Analytical Biological Services Inc.
701 Cornell Drive, Suites 1 – 7
Wilmington, DE 19801, USA

Date: March 02, 2019
Preliminary Report

Objective:

To evaluate cellular morphology of 2 rats liver tissues using H&E and Masson's trichrome special staining.

Methods/Materials:

- 2 rat liver from 12 week follow up
  - o #61: FNDP-(NV)
  - o #72: control

- Received in 70% EtOH
- Routine longitudinal orientation for embedding
- 5-micron sectioning onto plus slides
- Routine H&E
- Special stain Masson's trichrome

Analysis:

- The pathological evaluate was performed by Dr. Michael D'Andrea, and the criteria designed for the evaluation of liver analyses was supported by several peer-reviewed publications.[1-7]
- Criteria (gross estimates [semi-quantitative] and w/out image analysis) in Table 1:

Table 1. Features	Normal/None (0)*	Abnormal/Present (1-4)*
General architecture**		
Mitotic figures		
Lipidosis/Vacuolization		
Necrosis		
Inflammation		
Fibrosis		

*Based upon 4 random fields (200x mag: 20x objective/10x eyepieces) and ranked none (0), minimal (1), mild (2), moderate (3), and marked (4) pathological changes.
**Organization of hepatocytes, hepatocyte morphology, vasculature, etc.

Results:

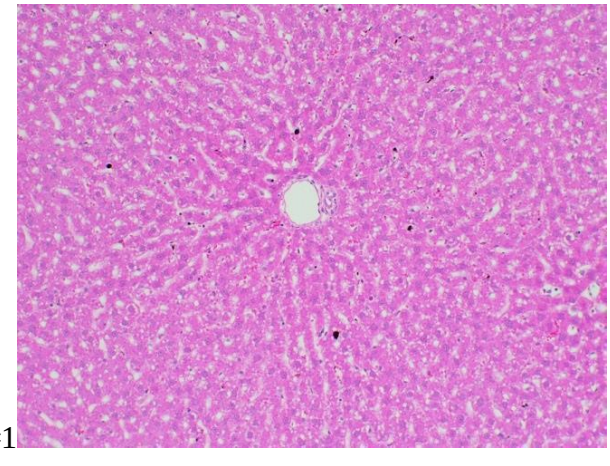
#61 Rat liver, normal: Normal morphology was observed in #61 rat liver tissue (Table 2). Representative H&E images below (Figs. 1-2) show normal radial patterns of the polygonal hepatocytes around the hepatic portal veins. Hepatocytes have normally appearing nuclei with prominent nucleoli and abundant euchromatin (denotes active transcription), and acidophilic cytoplasm. No evidence of pathology was observed (eg. inflammatory cells, necrosis, mitotic figures, or abnormal levels of vacuolization). Representative images (Figs. 3-4) of rat livers stained with Masson's trichrome staining showed normal distribution of blue-stained collagen fibers surrounding the vessels ruling out fibrosis.

Table 2. Tissue #61	Normal/None (0)*	Abnormal/Present (1-4)*
General architecture**	0	
Mitotic figures	0	
Lipidosis/Vacuolization	0	
Necrosis	0	
Inflammation	0	
Fibrosis	0	

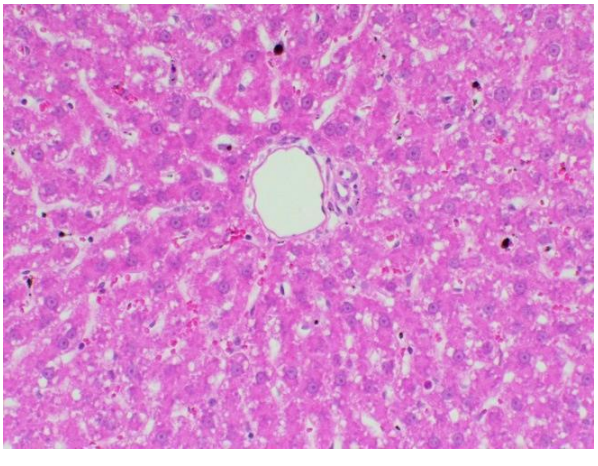
*Based upon 4 random fields (200x mag: 20x objective/10x eyepieces) and ranked none (0), minimal (1), mild (2), moderate (3), and marked (4) pathological changes.
**Organization of hepatocytes, hepatocyte morphology, vasculature, etc.

