June 7, 2022

Dockets Management Staff (HFA-305)
Food and Drug Administration (FDA)
5630 Fishers Lane
Room 1061
Rockville, MD 20852
Attn: Docket No. FDA-2021-D-1268

Re: Docket No. FDA-2021-D-1268: Use of Whole Slide Imaging in Nonclinical Toxicology Studies: Questions & Answers

Dear FDA Colleagues:

The Biotechnology Innovation Organization (BIO) thanks the Food and Drug Administration (FDA) for the opportunity to submit comments regarding the Draft Guidance for Industry, **Use of Whole Slide Imaging in Nonclinical Toxicology Studies: Questions & Answers.**

BIO is the world's largest trade association representing biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO's members develop medical products and technologies to treat patients afflicted with serious diseases, to delay the onset of these diseases, or to prevent them in the first place.

BIO greatly appreciates the opportunity to provide feedback on this guidance as it provides valuable information for sponsors seeking additional clarity on the proper usage, documentation, and retention of whole slide images (WSI) when used for nonclinical toxicology studies. However, BIO believes that the following modifications, provided in the table below, will enhance the guidance by ensuring its language is consistent with the terminology widely used in the field of toxicologic pathology today while offering additional detail that will increase the guidance's instructive value. At a high level, BIO suggests that highlighting whether a particular tool and/or process results in the generation of raw data could provide a useful method for clearly outlining the proper approach sponsors should take in handling/documenting various practices associated with the use of WSI for nonclinical toxicology studies, along with the rationale for doing so.

Sincerely,
/s/
Rachel Coe, MSPH, CPH
Manager, Science and Regulatory Affairs
Biotechnology Innovation Organization

SPECIFIC COMMENTS:

LINE NUMBER	ISSUE	PROPOSED CHANGE
I. INTRODUCTION		
Lines 18-20	"… used during histopathology assessment and/or pathology peer review performed for good laboratory practice (GLP)-compliant nonclinical toxicology studies using non-human specimens.[2] [2] We support the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible. We encourage sponsors to consult with us if it they wish to use a non-animal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method."	This sentence seems to state that whole slide imaging cannot be used for GLP-compliant tissue-cross-reactivity studies performed on frozen human tissues. Clarify whether this is the case. If use of frozen human tissues is acceptable to FDA, suggest: "… used during histopathology assessment and/or pathology peer review performed for good laboratory practice (GLP)-compliant nonclinical toxicology studies ~~using non-human specimens~~."
II. BACKGROUND		
Lines 40-43	"…includes an initial evaluation of glass histology slides by the study pathologist and a subsequent review (referred to as pathology peer review) by a second pathologist, group of pathologists, or Pathology Working Group."	Typically, the study pathologist conducts a primary evaluation, followed by a second pathologist who conducts the contemporaneous or retrospective pathology peer review. BIO highlights that conduct of an additional peer review by a group of pathologists or a Pathology Working Group is usually only performed if needed. Suggest: "… includes an initial evaluation of glass histology slides by the study pathologist and a subsequent review (referred to as either contemporaneous or retrospective pathology peer review) ~~by a second pathologist, group of pathologists, or Pathology Working Group.~~ A group of pathologists, or Pathology Working Group may review slides if needed."

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Footnote 3	"In the context of this guidance, the term histology slide refers to tissue mounted on a microscope slide, including organ sections and cell samples such as bone marrow and other cytological preparations."	BIO suggests providing examples or further clarifying the range of cytological preparations this guidance would apply to. For example, specify if GLP-compliant tissue cross reactivity sections/studies, tissue microarray screening, immunohistochemistry, etc., using cryopreserved tissue sections that may be whole mounted/stained.
III. QUESTIONS AND ANSWERS		
Q1: What is whole slide imaging?		
Lines 56-62	"Whole slide imaging includes the software and hardware used to generate a two-dimensional digital image[4] of a glass histology slide used for routine assessment in generation of the pathology report. The process includes four sequential parts: image acquisition (scanning), image processing, image file storage, and display of images. Due to inherent limitations of the current technologies used in the process that digitalizes the spatial and color information from the scanned histology slides, FDA does not consider the resulting digital image to be an exact copy of the glass slide. For example, the scanning systems have limited spatial and color resolution and loss of depth of field.[5]"	First, BIO concurs with FDA's statement that a whole slide image (WSI) should not be considered an "exact copy" of an original, glass histology slide because an exact copy must be verified against a precise reference and specimens are subject to change over time. However, BIO highlights that the original glass microscope slides have their own limitations for spatial and color resolution and depth of field. Paraffin embedded tissue sections often have stain variations, thickness variations, and other artifacts of histology production that the experienced pathologist reconciles. It is up to the study pathology and/or peer review pathologist to determine the "quality" and suitability of these original glass slides. These determinations made by experienced professionals are accepted, representing a longstanding and highly effective practice. **Therefore, BIO suggests that if the accountable study pathologist and/or peer review pathologist deem the WSI to be suitable, it should be considered "equivalent" for the purposes of nonclinical toxicology studies, same as applied to glass microscope slides.** This idea that a whole slide image should be considered "an equivalent" or "faithful image replica" of the original, glass histology slide is supported by several, reputable organizations including the Society of Toxicologic Pathology[1] and the

