-effect-levels, where appropriate, and the relevance of any extenuating circumstances (i.e., intercurrent disease, inordinate early deaths, etc). The final report represents the pathologist's best judgment regarding the relevant tissue changes and their interpretation in the context of the study.

10.0 Attribution of the Pathologist's Endeavors

10.1 The pathologist determines that his/her work is completed when he/she is completely satisfied 1) with every histopathologic diagnosis and description of findings for each animal on the study, 2) that all of the diagnoses and descriptions reflect the consensus of a peer review, if a peer review was done prospectively, 3) that the individual animal and tabular summary data output is an accurate representation of the findings, 4) that a NOEL or a NOAEL was determined from those findings, if appropriate, and 5) that the pathology portion of the final report accurately and completely presents the data and their interpretation.

10.2 Upon the completion of items listed previously, the pathologist is required to authorize the results with his/her signature on a pathology report that includes these data, or on the entire study report as a contributing scientist, author, or co-author.

10.3 Should errors, misdiagnoses, or the need for any changes surface after the pathologist has signed the final document signifying completion of the pathology portion of the report, an audit trail of any corrections or revisions to the data and/or the report is necessary.

11.0 Summary

This best practices guideline for toxicologic histopathology identifies the process elements necessary for high-quality histopathologic evaluation of toxicology studies. It is not intended to address the science of toxicologic histopathology, only procedural considerations that are necessary to produce valid study results. The qualifications, training, experience and effectiveness of the study pathologist, in the broadest context, clearly represent the most essential factors in determining the quality of the pathology evaluation and the interpretation.

References

Bancroft, J. D. and H. C. Cook (1994). *Manual of Histological Techniques and their Diagnostic Application.* Churchill Livingstone, Edinburgh.

Editorial. (1986). "Society of Toxicologic Pathologists' Position Paper on Blinded Slide Reading." *Toxicologic Pathology* **14**(4): 493–494.

Eighmy, J. J. (1996). "Study Pathologist Perspective of Pathology Peer Review." *Toxicol Pathol* **24**(5): 647–649.

Frantz, J. D. (1997). "Letter to the Editor." *Toxicol Pathol* **25**(3): 335–337.

Goodman, D. G. (1988). "Factors Affecting Histopathologic Interpretation of Toxicity-Carcinogenicity Studies." *Carcinogenicity: The Design, Analysis, and Interpretation of Long-Term Animal Studies*. ILSI Monographs, Springer-Verlag, New York.

House, D. E., E. Berman, J. C. Seely, and J. E. Simmons (1992). "Comparison of Open and Blind Histopathologic Evaluation of Hepatic Lesions." *Toxicol Lett* **63**: 127–133.

Iatropoulos, M. J. (1984). "Editorial." *Toxicol Pathol* **12**(4): 305–306.

Luna, L.G. (ed.). (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. McGraw-Hill, New York.

Mann, P. C. (1996). "Pathology Peer Review from the Perspective of an External Peer Review Pathologist." *Toxicol Pathol* **24**(5): 650–653.

McCullough, B., M. Valerio, G. Miller, M. Pino, and M. Mirsky (1997). "Letter to the Editor." *Toxicol Pathol* **25**(3): 337–338.

Newberne, P. M. and F. A. de la Iglesia (1985). "Editorial: Philosophy of Blind Slide Reading." *Toxicol Pathol* **13**(4): 255.

Peters, T. S. (1996). "Pathology Peer Review—A Concept for Consideration." *Toxicol Pathol* **24**(5): 654–656.

Position of the Society of Toxicologic Pathologists: Documentation of Pathology Peer Review. (1997). *Toxicol Pathol* **25**(6): 655.

Prasse, K., P. Hildebrandt, and D. Dodd (1986). "Letter to the Editor." *Vet Pathol* **23**: 540–541.

Sahota, P. S. (1997). "Letter to the Editor." *Toxicol Pathol* **25**(3): 337.

The Society of Toxicologic Pathologists. (1991) "Peer Review in Toxicologic Pathology: Some Recommendations." *Toxicol Pathol* **19**(3): 290–292.

The Society of Toxicologic Pathology's Recommendations on Statistical Analysis of Rodent Carcinogenicity Studies (2002). *Toxicol Pathol* **30**(3): 415–418.

Standardized System of Nomenclature and Diagnostic Criteria. *Guides For Toxicologic Pathology.* STP/ARP/AFIP. Washington, DC.

Thompson, S. M. and R. D. Hunt (1966). *Selected Histochemical and Histopathological Methods.* Charles C. Thomas, Springfield, IL.

Ward, J. M., J. F. Hardisty, J. R. Hailey, and C. S. Streett (1995). "Peer Review in Toxicologic Pathology." *Toxicol Pathol* **23**(2): 226–234.