H&E:

Low mag (10x objective)

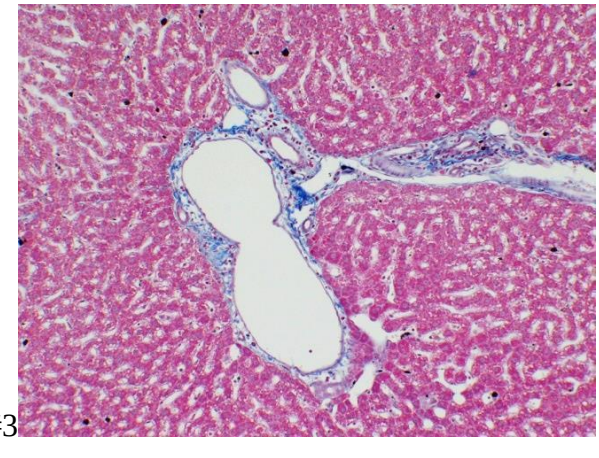
#1 

Higher mag (20x objective)

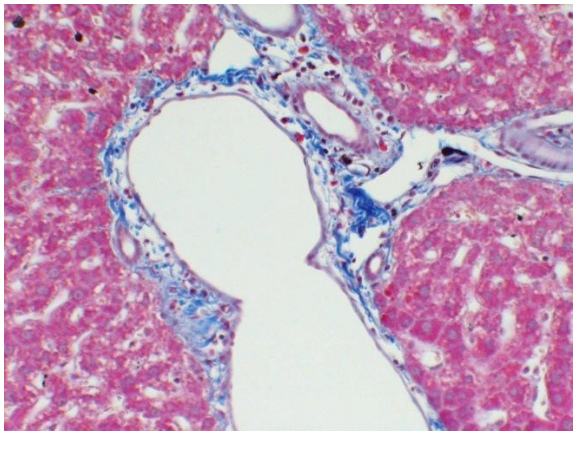
#2 

Masson's trichrome:

Low mag (10x objective)

#3 

Higher mag (20x objective)

#4 

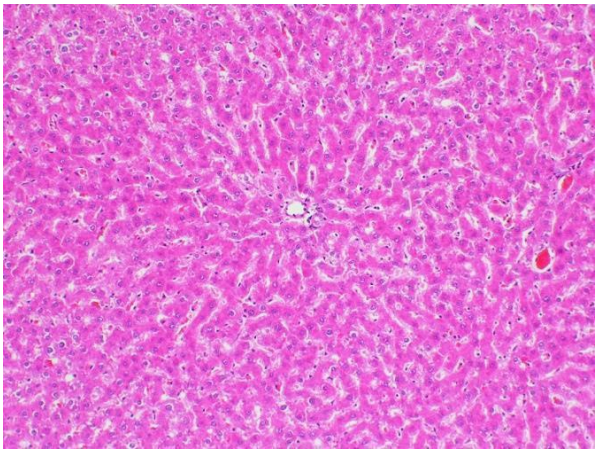
#72 Rat liver, normal: Normal morphology was observed in #72 rat liver tissue (Table 3). Representative H&E images below (Figs. 5-6) show normal radial patterns of the polygonal hepatocytes around the hepatic portal veins. Hepatocytes have normally appearing nuclei with prominent nucleoli and abundant euchromatin (denotes active transcription), and acidophilic cytoplasm. No evidence of pathology was observed (eg. inflammatory cells, necrosis, mitotic figures, or abnormal levels of vacuolization). Representative images (Figs. 7-8) of rat livers stained with Masson's trichrome staining showed normal distribution of blue-stained collagen fibers surrounding the vessels ruling out fibrosis.