[1] Forest T, Aeffner F, Bangari DS, et al. Scientific and Regulatory Policy Committee Points to Consider: Primary Digital Histopathology Evaluation and Peer Review for Good Laboratory Practice (GLP) Nonclinical Toxicology Studies [published online ahead of print, 2022 Jun 3]. *Toxicol Pathol*. 2022;1926233221099273. doi:10.1177/01926233221099273

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		Organisation for Economic Co-operation and Development (OECD)[2] in addition to numerous peer-reviewed publications.[3,4] This sense of equivalence is essential to validate scanning platforms. In the absence of an accepted view that microscope slide and the WSI have equivalence for purposes of nonclinical toxicology, it is not possible to achieve "validation" of a digital scanning platform. Given the likely evolution of whole slide imaging technology, there is value in establishing what whole slide images used in GLP studies should comprise. Furthermore, as WSIs are used by MD pathologists to make critical evaluations and decisions that impact patients, WSI for toxicologic pathology purposes should not have greater restrictions in nonclinical application. This was recently addressed in Schumacher et al, who stated "the ultimate responsibility for assessing that the image is adequate for the intended purpose lies with the pathologist." Lastly, BIO points out that some new technologies allow for much greater (in some cases 3D) depth of field than is indicated by the broad statements included in this response. Given these considerations, we suggest the following: "Although Whole Slide Images (WSI) can be used to generate raw data, they do not strictly fulfil the definition of specimens per se since they are derived from specimens, namely glass histology slides, and not from a test system. Whole slide images are rather a faithful representation of the glass slides (including stained artefacts and labels) generated by validated and fit for purpose computerized systems. (Schumacher 2021) as verified by a pathologist comparing the digital to the glass slide under a microscope.

[2] https://www.oecd.org/chemicalsafety/testing/glp-frequently-asked-questions.htm

[3] Schumacher VL, Aeffner F, Barale-Thomas E, et al. The Application, Challenges, and Advancement Toward Regulatory Acceptance of Digital Toxicologic Pathology: Results of the 7th ESTP International Expert Workshop (September 20-21, 2019). *Toxicol Pathol*. 2021;49(4):720-737. Doi:10.1177/0192623320975841

[4] Jacobsen M, Lewis A, Baily J, Fraser A, Rudmann D, Ryan S. Utilizing Whole Slide Images for the Primary Evaluation and Peer Review of a GLP-Compliant Rodent Toxicology Study. *Toxicol Pathol*. 2021;49(6):1164-1173. doi:10.1177/01926233211017031

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		Whole slide imaging includes the software and hardware used to generate a ~~two-dimensional~~ digital image of a glass histology slide used for routine assessment in generation of the pathology report. The process includes four sequential parts: image acquisition (scanning), image processing, image file storage, and display of images. ~~Due to inherent limitations of the current technologies used in the process that digitalizes the spatial and color information from the scanned histology slides.~~ Though FDA does not consider the resulting digital image to be a verifiable, exact copy of the glass slide, ~~For example, the scanning systems have limited spatial and color resolution and loss of depth of field.~~ it is a faithful image replica, which contains all the information in the glass histology slide that is useful in forming a diagnosis or another opinion relevant to the use case."[5]
Q2: Should whole slide image files be retained?		
Line 67-71	"If whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or pathology peer review performed for GLP-compliant nonclinical toxicology studies, the whole slide image files should be retained as study records and archived after study finalization."	BIO agrees that if the primary histomorphologic evaluation by the study pathologist was performed using whole slide images (WSI) in lieu of the original glass histology slides ***and generates raw data***, the whole slide images should be appropriately retained. However, WSI that are generated for the singular purpose of contemporaneous peer review should not be archived as these WSI ***are not used to generate raw data,***[5] do not reconstruct the study, and therefore, do not need to be retained according to the definition of "raw data" per 21 CFR Part 58, 58.3 (k): ***(k)*** *… any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a nonclinical laboratory study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. Raw data may include photographs, microfilm or*

[5] Tuomari, D.L., Kemp, R.K., Sellers, R., Yarrington, J.T., Geoly, F.J., Fouillet, X.L., Dybdal, N. and Perry, R., 2007. Society of Toxicologic Pathology position paper on pathology image data: compliance with 21 CFR Parts 58 and 11. *Toxicologic Pathol.* 35(3), pp.450-455.

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		microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments. Moreover, given the resource requirements for storage of high-quality whole slide images, the need to retain whole slide images regardless of use will discourage adoption of the technology and/or the **contemporaneous peer review** within the GLP quality system. **We disagree with this recommendation as the retention of WSI for this purpose is not necessary and expends resources which add no value for GLP-compliance purposes**.[3] Suggest: "If whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or retrospective pathology peer review performed for GLP-compliant nonclinical toxicology studies, resulting in the generation of raw pathology data; the whole slide image files should be retained ~~as~~ with study records and archived after study finalization. However, as contemporaneous histopathology peer review does not create raw histopathology data, in the scenario where the study pathologist evaluates glass mounted tissue specimens and a concurrent peer review is conducted with WSI, archiving the WSI should not be required. Consideration should be given to ensure that archived digital images remain viewable as software/hardware updates/versions are implemented."
Q3: If the whole slide image files are retained, should the glass slides also be retained?		
		No comments. BIO concurs.
Q4: What should be retained with respect to the whole slide image file? Should modified whole slide image files be retained?		
Line 82-85	"The whole slide image files assessed by the pathologist for histopathology assessment and/or pathology peer review (i.e., files containing all image data captured by the sensor and documentation of any modifications), referred to here as	BIO suggests that a whole slide image used in a GLP study should be a faithful replica of the glass histology slide, and thus considered "equivalent" for the purposes of histopathology review. Whole slide images used to create raw data should be retained based on the rationale expressed with respect to Q2. Suggest: "The whole slide image files assessed by the pathologist for histopathology assessment and/or retrospective pathology peer review (i.e., files containing all image data captured by the sensor and documentation of any modifications), referred to here as

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	the original whole slide image files, should be retained."	the original whole slide image files, should be retained if they are used to generate raw data.
Lines 87-90	"Viewing software should not allow the original whole slide image files to be changed. Simple adjustments made by the pathologist using the image viewing software during whole slide image evaluation (e.g., brightness, contrast, arrows pointing to regions of interest) do not need to be documented or retained."	It is conceivable that future technology may allow the study pathologist to manipulate whole slide images during viewing to increase the utility of the image for discerning findings or ascertaining quality. Therefore, BIO recommends that the study pathologist **should be allowed to modify** an image if an exact copy of the original image is retained, the process for manipulating the image is documented in a standard operating procedure or the study plan, an exact copy of end-product of the manipulation is retained, and the activity otherwise complies with GLP. To prevent accidental, permanent alteration of an *original* image while viewing, the guidance could suggest that a best practice would be for pathologists to save a separate copy of the original image prior to making any modifications. This is in line with standard best practices for GLP studies. Additionally, BIO notes that this answer does not mention annotations, which are commonly created as a digital overlay on the WSI to help the Study Pathologist identify regions of interest or variations that the Peer Review Pathologist has identified (positive findings, controls with the same findings as test article groups, variation in characteristics or severity of findings). These annotations aid peer review and facilitate the resolution of any differences in opinion. However, like peer review working notes, annotations do ***not*** generate raw data and thus should not be retained. **BIO suggests FDA specifically reference annotations in the revised guidance to provide additional clarity to sponsors.** Finally, BIO recommends FDA reiterate or reference any format-specific information contained within the final guidance, Pathology Peer Review in Nonclinical Toxicology Studies: Questions and Answers Guidance for Industry, to provide additional clarity and consistency. Given these considerations, we suggest the following: "~~Specifically, any technical image processing modifications made to whole slide image files prior to being provided to the pathologist (e.g., smoothing, color manipulation) should be documented and retained. Viewing software should not allow the original whole slide~~