Table 3. Tissue #72	Normal/None (0)*	Abnormal/Present (1-4)*
General architecture**	0	
Mitotic figures	0	
Lipidosis/Vacuolization	0	
Necrosis	0	
Inflammation	0	
Fibrosis	0	

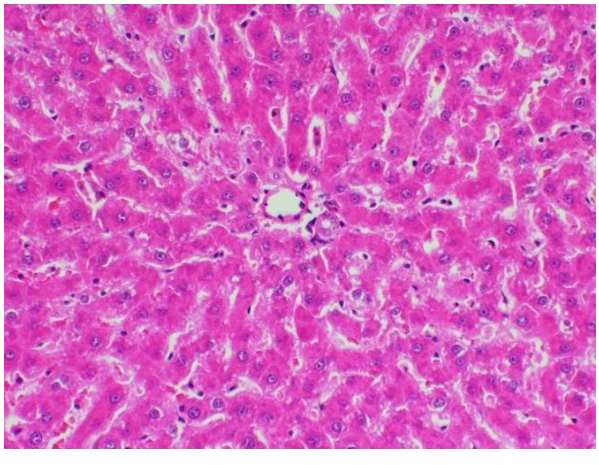
*Based upon 4 random fields (200x mag: 20x objective/10x eyepieces) and ranked none (0), minimal (1), mild (2), moderate (3), and marked (4) pathological changes.
**Organization of hepatocytes, hepatocyte morphology, vasculature, etc.

H&E:

Low mag (10x objective)

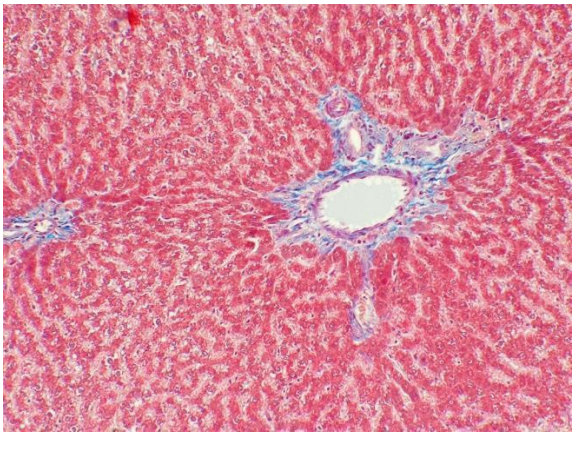
#5 

Higher mag (20x objective)

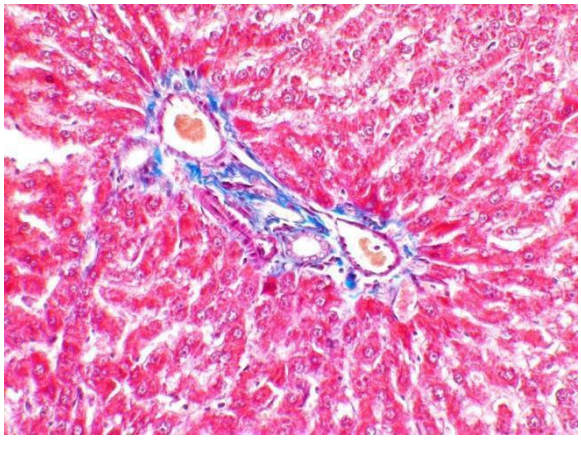
#6 

Masson's trichrome:

Low mag (10x objective)

#7 

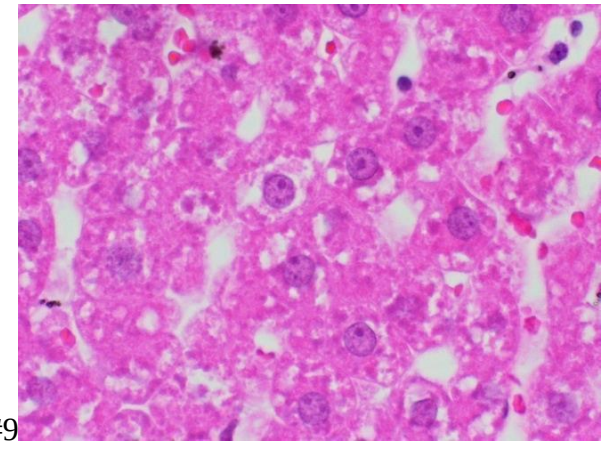
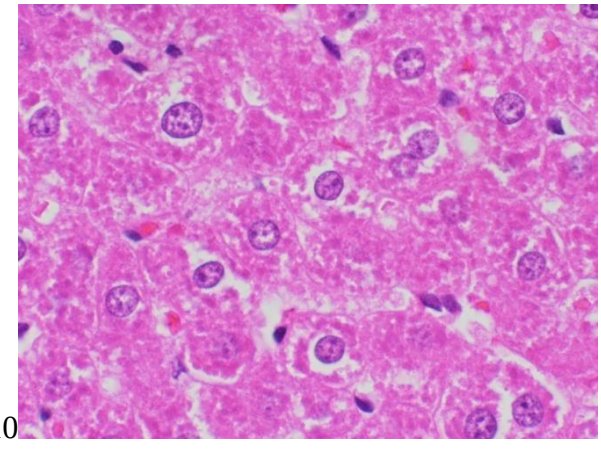
Higher mag (20x objective)

#4 

Conclusions:

The 2 liver specimens, one control (rat #72) and one FNDP-NV treated (rat #61) appear morphologically normal based on established criteria.[1-6] To elaborate, examples of reported pathological features of the liver would include disorganization of the overall hepatic architecture, hepatocyte atrophy (including nuclear degeneration/pyknosis), steatosis as presented by prominent intracellular vacuoles, presence of inflammatory cells, hemorrhage, degeneration of hepatic portal vein walls, proliferative hepatocytes, and necrosis[1-6], none of which were observed in the 2 rat liver tissues (#61, #72).
In addition, the Masson's trichrome staining is among the most common special stains applied to liver specimens. The stain imparts a blue color to collagen against a red background of hepatocytes and other structures. It stains type 1 collagen that is normally present in the portal tracts and vessel walls, but also highlights the presence and distribution of reactive fibrosis as a result of liver injury. It is used for staging of chronic liver diseases, and helps to delineate patterns of injury, such as the perisinusoidal fibrosis associated with steatohepatitis and periductal fibrosis in primary sclerosing cholangitis.[7] However, none of these pathological changes were observed in the 2 rat liver tissues (#61, #72).

Technical considerations.

#9  #10 

I noticed the appearance of glycogen accumulation within the hepatocytes, which is normal. They appear as clear areas around the hepatocyte nuclei (Fig. 9 #61; Fig. 10 #72). It is recommended to try and sacrifice all animals around the same time of the day for similar comparisons as animals from the same study in this reference showed differential staining in the hepatocytes.8 Please also note that this is not a pathological change or worth recording, but is a factor to control (time of the day for the necropsies). Also, if possible, control feeding time among the animals, which is another variable to standardize especially for analyzing livers as a fasting liver will have very low levels of glycogen in the liver (appears are clear spaces perinuclear) while an animal who just ate even 2 hours before fixing the liver, will have very high levels of glycogen.[9]

References:

1. Awaad A. Histopathological and immunological changes induced by magnetite nanoparticles in the spleen, liver and genital tract of mice following intravaginal instillation. J Basic & Applied Zoology. 2015;71:32-47.
2. El-Daly A. The Histopathological, ultrastructural and immunohistochemical effects of intraperitoneal injection with titanium dioxide nanoparticles and titanium dioxide bulk on the liver of the albino mice. J Animal Health and Behavioural Science. 2017;1:1.
3. Ennulat D, et al. Diagnostic performance of traditional hepatobiliary biomarkers of drug-induced liver injury in the rat. Tox Sci. 2010;116(2):397-412.
4. Hegazy AA, et al. Changes in rats' liver structure induced by zinc oxide nanoparticles and the possible protective role of vitamin E. International J Human Anatomy. 2018;1(3):1-16.
5. Ibrahim KE, et al. Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. Molecules. 2018;23:1848.
6. Parang Z, Moghadamnia D. Effects of silver nanoparticles on the functional tests of liver and its histological changes in adult male rats. J Nanomed Res. 2018;3(3):146-153.
7. Krishna. Role of special stains in diagnostic liver pathology. Clinical Liver Diseases. 2013;2(S1):S8-S10.
8. https://ntp.niehs.nih.gov/nnl/hepatobiliary/liver/hglycoacc/index.htm (accessed 3/2/19)
9. http://www.vivo.colostate.edu/hbooks/pathphys/digestion/liver/histo_hcytes.html (accessed 3/2/19)

If interested in normal hepatic histology, visit this nice presentation on the internet:
http://www.vivo.colostate.edu/hbooks/pathphys/digestion/liver/histo_lobule.html

And if you have interest in the pathological NIH guidelines:
https://ntp.niehs.nih.gov/nnl/guide/index.htm

--END OF REPORT--