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		~~image files to be changed.~~ Simple adjustments or annotations made by the pathologist using the image viewing software during whole slide image evaluation (e.g., brightness, contrast, arrows pointing to regions of interest) do not need to be documented or retained." The peer-review statement should be signed and dated by the peer-review pathologist(s) and include the following information: What format (e.g., glass slides or whole slide images).[1] [1] See FDA Guidance on Pathology Peer Review in Nonclinical Toxicology Studies: Questions and Answers Guidance for Industry (Dec 2021).
Q5: Should written procedures for whole slide imaging processes be in place?		
Lines 94-97	"Yes, written procedures for whole slide imaging processes should be in place. These may include slide scanning, validation, training, maintenance, software..."	Further clarification regarding "written procedures" would be useful as they possibly relate to formal standard operation procedures (SOPs), work instructions, best practices, etc. Suggest: "Yes, written procedures for whole slide imaging processes (e.g., documented standard operating procedures and/or work instructions with audit trails) should be in place. These may include slide scanning, validation, training, maintenance, software…"
Q6: Should the whole slide imaging system be validated?		
Lines 101-103	"If the whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or pathology peer review performed for GLP-compliant nonclinical toxicology studies…"	BIO notes that this draft answer erroneously includes contemporaneous peer review, yet this type of peer review **does not generate raw data**. BIO suggests that whole slide images *used to generate raw data* should be retained based on the rationale expressed with respect to Q2. Given the resource requirements for establishing and maintaining a GLP validated whole slide imaging workflow, and the resource requirements for retaining whole slide images used to create raw data, many organizations may hesitate to adopt or discontinue use of this technology if whole slide image-based peer review must be a GLP-compliant endpoint and all whole slide images are retained regardless of use. Suggest: "If the whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or retrospective pathology peer review ~~performed~~ to generate raw data for GLP-compliant nonclinical toxicology studies…"

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Lines 103-105	"…GLP-compliant nonclinical toxicology studies, the whole slide imaging system (including software and hardware) should be validated and maintained in a manner specific to the intended use of the technology consistent with 21 CFR Part 58."	Additionally, while validation of whole slide imaging system should be required in cases where whole slide images are used to generate raw data for GLP-compliant nonclinical studies, BIO notes that OECD 16 identifies peer review as an optional activity that can be conducted in a non-GLP compliant environment if appropriately planned and documented. To that end, BIO suggests the guidance be written with increased flexibility to account for the potential use of whole slide images in a non-GLP compliant environment if properly planned and documented in the protocol of the study, especially for peer reviews. Suggest: "…for GLP-compliant nonclinical toxicology studies, the whole slide imaging system (including software and hardware) should be validated and maintained in a manner specific to the intended use of the technology, consistent with 21 CFR Part 58. Peer review may be conducted as a GLP endpoint using a validated whole slide imaging system, or as a non-GLP endpoint using a non-validated system. Peer review procedures should be planned and documented consistent with existing guidance, regardless of GLP compliance."
Q7: How should whole slide image files be protected, including when transmitted to external users?		
Lines 110-115	"If the whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or pathology peer review performed for GLP-compliant nonclinical toxicology studies, generation of a backup file, chain of custody, access controls, and securing data systems and data transmission should be performed following written procedures and processes in compliance with an electronic record under 21 CFR Part 11 to maintain whole image file integrity."	While generating backup files of whole slide images is prudent risk mitigation in the current state of the art for retention of computer files, technology may change. Moreover, in the specific case of whole slide images, the glass histology slides from GLP studies are retained as specimens and can be used to reconstruct the study, which decreases the impact of loss of whole slide images. BIO notes that this draft answer erroneously includes contemporaneous peer review, yet this type of peer review does not generate raw data. Including Peer Review under this premise creates administrative burden which is unnecessary and arbitrarily restricts the free flow of information between Study Pathologist and Peer Review Pathologist for no value. BIO suggests that whole slide images used to create raw data should be retained based on the rationale expressed with respect to Q2. Suggest: If the whole slide images are assessed in lieu of the original glass slides ~~during histopathology assessment and/or pathology peer review performed~~ to generate raw data for GLP-compliant nonclinical toxicology studies, they should be retained in the study file

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		and measures should be taken to prevent loss of data, such as generation of a backup file, maintaining chain of custody and access controls, ~~and~~ securing data systems and data transmission. These steps should be performed following written procedures and processes in compliance with an electronic record under 21 CFR Part 11 to maintain whole slide image file integrity.
Q8: Should the signed pathology report/peer review statement state that whole slide images were evaluated in lieu of glass slides?		
		No comments. BIO concurs.